FY22 Awards Public (Lay) Abstracts

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*Transforming Healthcare through Innovative and **Impactful** Research*

CDMRP
DEPARTMENT OF DEFENSE
CONGRESSIONALLY DIRECTED MEDICAL RESEARCH PROGRAMS

Updated 11Oct2023

Proposal Title:	Cellular Delivery of Protein Therapeutics for the Treatment of Amyotrophic Lateral Sclerosis Using Endogenous Retroviruses
Log Number:	AL220004
Current PI Name:	Tim Luetkens
Award Number:	HT9425-23-1-0195
Current Contracting Organization:	Maryland, University of, Baltimore
Current Performing Organization:	Maryland, University of, Baltimore
Web Approval Date:	03-02-2023

Applicability of the research: Amyotrophic lateral sclerosis (ALS) is a currently incurable chronic disease that results from the death of nerve cells that are needed to transmit signals from the central nervous system to muscle cells. As a result of this process, patients increasingly lose control over their voluntary muscles and most patients eventually die from the disease within 3-5 years. There are currently only two approved treatments for patients with ALS and neither increase the patient's survival. Novel therapies are urgently needed.

While the causes of ALS differ substantially from patient to patient, 97% of ALS patients share one common disease process: The excessive build-up of proteins inside the nerve cells that can cause the cells' death. Other groups have previously developed drugs that when artificially delivered into the diseased cells are able to efficiently reduce this build-up and increase cell survival in the lab setting. However, to date no efficient methods exist to deliver these drugs into diseased nerve cells in patients.

Using our extensive expertise in the engineering of cell therapies, we are now developing a new cell therapy for patients with ALS to deliver these previously developed drugs to the affected nerve cells. In our approach the engineered cells will act as living drug factories that are constantly producing and delivering the drugs to reduce protein build-up to stop or delay disease progression. Over the course of this project we will (1) establish that this new cell-based therapy is effective at reducing protein build-up and able to stop or delay disease progression and (2) carry out essential engineering work to enable efficient manufacturing of this new therapy.

Cell therapies have had remarkable clinical success in other diseases over the past decade not least due to their ability to carry out their therapeutic functions in the patient's body for years. We hypothesize that this ability of the engineered cells to persist for long periods of time will be particularly useful in the case of ALS as it allows the persistent targeting of an also persistent central disease process.

What type of ALS patient will the research help and how will it help them? We anticipate that this new treatment will be particularly effective for patients with a more recent diagnosis of ALS because it has been shown that, in later stages of the disease, ALS cells can acquire additional defects that eventually prevent them from efficiently removing the built-up proteins. Similar to treatments for other neurodegenerative diseases, we envision a disease-modifying mechanism of action for our approach, that will delay or even stop symptom onset and/or disease progression by reducing or preventing death of nerve cells.

What are the potential clinical applications, benefits, and risks? There are different options for the clinical application of our approach. Over the past 10 years, over 1,000 clinical cell therapy trials have been performed in the U.S., each relying on the clinical-grade production of the respective cell therapy product. Manufacturing processes for these trials were either centralized to be performed by a relatively small number of dedicated manufacturing sites throughout the country or decentralized using specialized equipment allowing cell therapy production at individual hospitals. As, at its core, our new approach represents a

conventional cell therapy engineered using the same processes needed for production of other cell therapy products, we envision clinical translation to be straightforward, requiring relatively minimal work to develop clinical trial protocols and establish/ scale up clinical-grade production. If successful, we believe that this approach will result in few toxicities as it specifically targets ALS-associated protein build up and that it has the potential to delay or stop symptom onset and/or disease progression.

Projected time to achieve a patient-related outcome? If successful, at the conclusion of this project, we will have developed a proof-of-concept including demonstrated efficacy of our new cell therapy approach for the treatment of ALS patients. In addition, we will have established a workflow for the production of this new cell product. A streamlined approach for the initiation of clinical testing of cell therapies for the treatment of cancer generally allows the start of clinical trials within approximately one year following proof-of-concept and toxicology studies. As our approach relies on some elements that have not yet been tested in clinical trials, additional toxicology work will likely be requested by the Food and Drug Administration, prior to clinical testing.

Contributions of this study in advancing the development of therapeutics: If successful, this project will provide the basis for a novel form of cell therapy targeting a central disease process in patients with ALS as well as establish key data for its manufacturing and subsequent clinical translation.

Proposal Title:	CHMP7 ASO Therapy: Repair of Nuclear Pore and TDP43 Dysfunction in Sporadic and Familial ALS
Log Number:	AL220009
Current PI Name:	Jeffrey Rothstein
Award Number:	HT9425-23-1-0136
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	08-31-2023

Amyotrophic Lateral Sclerosis (ALS) is a devastating and universally fatal neurodegenerative disease. While multiple genetic mutations have been identified as causative of ALS, the vast majority of cases remain sporadic in nature. Additionally, the precise molecular events leading to neurodegeneration in ALS and related diseases remain largely unknown. Defects in an essential cellular process which maintains communication between nuclear and cytoplasmic compartments of the cell (nucleocytoplasmic transport, NCT) have recently emerged as a prominent pathomechanism underlying ALS and other neurodegenerative diseases. The nuclear pore complex (NPC) is made up of multiple copies of approximately 30 individual proteins (Nups) and tightly regulates NCT. However, the mechanisms leading to alterations in NPC composition and function remain understudied. We recently identified a reproducible subset of Nups that are altered as an early event in disease pathogenesis by using patient derived induced pluripotent stem cells and validating these results in actual patient autopsy tissue.

Furthermore, we discovered the underlying mechanism for this early cellular event, the abnormal nuclear localization of the nuclear pore surveillance protein CHMP7. Using antisense oligonucleotide (ASO) treatment, we can reliably, specifically and effectivity repair this problem, setting the stage for the development of a CHMP7 ASO human therapy. The current program will advance the CHMP7 ASO through the necessary steps to eventually make it ready for human ALS trial in sporadic patients by our collaborating pharma company, Ionis Pharmaceuticals, and their expert team. The plans also include the development of drug efficacy biomarker, so that following therapy one can determine if the drug is, in fact, repairing the abnormal brain cell functions characteristic of ALS. The DOD support will ensure that the therapy can be readied for human trials in a relatively short timeline. Most importantly, this therapy will be targeted for sporadic and familial ALS patients.

Proposal Title:	Targeting Pathogenic Beta-6/Beta-7 Loop Epitope of Misfolded SOD1 to Treat Amyotrophic Lateral Sclerosis (ALS)
Log Number:	AL220010
Current PI Name:	Stanislav Engel
Award Number:	HT9425-23-1-0282
Current Contracting Organization:	Ben-Gurion University of the Negev
Current Performing Organization:	Ben-Gurion University of the Negev
Web Approval Date:	08-31-2023

Amyotrophic Lateral Sclerosis (ALS) is the most common type of motor neuron disease. It is a progressive disease, with patients becoming increasingly dependent on caregivers and physicians as time from the onset increases, and ultimately, it is fatal. ALS can be split into two origins: hereditary, which is known as fALS (familial ALS, ~10% of cases), or idiopathic, known as sALS (sporadic ALS, ~90% of cases). Currently, the only treatment options for ALS are U.S. Food and Drug Administration (FDA)-approved Rilutek and Radicava/Radicut, neither of which can stop nor significantly slow the progression of the disease. At best, these drugs may increase a patient's life span by up to 6months. Therefore, there is a pressing need for more effective therapeutics for ALS worldwide.

Our aim is to develop treatment/prophylactic strategy for fALS patients caused by mutations in the gene encoding for SOD1 protein (approximately 20% of familial cases). SOD1 is a major enzyme responsible for the body's defense against oxidative damage. Mutations cause SOD1 protein to undergo noxious structural transformation, so-called misfolding, rendering it toxic to motor neurons. Current approaches to mitigate SOD1 toxicity in ALS relay on blocking the production of SOD1 proteins in nervous cells. These approaches, being indiscriminative between misfolded and healthy SOD1 proteins, may have a significant drawback of depriving nervous cells of their essential antioxidant potential, crucial for the normal functioning of the nervous system. We, for the first time, demonstrated that blocking a unique surface feature (epitope) exposed exclusively on toxic, but not on healthy SOD1 proteins, with a specific anybody, neutralizes toxic SOD1 species, thus rescuing motor neurons, strongly delaying disease onset and extending survival in animal model of ALS. Our approach is expected to overcome the limitations of the current indiscriminative methods, and minimize the potential adverse effects of anti-SOD1 therapy by targeting only misfolded (toxic) forms of SOD1, while sparing intact proteins, thereby leading to more efficient and safe ALS therapeutics.

The aim of the proposed research is to fully explore the therapeutic potential of anti-SOD1 ALS therapy relying on targeting the specific pathogenic epitope on misfolded SOD1. We will improve the pharmacological properties of the original antibody, to make it more efficient in neutralizing the toxic SOD1 species, and test its performance, once applied at different disease stages, in ameliorating disease in an animal model of ALS. These experiments are crucial for assessing the feasibility of using the proposed anti-SOD1 therapy for the treatment of diagnosed ALS patients. In addition, we will explore a new modality of targeted anti-SOD1 therapy, which is designed to prevent spreading of the toxic misfolded SOD1 proteins among healthy nervous cells, and thus attenuate disease progression. For this purpose, we will develop a vaccination approach based on the FDA-approved mRNA technology, in which the body itself would be encouraged to produce an array of neutralizing antibodies against the pathogenic epitope of misfolded SOD1, and thus help fighting disease.

We anticipate that our therapy has a potential to be effective in the careers of a mutated version of SOD1 gene if applied at the very early disease stages, when substantial damage to the nervous tissue has not been made yet. Moreover, because of the possibility that also SOD1 proteins without mutation may undergo

misfolding, as a result of poorly characterized environmental stresses, and thus contribute to ALS pathogenesis of a significant portion of sporadic ALS cases, our anti-SOD1 therapy may prove effective in a much broader population of ALS patients than just fALS cases with mutated SOD1 gene. Moreover, in recent years, the paradigm of ALS treatment has begun to shift - as a result of advances in genomic sequencing -- toward disease prophylactics, a strategy particularly useful for the carriers of mutated SOD1 gene. Thus, known asymptomatic carriers of mutated SOD1 gene in affected families might be an appropriate population to apply the proposed anti-SOD1 therapy to prevent disease appearance.

Since all methods of the delivery of the therapeutic agents to the nervous system used in the proposed study are FDA-approved clinal tools, the outcome of this study, if successful, might be directly translated into therapeutic application(s), with proceeding to a clinical trial using a limited number of fALS patients with a mutated version of SOD1 gene at the very early disease stage.

Proposal Title:	Platelet-Derived Extracellular Vesicles to Restore Neurovascular Integrity in ALS
Log Number:	AL220022
Current PI Name:	Amit Srivastava
Award Number:	HT9425-23-1-0138
Current Contracting Organization:	Thomas Jefferson University
Current Performing Organization:	Thomas Jefferson University
Web Approval Date:	05-01-2023

Critical Need for ALS Treatment. Amyotrophic Lateral Sclerosis (ALS) is a neurological disease that is driven by the death of nerve cells in the brain and spinal cord. ALS is prevalent in both men and women who are 55 years or older, military Veterans, or may have had prior brain injuries or infections. Initially, patients with ALS experience muscle weakness or stiffness and eventually progress to a loss of voluntary muscle movement, including chewing, walking, talking, and even breathing. Currently, there are no effective treatments to stop the disease from progressing. Most people with ALS die from respiratory failure within 5 years of diagnosis. Recently, it was discovered that the barrier between the circulating blood and the nervous system (including the brain and spinal cord) breaks down very early in ALS. In fact, this breakdown occurs even earlier than the death of nerve cells. The barrier between the blood and nervous system is made of a semipermeable border of cells called endothelial cells. This barrier is essential for maintaining healthy brain and spinal cord tissues and preventing the entry of unwanted particles into the nervous system where nerve cells reside. We have demonstrated that platelet-derived extracellular vesicles (PEVs), which are very small particles secreted from platelets, can repair damaged endothelial cells and improve barrier function. In this project, we will test PEVs in animal models of ALS to determine whether it can reduce or stop nerve cell death and disease progression by repairing the damaged blood-nervous system barrier. Results generated from this project will lay the groundwork for clinical trials to determine whether PEVs can enhance quality of life and potentially help ALS patients survive longer.

Potential Benefits and Risks of PEVs. Therapy using platelet transfusion has been successfully and safely used in many clinical trials and hospital setting. PEVs are very small particles that are naturally secreted from platelets and offer additional benefits to platelets. For example, PEVs are easily soluble in saline, can be stored at low temperatures for extended periods of time, and are easily transportable. Unlike platelets and other cell-based therapy, PEVs do not get trapped in the lung after injection and can reach the nervous system by crossing the barrier between the blood and nervous system. These characteristics offer PEVs as a promising candidate for future translational clinical trials. Clinically, potential therapeutic benefits of PEVs in early-stage ALS patients may include the prevention of disease progression and an overall higher quality of life compared to those without intervention. In late-stage ALS patients, PEVs may potentially reverse neural degeneration, aid in the recovery of motor function, and renew life. Thus, we see little if any risk associated with the use of this drug in treating ALS patients.

Time to Patient Benefit. This project will provide proof-of-concept data that will allow us to initiate human clinical trials in ALS patients soon. It is anticipated that the clinical trials will be supported by the ALS Foundation to assist in patient recruitment and considered for orphan designation in the U.S. and Europe with FDA approval for a clinical trial coming as early as 2027.

Study Contributions to ALS and Other Degenerative Neurological Diseases. PEVs offer a new therapy that can repair the damaged blood-nervous system barrier and provide an innovative approach for treatment of ALS. The efficacy of PEVs in repairing damaged endothelial cells and improving neurological function in ALS animal model will lay the groundwork for clinical trials in ALS patients. As disruption of blood-

nervous system barrier function is also observed in other conditions such as stroke, multiple sclerosis, brain injury, and Alzheimer’s diseases, these studies will also provide a blueprint for the use of PEVs for treatment of other neurodegenerative diseases.

Proposal Title:	Targeting Excessive Sphingolipid Synthesis for Treatment of ALS
Log Number:	AL220025
Current PI Name:	Teresa Dunn
Award Number:	HT9425-23-1-0375
Current Contracting Organization:	Henry M. Jackson Foundation
Current Performing Organization:	Uniformed Services University of the Health Sciences (USUHS)
Web Approval Date:	08-31-2023

Ultimate applicability of the research: A cohort of patients with juvenile ALS have mutations in SPTLC1, which is a subunit of SPT, the enzyme that catalyzes the first step in the synthesis of sphingolipids.

The mutations cause unrestrained SPT activity that leads to excessive accumulation of sphingolipids (SLs). This understanding of the fundamental problem points to an obvious target for a therapy, reducing SPT activity back to the levels seen in unaffected people. Fortunately, inhibitors of SPT are available, including some that have already proven useful in the treatment of mice that have elevated SLs for other reasons. In addition, we have generated cell and animal models of juvenile ALS that will be used in the proposed preclinical studies aimed at identifying the most promising SPT inhibitor(s) for eventual use in clinical trials.

What type of ALS patients will it help and how will it help them? The specific patients most likely to benefit from these studies are the cohort with SPTLC1-related juvenile ALS. Although the SPTLC1-related ALS patient cohort is very small, if preliminary findings of elevated SLs in a broader class of ALS patients holds up, these studies could significantly change the landscape with regard to strategies for treating this intractable disease.

What are the potential clinical applications, benefits, and risks? The discovery that juvenile ALS patients with mutations in SPTLC1 have elevated SPT activity suggest inhibition of SPT as a potential therapy. The cell and animal models that we have developed will be used in the proposed preclinical studies to determine whether this is the case. If we are successful in restoring normal SL levels in the animal models, the stage will be set for moving forward with clinical trials. Because SPT is an essential enzyme, there are risks associated with reducing activity too much, which is why it will be important to establish safe but efficacious dosing in the mouse models. In addition, as with any drugs, there are risks of off-target effects.

What is the projected time it may take to achieve a patient-related outcome? If the SPT inhibitor D-cycloserine (already U.S. Food and Drug Administration-approved for treatment of tuberculosis) or dietary modifications (e.g., high alanine) prove beneficial in the mouse models, clinical trials might follow relatively quickly. In addition, although myriocin is not approved for human use, extracts of the fungus, Cordyceps, used in traditional Chinese medicine, contain myriocin. Significantly, commercially available extracts of Cordyceps are routinely used as a supplement and treatment of mice with comparable doses have proven effective in the treatment of metabolic diseases (diet-induced obesity) associated with excessive SLs. However, it is important to point out that the SPTLC1 ALS patient population is very small, which will confound clinical trials. Of course, if our studies indicate that elevated SLs underlie pathophysiology in a broader class of ALS patients, this will not be an issue.

If the research is too basic for clinical applicability, describe the interim outcomes. These studies are necessary preclinical studies that take advantage of the robust cell and animal models of SPTLC1-ALS we have generated. We hope to unequivocally demonstrate that one or more of the SPT inhibitors is both safe and efficacious for reducing SLs in mice. As mentioned above, numerous neurodegenerative and metabolic diseases are associated with perturbations in SLs, and thus these studies which are focused on strategies for regulating SLs will also impact other important areas of translational research.

What are the likely contributions of this study in advancing the development of therapeutics for ALS? These studies take advantage of new cell and animal models for testing therapeutics for the treatment of SPTLC1-related juvenile ALS. They will be pivotal to identifying mechanism-based treatments for the juvenile ALS patients. A frustrating limitation in the field of ALS research is the lack of a unifying mechanism of pathophysiology across the myriad of ALS disease genes. If this work were to establish perturbed SL synthesis as a common mechanism underlying ALS in other patients, the impact will regard to advancing therapeutics would be enormous.

Proposal Title:	Development of a Novel Treatment Strategy and Unique Methods of Delivery to the CNS for Amyotrophic Lateral Sclerosis
Log Number:	AL220027
Current PI Name:	Sarah Rea
Award Number:	HT9425-23-1-0411
Current Contracting Organization:	Murdoch University
Current Performing Organization:	Murdoch University
Web Approval Date:	08-31-2023

Background information: Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease that is characterised by the loss of motor neurons. In 97% of cases, abnormal aggregates of a protein called TDP-43 are found in post- mortem tissue. ALS researchers believe that the aggregation of TDP-43 leads to motor neuron death and therefore development and progression of disease.

Rationale and feasibility of the proposed research project: We have identified a second protein, p62, that is over abundant in ALS and show that increased levels of p62 causes TDP-43 to aggregate in a manner reminiscent of that seen in ALS-affected neurons. We found that a specific region of p62 is required for this aggregation to occur. We have developed gene-targeted therapeutic strategies that remove this region, and predict that this will prevent abnormal TDP-43 aggregation. Our project will further develop these gene therapies by testing them in patient-derived motor neurons and a TDP-43 mouse model of ALS. We have also identified several compounds that clear TDP-43 aggregates via activation of an intrinsic garbage removal system that is present in all cells, autophagy. Our project will test these autophagy-activating compounds in combination with our gene therapies to definitively establish that our gene therapies, either alone or in combination with these compounds, prevents and rescues the TDP-43 pathology associated with ALS. Lastly, while gene therapies are increasingly being developed for neurodegenerative disorders, delivery of these therapies to the brain and spinal cord (central nervous system, CNS) remains a significant limiting factor in the efficacy of these therapies. Thus, we will test a series of modifications to our gene therapies to identify those that significantly enhance delivery to the CNS.

Applicability of the research: One of the issues with gene therapy for neurodegenerative diseases is that delivery to the brain and spinal cord is minimal due to the presence of the blood-brain-barrier, designed to protect the brain from toxins and pathogens. In this research project we have designed a suite of variations to apply to gene therapies that we envisage will enhance delivery to the CNS. These modifications can then be applied to any gene therapies developed for various neurodegenerative disorders, including ALS.

Beneficiaries of the research: The main intended beneficiaries of the research are individuals with sporadic ALS, that is patients without a known family background or genetic component to their disease. Only 10% of ALS cases are familial, so our research will benefit almost all ALS patients. Our research has highlighted a genetic target that can be manipulated leading to a decrease in the TDP-43 pathology that is present in 97% of ALS cases. It is likely that this will be most beneficial to patients that are treated early following diagnosis. Unfortunately, our therapeutic strategy will not be able to reverse the damage already done as it cannot replace lost motor neurons. However, research by other investigators is underway to identify ways to replace lost motor neurons. Therefore, in future our treatment strategies could be used in conjunction with therapies that are developed to replace those motor neurons.

Potential clinical applications, benefits, and risks: Currently gene therapies like ours are administered directly via injection into the spinal cord, an administration route that has significant potential risks. Our research aims to identify modifications that will allow gene therapies to be administered by injection into the

bloodstream. The outcomes of our work will be applicable to existing and future gene therapies developed for neurodegenerative diseases to enhance their efficacy. Slowing the progression of ALS will extend the productive life of patients, improve their well-being, and reduce the burden on the health care system. Gene therapies can cause toxicity and this needs to be clinically managed. In testing modifications to enhance delivery to the CNS, we may also identify modifications that reduce toxicity.

Projected time to achieve a patient-related outcome? The current project will progress our research to the stage of patenting our gene therapy strategies. Clinical trials could begin as early as 2025. Likely contributions of this study in advancing the development of therapeutics for ALS. Our work will further develop one of our targeted gene therapies that may be of clinical use in up to 97% of ALS cases. Our work identifying modifications to gene therapies that increase delivery to the CNS could be applied to all existing gene therapies and those yet to be developed. The outcomes of our research have the potential to greatly increase the efficacy and cost effectiveness of ALS treatment strategies.

Proposal Title:	Development of Lovastatin or Related Compounds as a Therapy for ALS
Log Number:	AL220032
Current PI Name:	Timothy Miller
Award Number:	HT9425-23-1-0154
Current Contracting Organization:	Washington University in St Louis
Current Performing Organization:	Washington University in St Louis
Web Approval Date:	08-31-2023

Amyotrophic lateral sclerosis (ALS) or Lou Gehrig's disease is a condition in which motor neurons of the brain and spinal cord degenerate. The disease worsens over time, resulting in loss of muscle control and eventual death. One way to better understand ALS is to use information from anonymous health records to see what medical factors are related to whether someone develops ALS and how long they survive with ALS if they get the disease. Using a large Medicare dataset with several years of billing records from anonymous patients with or without ALS, we identified medications that appear to help prevent ALS. We then tested some of these medications in mice. We discovered that one of these medications -- a statin used to treat high cholesterol -- appeared to benefit mice who are genetically predisposed to develop a condition similar to ALS. The mice given the statin lived longer and lost motor neurons slower than untreated mice. In order to better understand whether this medication, or a new similar medication, might help prevent or treat ALS, more research is needed to understand how the drug works to protect the neurons that are damaged in ALS.

In this proposal, we will expand on our prior work to include more anonymous health records with information about patients' cholesterol levels and evaluate cholesterol lowering mechanisms in mice, including mice with different genetics than our original tests. This work will help us understand which target in the body affected by the drug is responsible for motor neuron protection. Our goal for this project is to better understand whether and why the tested medications might be suitable to use in humans to prevent or slow ALS.

With the successful completion of these studies, we will have defined a new target for designing medications to treat all or some types of ALS.

Proposal Title:	Plasma Biomarkers in Amyotrophic Lateral Sclerosis
Log Number:	AL220035
Current PI Name:	Katheryn Cousins
Award Number:	HT9425-23-1-0137
Current Contracting Organization:	Pennsylvania, University of
Current Performing Organization:	Pennsylvania, University of
Web Approval Date:	08-31-2023

Applicability of the research: For people with amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, neurons in the brain (upper motor neurons or UMN) and the spinal cord (lower motor neurons or LMN) progressively degenerate, causing weakness of the muscles and a loss of function. The biology and symptoms of ALS, as well as how quickly the disease progresses, differ from individual to individual. Moreover, there is overlap with related disorders like primary lateral sclerosis, progressive muscular atrophy, and frontotemporal dementia. This diversity in the cause and manifestation of ALS is a major hurdle in the search for a cure and disease-modifying treatments: It can be difficult to disentangle a person's natural disease course from treatment effects. Biomarkers can provide important insights into an individual's disease for clinicians and researchers by helping to define biological subtypes of ALS and to predict long-term clinical outcomes.

Recent technological advancements have made blood-based biomarkers, including plasma, accurate and reliable. Foremost among these blood-based biomarkers in ALS is neurofilament light chain (NfL). NfL levels increase in the plasma even at very early stages of the disease, up to a year before clinical symptoms appear, and have proven useful for predicting when clinically manifest disease will emerge.

Here, we propose to investigate a new blood-biomarker of ALS: plasma phosphorylated tau 181 (p-tau181). While much research has proven the utility of plasma p-tau181 in another neurodegenerative disease, Alzheimer's disease, we find new and robust evidence that plasma p-tau181 is increased with LMN dysfunction in ALS. In this proposal, we evaluate and validate the utility of plasma p- tau181 in ALS. We test performance of plasma p-tau181, both alone and in combination with plasma NfL.

We hypothesize that these two plasma biomarkers used in combination may boost our ability to differentiate between different subtypes of ALS and to predict long-term clinical outcomes. We test these hypotheses in three specific aims:

(1) Classify clinical subtypes of ALS and related disorders using plasma p-tau181 and NfL.

(2) Track how plasma p-tau181 changes with time, and test use of p-tau181 and NfL to predict ALS outcomes.

(3) Evaluate p-tau181 as an early biomarker in individuals at risk for ALS, who do not yet show symptoms.

What types of ALS patients will it help and how will it help them? Plasma p-tau181 is associated with LMN dysfunction and degeneration in ALS. Plasma p-tau181 may be used to track or predict spread of LMN symptoms, and to differentiate ALS and related disorders that have LMN predominant disease. In addition, there is some evidence that plasma p-tau181 is especially elevated in the genetic form of ALS due to a SOD1 point mutation.

What are the potential clinical applications, benefits, and risks? We propose to test plasma p-tau181 in combination with plasma NfL. These two biomarkers are sensitive to distinct aspects of ALS disease processes: While plasma p-tau181 is associated with LMN disease, NfL indicates overall disease severity

and is associated with UMN disease and bulbar signs. This means p-tau181 and NfL may provide different but complimentary information about ALS, and that their combination may have potential clinical application to better characterize the spectrum of ALS and related disorders. Benefits of plasma-based biomarkers include that they are relatively less expensive and less invasive than other biomarker modalities, and that new automated and ultra-sensitive assays have made quantification reliable. Moreover, blood draw can be completed at clinic or via at-home visits, enabling ease of longitudinal monitoring. Risks associated with blood-draw are minimal, most commonly pain or bruising at the site of needle injection.

What is the projected time it may take to achieve a patient-related outcome? Clinical biomarkers have four phases of development, including (1) discovery, (2) assay measurement and technical validation, (3) clinical assessment and validation to demonstrate clinical utility, and (4) clinical application. While plasma p-tau181 is a novel biomarker for ALS, it is well-established in other neurodegenerative diseases; thus we are in Phase 3 for ALS. Based on the U.S. Department of Health and Human Services 2018 Biomarkers report, Phase 3 for prognostic biomarkers can take an estimated 1.5 to 10 years to reach Phase 4 to achieve a patient-related outcome.

What are the likely contributions of this study to accelerating progress toward eradicating deaths and suffering from ALS? We propose to study novel biomarker strategies in ALS to test utility of plasma p-tau181 and NfL for classifying biomarker-defined subtypes within the spectrum of ALS and for predicting long-term clinical outcomes in ALS. Findings may help to refine clinical trial design by informing eligibility criteria and by modeling long-term trajectories to better estimate treatment-related changes.

Proposal Title:	A Brain-Computer Interface for Voice Synthesis in People with ALS
Log Number:	AL220043
Current PI Name:	Sergey Stavisky
Award Number:	HT9425-23-1-0153
Current Contracting Organization:	California, University of, Davis
Current Performing Organization:	California, University of, Davis
Web Approval Date:	08-31-2023

Having the ability to communicate quickly and with depth and nuance is important for a high quality of life, and retaining or regaining this ability is a high priority for people who are losing or have lost their speech due to ALS. Today, patients living with ALS can use existing augmentative and alternative communication devices that use remaining motor functions such as eyeblinks or a sip and puff system. But these devices are slow, cumbersome, and require substantial effort by both the user and caregiver to work reliably. They also do not capture the full range of expression possible by speech. Communicating just by trying to speak would be much faster and easier; we are trying to build a neuroprosthesis that enables people to do just that.

This can potentially be achieved using an intracortical brain computer interface (iBCI): A device that links the brain to external devices. This emerging type of medical technology essentially bypasses the damaged parts of the nervous system and connects healthy parts of the brain to a computer. In this project, we will place hundreds of tiny electrodes into areas of the cortex that are trying to send commands to the muscles of the lips, tongue, jaw, and voice box that would normally produce speech. This computer runs advanced machine learning algorithms that "decode" what the measured brain signals mean; we will develop deep learning techniques that translate brain activity patterns into a voice output that is immediately played out by the computer's speakers. This synthesized voice would be audible to both the iBCI user and whoever they are conversing with so that the speech iBCI user can have natural conversations.

Being able to immediately hear this output also allows the user to practice controlling their new artificial voice. One of the things we will study is whether our participants get better at speaking through their speech iBCIs with time, much like how many people with speech-impairing injuries gradually improve their ability to speak thanks to the brain’s ability to learn and adapt.

The group conducting this study is a consortium of academic research groups called BrainGate, which is the largest and longest running iBCI clinical trial. Previous neuroprostheses developed by this team have successfully provided point-and-click and handwriting communication: clinical trial participants with paralysis of their arms attempted to move their hand either as if moving a computer mouse, or as if handwriting. Our algorithms decoded their neural activity to make character selections displayed on a computer screen with speeds of up to nineteen words per minute. This communication rate represents the best iBCI performance to date, but natural speech is even faster: 150 words per minute. Here we will test whether a similar approach, now applied to directly decoding what the person is trying to say from high precision brain signals provided by these implanted electrodes, can allow patients to speak at speeds approaching this rate. Potential users report high interest in such an assistive technology even though it requires surgery. Thus far this type of implanted brain sensors has an impressive safety and reliability record, and the devices work well for many years. One of the goals of the present study is to collect additional data about the safety of these brain implants, and to track how long they provide useful signals.

Our hope is that the speech iBCIs we will develop will be able to help almost all ALS patients who are going to lose their ability to speak -- or even already have lost this ability. While it is more difficult to build a system to restore speech without having examples of that person’s actual speech and their corresponding brain activity, we have several reasons to believe this is possible. A recent study using a different type of technology that records from the brain’s surface rather than inside the brain found that the neural signals of a

person who had been unable to speak for sixteen years still contained information about what he was trying to say. Furthermore, our team's past work decoding attempted arm and hand movements was all performed with clinical trial participants who were already paralyzed, and yet still achieved very high computer cursor control and writing performance.

Thus, we believe that this project can develop a widely useful speech neuroprosthesis that would allow people living with ALS to fluently perform cherished activities like talking or even singing.

Proposal Title:	Targeting ALS-FUS Aggregation with Proteasome Inhibitors
Log Number:	AL220058
Current PI Name:	Reut Shalgi
Award Number:	HT9425-23-1-0319
Current Contracting Organization:	Technion Research and Development Foundation
Current Performing Organization:	Technion Research and Development Foundation
Web Approval Date:	08-31-2023

Here we propose to examine a specific class of molecules, proteasome inhibitors, as potential disease-modifying therapy for ALS-FUS.

The vast majority of the known ALS drivers have been shown to generate intracellular structures called aggregates, that accumulate in affected neurons and lead to their degeneration. Moreover, it is well known that many of the ALS drivers co-aggregate in different ALS subtypes, including ALS-C9orf72 and ALS-TDP-43; however, FUS-ALS forms distinct aggregates that do not sequester other major ALS drivers, and vice-versa. These observations suggest that the molecular properties of the ALS driver FUS, as well as the molecular properties of FUS, aggregates in the disease, are different than those of other ALS drivers. This notion calls for therapeutic strategies that are specific for ALS-FUS.

Using a highly quantitative molecular readout for mutant FUS aggregation in cell models, the Shalgi lab has found that, in sharp contrast to other ALS drivers, as well as to other neurogenerative disease aggregating proteins, treatment with a class of small molecule drugs, namely proteasome inhibitors, leads to a marked reduction in the aggregation of FUS harboring ALS-associated mutations.

This class of molecules is known to primarily inhibit the process of protein degradation in cells. Accordingly, the current dogma in the field stated that treatment with proteasome inhibitors should in fact enhance aggregation, rather than inhibit their formation. Nevertheless, in contrast to the dogma in the field, and to other aggregating proteins, our observations of reduced mutant FUS aggregation following proteasome inhibitor treatment recapitulated for a number of different types of these molecules.

Thus, in the current project, we propose to take these initial observations to the next level, and to establish the potential of proteasome inhibitors as specific disease-modifying therapy for ALS-FUS, with the ultimate goal of preventing FUS aggregation, and thereby slowing down disease progression.

Importantly, one of the strengths of the proposed study is that it will focus both on efficacy studies, in model systems and in animal models, as well as on unraveling the mechanism underlying this specific aggregation prevention effect on mutant FUS, examining a variety of mutations. Thus, a successful outcome of this study bears great therapeutic potential, as it will offer a proof of concept for efficacy as well as mechanism-driven therapy.

While the current study is aimed at examining various sub-classes of proteasome inhibitors in neuronal models and in animal models of ALS-FUS, several derivatives have been shown to cross the blood-brain barrier and effectively penetrate into neurons. Importantly, some of these derivatives are currently in phase-III clinical trials for other diseases. Therefore, a successful outcome of the proposed research will pave the way for potential drug repurposing, thereby providing a relatively faster track for moving forward into the clinic, in order to offer a therapeutic avenue for patients with ALS-FUS.

Proposal Title:	A 13-week GLP toxicology study with SPG302 in Beagle dogs
Log Number:	AL220059
Current PI Name:	Vincent Simmon
Award Number:	HT9425-23-1-0580
Current Contracting Organization:	Spinogenix Inc.
Current Performing Organization:	Spinogenix Inc.
Web Approval Date:	08-31-2023

There is a great unmet need for innovative new therapeutics for ALS (Lou Gehrig's disease), a degenerative disease of motor neurons in the brain and spinal cord that is almost invariably fatal within 3-5 years of diagnosis. The therapies that are currently approved for ALS provide very modest extension of life of several months and are not well tolerated by all patients. The goal of this proposal is to advance the development of a novel small molecule, SPG302, as a new potential therapeutic for patients with ALS by completing required animal toxicology studies as agreed to by the FDA. SPG302 has a unique mechanism of action wherein it induces an increase in synapses, the key connections between neurons that allow us to think, plan, remember, and control motor functions, faculties that are diminished in neurodegenerative diseases including ALS. Recent data indicate that a loss of dendritic spine synapses is an early and progressive feature of ALS pathogenesis that may contribute to both motor and cognitive symptoms. Spinogenix has developed drugs that regenerate these lost synapses. These drugs improve motor function and survival in an aggressive mouse model of ALS, as well as neural network function in ALS patient-derived induce pluripotent stem cells and respiratory motor function in a rat model of spinal cord injury. Biomarker evaluation of a synapse-specific PET imaging tracer is ongoing in preclinical models, which if successful, can easily be translated into patient trials. To our knowledge, this is the only current therapeutic under evaluation to regenerate synapses lost in ALS. Spinogenix's novel approach has the potential to demonstrate that replacing lost synapses can provide a meaningful benefit for patients with ALS. Therefore, our lead compound could be used as a monotherapy and, in principle, as a co-therapy with any among the large and diverse set of emerging therapeutics aimed at inhibiting neurodegeneration in ALS patients.

Proposal Title:	Identification of a Novel Therapeutic Target and Strategy for C9-ALS
Log Number:	AL220061
Current PI Name:	Nicolas Charlet-Berguerand
Award Number:	HT9425-23-1-0679
Current Contracting Organization:	Ctre Europeen de Recherche en Biologie et en Medecine
Current Performing Organization:	Ctre Europeen de Recherche en Biologie et en Medecine
Web Approval Date:	08-31-2023

Amyotrophic lateral sclerosis, also known as Lou Gehrig's disease, is a devastating neurodegenerative disease leading to muscle weakness and paralysis leading to a fatal conclusion, typically in 3 to 5 years after the age of onset.

The most common cause of ALS is a genetic mutation in a gene known by its acronym: C9ORF72. This mutation is present in 50% of ALS families in North America and Europe and is highly atypical as it consists of hundreds to thousands of repetitions of a small DNA sequence (GGGGCC).

Equally unusual is that this mutation is toxic through the non-canonical translation of these DNA repeats into novel proteins composed of repeated motifs and known by another acronym: DPR proteins. These DPR proteins are highly toxic for neurons.

We and others recently refined the mechanism of production and the toxic characteristics of these DPR proteins. Importantly, these data unveiled novel pathogenic sequences that can be used as therapeutic targets. In that proposal, we plan to take advantage of these findings to test new molecules (antisense DNA oligonucleotides) aimed at blocking the expression of these toxic DPR proteins in human neuronal cells and pre-clinical animal models.

In short, we uncovered novel sequences that could be of medicinal interest in ALS. Thus, in this funding request, we propose to generate pre-clinical data in cell and animal models to test therapeutic approaches for this devastating neurodegenerative disease .

Proposal Title:	Small-Molecule-Mediated Upregulation of G3BP1 as a Therapy for ALS
Log Number:	AL220062
Current PI Name:	Dominque Cheneval
Award Number:	HT9425-23-1-0262
Current Contracting Organization:	Novaton Pharmaceuticals, Inc.
Current Performing Organization:	Novation Pharmaceuticals Inc.
Web Approval Date:	08-31-2023

Amyotrophic lateral sclerosis (ALS) also known as Lou Gehrig's Disease, is a neurodegenerative disease for which there is no effective treatment. The disease affects motor neurons, which are nerve cells that control the voluntary movement of muscles. These cells are under constant stress in ALS patients and eventually die, resulting in paralysis and death. An ancient and critical cellular response is to protect cells by temporarily halting the production of proteins (functional components of cells). This is accomplished by the formation of small containing cytoplasmic particles, called stress granules. Disruption in the formation or disassembly of these granules results in cell death, including the types of cells that are lost in ALS, and this process may be defective in ALS. Prior work suggests that the redistribution of TDP-43 from its usual location in the nucleus to the cytoplasm is associated with an impaired stress granule response in neurons. This proposal aims to restore this essential cell survival mechanism using small molecules as a potential therapeutic strategy for ALS by exploiting recent findings of how it is regulated. Since TDP-43 mislocalization happens in the majority of cases, it is anticipated that this small molecule drug approach would be broadly applicable to the majority of people affected by ALS. This proposal will enable lead selection and provide a critical appraisal of whether targeting this stress response could be a viable therapeutic strategy using mouse models, and thus pave the way for downstream studies to move this approach closer to clinical trial.

Proposal Title:	Preclinical Evaluation of ASK1 Inhibitor SRT-055 as a Potential Development Candidate for ALS
Log Number:	AL220063
Current PI Name:	Johannes Grosse
Award Number:	HT9425-23-1-0182
Current Contracting Organization:	SEAL ROCK THERAPEUTICS, INC.
Current Performing Organization:	SEAL ROCK THERAPEUTICS, INC.
Web Approval Date:	08-31-2023

Amyotrophic lateral sclerosis (ALS) is a devastating neurological disease that affects primarily the ability to move the limbs, later followed by problems with speaking and swallowing. Failure of the muscles needed for breathing leads eventually to death. The disease begins typically in the sixth or seventh decade. Most patients die within 3 to 4 years after the first symptoms are recognized.

Genetic and environmental risk factors together with aging trigger the disease onset: The genetic causes of approximately 20% of ALS cases are known, which has enabled the development of reliable mouse models of the disease. In addition, cells obtained from ALS patients can be reprogramed to become stem cells. These induced pluripotent stem cells, or iPSC, can be differentiated in cell culture dish to become motor neurons, the critical nerves that control muscles and are lost as the disease progresses. These iPSC-derived motor neurons provide another important model to study the disease and assess the prospects of potential ALS treatments. Both disease-causing mutations as well as normal aging lead to severe oxidative stress in these motor neurons, irreparably damaging proteins, cellular organelles such as the mitochondria and the DNA in the cell nucleus over time. This explains why the mutations do not immediately cause problems in young people. Only the combined hit from the mutations together with the normal aging process leads to the demise of these cells.

ASK1 drives the death of motor neurons, and its inhibition is a promising therapeutic approach: This cell death is a tightly regulated process in which a protein known as apoptosis signal-regulating kinase 1 (ASK1) plays a central role. We have developed a small molecule drug, SRT-055, which inhibits the signaling function of ASK1. We have tested SRT-055 in simpler cellular models where the oxidative stress was induced with hydrogen peroxide. Encouragingly, the SRT-055 treated cells were well protected from the hydrogen peroxide induced cell death. Now, as part of the proposed study, we aim to test this protective effect of SRT-055 directly in ALS patient iPSC derived motor neurons. In this case the mutation causing the disease is triggering the oxidative stress.

To enable testing of SRT-055 in a mouse model of ALS as a key next step in the study proposal, we will first evaluate different solubility and formulation conditions of SRT-055 to enable optimal absorption after oral administration. Administering SRT-055 to mice, we will measure how much is absorbed from the bowel, how long it stays in the organism, and, importantly, whether it gets into the brain and spinal cord, where the motor neurons are found.

These key pharmacokinetic studies will enable the identification of an appropriate dosing schedule for the next experiment, testing the efficacy in a mouse model of ALS. These mice express the mutant version of a protein which causes ALS in humans. The mice start to show weakness in their limbs at an age of 12 weeks and decline for the next 6 weeks eventually leading to death. During this period SRT-055 will be given daily and, as a key indicator of efficacy, we will monitor whether SRT-055 treatment can slow down the decline in muscle strength and extend survival of the mice.

Next steps toward a therapy for ALS after this project is completed: If, in the project described here, SRT-055 turns out to be efficacious by slowing muscle weakness progression and extending animal survival, the compound will be validated and moved forward to an even more rigorous and comprehensive test program where broader and more in-depth pharmacokinetic characteristics and toxicity effects will be studied. This preclinical development phase is anticipated to take about 1 year, after which SRT-055 or a similar compound could enter clinical studies in ALS patients with the aim of slowing or even completely halting the human disease progression.

Proposal Title:	Senotherapy for ALS
Log Number:	AL220064
Current PI Name:	Davide Trotti
Award Number:	HT9425-23-1-0258
Current Contracting Organization:	Thomas Jefferson University
Current Performing Organization:	Thomas Jefferson University
Web Approval Date:	08-31-2023

Evidence is emerging that brain senescence associated with progressive brain inflammation is one of the mechanisms underpinning neurodegenerative diseases, including ALS. Hallmarks of brain senescence are present in ALS models and patient post-mortem tissues. Although there remains a gap in knowledge between the observations of brain senescence and its direct causative role in neurodegeneration, harnessing the therapeutic potential of targeting senescence to stop disease progression could address the urgent clinical need for treatments. Indeed, it was recently demonstrated that this approach could attenuate the cognitive decline seen in an Alzheimer's preclinical model. In C9orf72 linked to ALS/FTD, the extent and contribution to the disease of senescence have not yet been tested. We propose to characterize and validate a C9orf72 ALS /FTD cell-based model in which we can study the basic biology of senescence.

Several therapeutic strategies, known as senotherapies, have been developed to eliminate tissue-specific cells undergoing senescence or senescent cells. However, almost none have been tested in models of ALS. Senescent cells provide ideal targets for cell-based immunotherapy (a.k.a. CAR-T cell immunotherapy). CAR-T cell immunotherapy was first applied for cancer. Several CAR-T cell immunotherapies are now approved by the U.S. Food and Drug Administration for different types of cancer, and several are being tested in clinical trials. We will build upon our expertise in CAR-T cell design and cancer applications to directly measure the ability of this approach to clear senescent cells for C9orf72-ALS/FTD therapy, in combination with IFNgamma and IL-6 inhibitors to preserve CAR-T cell efficacy while suppressing the potential cytokines side effects. Several small molecules have already been identified to exhibit anti-senescence activity, but most lack potency and produce substantial side effects. Here, we try the therapeutic concept that CAR-T cells targeting senescent cells can be an effective anti-senescence therapy.

CAR-T cell research has advanced rapidly in the clinic for different types of cancer. It is back to the bench, with trial results informing new mechanisms of efficacy, toxicity, and resistance and catalyzing the search for new targets and application of novel technologies. Innovation in CAR-T design and methodologies are bound to lead to improved responses and transform the treatment of patients, potentially including patients with neurodegenerative conditions. Should this study provide promising results, we will seek to advance the CAR-T cell therapeutic approach to different pre-clinical models of ALS and C9orf72 ALS/FTD.

This project is focused on a fast-to-patient development program. After developing a preclinical proof of concept, a CAR-T cell product will be advanced into a subsequent Phase I clinical trial in patients with C9orf72-ALS/FTD, with objectives establishing that this new approach is safe and well-tolerated. Validation of the CAR-T cell anti-senescence therapeutic approach could be extended to other forms of ALS, including sporadic ALS, as a next step of this project. Proceeding directly to clinical testing in ALS patients is also appropriate. CAR-T cell approaches have already been tested for different brain cancers, including certain lymphomas of the central nervous system. The Weinberg ALS Center, in which this study will be conducted, is also an appointed member site for NEALS, a nation-wide consortium for centralized ALS clinical trials, whose member sites are medical institutions equipped and set up to perform large ALS clinical trials. This arrangement will allow for a rapid translation of scientific advances from this project into clinical research and new treatments for people with ALS.

Proposal Title:	Development of Clinical Biomarkers for Molecular Subtypes of Sporadic ALS
Log Number:	AL220065
Current PI Name:	Molly Gale Hammell
Award Number:	HT9425-23-1-0198
Current Contracting Organization:	Cold Spring Harbor Laboratory
Current Performing Organization:	Cold Spring Harbor Laboratory
Web Approval Date:	08-31-2023

Amyotrophic Lateral Sclerosis (ALS) is a complex disease. Several inherited gene mutations are known to cause ALS. However, for 90% of those living with ALS, they have no close family members with ALS or known gene mutations that caused the disease. Despite this variation in genetic contributions to ALS, several other commonalities have been identified. For example, nearly all patients show accumulations of a protein called TDP-43 in the tissues affected by this disease (brain and spinal cord). Moreover, work from our own laboratories as part of the New York Genome Center (NYGC) ALS Consortium has shown that there are clear patterns of RNA and protein changes that happen in the brain tissues of people with ALS (pALS). Rather than a single pattern of RNA and protein changes for all pALS cases, we have shown that there are three characteristic patterns seen in brain tissues. These three characteristic patterns of RNA and protein changes have been called ALS sub-groups. ALS sub-groups can be defined by whether the brain tissues display signatures of mitochondrial dysfunction and oxidative stress (ALS-Ox), activation of pro-inflammatory cells (glia) and neuroinflammation (ALS-Glia), or dense TDP-43 pathology and associated elevation of transposable elements (ALS-TE).

Transposable elements are viral-like pieces of our genome that are normally silent, but can become activated in response to stress. The patterns identified in the NYGC ALS patient samples (mitochondrial dysfunction, inflammation, and TDP-43 pathology) have been previously implicated in ALS disease by many different groups. Many current ALS therapeutics are directed toward one or more of these dysfunctional processes. This raises the possibility that patients of a given ALS sub-group might respond better to targeted therapeutics that address the underlying processes at play in their specific subset of the disease. However, the ability to stratify patients into these ALS sub-groups requires identifying clinical biomarkers from tissues or fluids that would be available at diagnosis, for example, blood, cerebrospinal fluid (CSF), or muscle biopsies. In order to identify the best tissues or fluids for biomarkers, one must first collect matched samples from the same patients: First, from tissues where the ALS sub-groups are known to be identifiable (brain tissue), and, second, from tissues or fluids that would be available at diagnosis (muscle, CSF, and/or blood). This project has identified several biobanks and repositories that have those matched samples from dozens of pALS cases. We will leverage these resources to identify biomarkers that can stratify pALS cases into ALS sub-groups.

Proposal Title:	RP-115: A Novel Biomarker of Astrocyte Dysfunction and Glutamate Dysregulation in Brain and Spinal Cord in ALS
Log Number:	AL220073
Current PI Name:	Michael OSullivan
Award Number:	HT9425-23-1-0465
Current Contracting Organization:	University of Queensland
Current Performing Organization:	University of Queensland
Web Approval Date:	09-01-2023

Amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease or more often referred to in some countries as Motor Neuron Disease) is a progressive, degenerative neurological condition that affects the limbs and the muscles that control breathing and swallowing. In some individuals, other parts of the brain are also affected leading to a form of dementia. ALS involves selective degeneration of nerve cells that control movement. One pathway to cell death in ALS is excessive stimulation of motor neurons, or excitotoxicity. The major excitatory transmitter in the nervous system is called glutamate and glutamate levels are controlled by a transporter protein called EAAT2. There is currently no type of scan that detect these specific biochemical changes. This is a limitation not only for the diagnosis of ALS but also for the development of new treatments. Rio Pharmaceuticals have discovered a molecule called RP-115 which can be turned into a dye to show the levels of EAAT2 in the nervous system. This project will test whether RP-115 can be used as a marker of critical changes to cells in the brain and spinal cord in ALS.

The project will implement and test the utility of RP-115 imaging with PET as a disease biomarker in ALS. Ultimately, this project will accelerate the development of transformative new therapies for ALS. It will develop a new specific imaging biomarker that can be used to select the most promising candidates for full-scale trials.

All types of patient with ALS are potential beneficiaries of a more rapid and efficient pipeline for therapeutic discovery. By providing an objective marker of disease RP-115 will reduce disparities in access to trials, thereby benefiting minority groups. An imaging biomarker is particularly well-placed to help patients with early memory and cognitive symptoms by providing objective insight into the pattern of damage in the brain. The potential clinical applications also include diagnosis, with the benefit of enhanced diagnostic certainty in some subtypes of ALS. There are no major risks.

The current project will take 2 years to establish the utility of this biomarker for use in patients with ALS, producing evidence of safety and acceptability, preliminary data to design larger studies and trials and an emerging trans-Pacific network to support those trials. Emergence of a new therapy, first tested with RP-115, is expected within 5 years of beginning this program.

Eradication of death and suffering from ALS requires transformative new therapies. The contribution of this project will be to drive the selection of the most promising candidates, reducing the number of failed trials, and potentially reducing the time to transformative therapies by many years.

Proposal Title:	Clinical Development of ISGylation Diagnostic Biomarker for ALS
Log Number:	AL220079
Current PI Name:	Shyamal Desai
Award Number:	HT9425-23-1-0257
Current Contracting Organization:	Louisiana State University Health Sciences Center
Current Performing Organization:	Louisiana State University Health Sciences Center
Web Approval Date:	09-01-2023

This grant aims to establish a new non-invasive biomarker for the quick diagnosis of amyotrophic lateral sclerosis (ALS), traumatic brain injury (TBI), or both in veterans and civilians, as well as two distinct prototype assays (WesTM and sandwich ELISA) for measuring this biomarker in clinical settings. ALS is also known as Lou Gehrig's disease. It is a rare and incurable neurodegenerative disease caused by the slow deterioration and death of motor neurons, which are specialized cells in the brain and spinal cord that control the muscles throughout the body that mediate voluntary movements, including chewing, walking, and talking. Permanent motor neuron loss leads to paralysis and eventual death, generally from respiratory failure. There are two forms of ALS: sporadic ALS (approximately 90% of cases) and familial ALS (approximately 5%-10% of cases). In both forms, the symptoms are the same (consequences of motor neuron loss) and the average life expectancy is about 2 to 5 years from the time of diagnosis. Although ALS is rare in the general population, research supported by the U.S. Department of Veterans Affairs (VA) and Department of Defense (DoD) has revealed that military Veterans are at a nearly 60% greater risk of ALS than people with no history of military service. The causes of ALS are not fully understood in civilians or Veterans. However, TBI has been identified as a risk factor for ALS in Veterans. TBI due to traumatic incidents (e.g., falls, assaults, car accidents, etc.) is a major cause of death and disability among American civilians and Veterans. For both populations, ALS-like symptoms have been detected in those afflicted with TBIs.

Although there is no cure for ALS, two U.S. Food and Drug Administration-approved medications, riluzole (Rilutek) and edaravone (Radicava), prolong life by a few months in some patients and, most importantly, slow disease progression best in patients who are in the early stages of disease. Hence, there is an urgent need to find diagnostic markers that can be used for the early diagnosis of ALS in Veterans and civilians unexposed/exposed to TBI to begin treatment earlier and slow the disease progression. Excitingly, my group has identified a potential diagnostic biomarker known as "ISGylation" -- a biological process wherein a small protein called ISG15 (Interferon-Stimulated Gene 15) is attached to various other proteins and alters their normal cellular functions -- that could help inform the diagnosis of ALS (without the previous history of TBI) and TBI- ALS (TBI-exposed Veterans who are later diagnosed with ALS) in Veterans. We have found that ISGylation is elevated in the spinal cords and brains of individuals with ALS and TBI-ALS. More recently, we found ISGylation circulating in the cerebrospinal fluid (CSF), a colorless body fluid that surrounds and protects the brain and spinal cord, and blood and urine samples of ALS patients. Together, these findings suggest that ISGylation is a promising potential liquid biopsy biomarker for the diagnosis of ALS and TBI-ALS Veterans and civilians. We do not know if ISGylation can detect a TBI exposure in non-ALS Veterans or civilians. In the current grant, we aim to explore the clinical utility of using ISGylation biomarker for ALS, TBI-ALS, and TBI conditions using liquid biopsies (CSF, blood and urine) archived in biorepositories and/or collected from living patients.

Our ultimate goal is to develop and commercialize an ISGylation biomarker assessment tool to advance ALS patient care. We have already established a quantitative 3-hour automated test (WesTM assay) to measure ISGylation in liquid biopsies. In the current application, we propose to develop a 15-min specialized assay called a sandwich ELISA assay for measuring ISGylation in liquid-biopsies. For that, I have assembled an outstanding team of investigators with a track record of expertise in the clinical development of a rapid 15-min blood test using a hand-held portable device called Abbott i-STATTM to measure two TBI biomarkers.

The sandwich ELISA assay that will be developed using this grant, can serve as a precursor in the development of a similar i-STATTM test for ALS and TBI-ALS, and plausibly TBI diagnosis. We will obtain/collect liquid biopsies from men and women Veterans/civilians diagnosed for ALS (with distinct genetic mutations and disease stages) and following acute/chronic TBI and 4 hours to 4 days post-TBI, for ISGylation assessment using WesTm and sandwich ELISA assays. This study plan would help us to determine if an ISGylation biomarker could be used for early diagnosis, progression, and follow up of ALS, TBI-ALS, and TBI conditions in Veterans and civilians. When successful, this early diagnosis may help physicians to start riluzole and edaravone therapies in the early stages of the ALS disease and guide TBI treatment decisions, when they are expected to have their greatest impact. Moreover, it may help ALS patients prepare for the upcoming challenges by proactively seeking out appropriate resources and care. In summary, this study will develop a new ISGylation biomarker for the quick diagnosis of ALS, TBI-ALS, and TBI conditions in Veterans and civilians, as well as two distinct prototype assays (WesTM and sandwich ELISA) for measuring ISGylation in clinical settings. This line of research would, therefore, significantly impact the clinical diagnosis of ALS and TBI-ALS, and TBI in Veterans and civilians.

Proposal Title:	Characterizing Microbial Markers Predictive for ALS Onset and Progression
Log Number:	AL220081
Current PI Name:	Michael Morrison
Award Number:	W81XWH-22-0-AL220081
Current Contracting Organization:	LAWRENCE LIVERMORE NATIONAL SECURITY, LLC
Current Performing Organization:	Lawrence Livermore National Laboratory
Web Approval Date:	08-31-2023

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with progressive loss of motor neurons. No effective neuroprotective therapy exists, and individual outcomes show considerable variation. ALS is a recognized military service-related condition; rates of ALS are higher among U.S. military Veterans, who are exposed to intense physical, emotional, and environmental experiences. Deployed Veterans are at greater risk than non-deployed Veterans. Significant heterogeneity in disease onset and progression suggests environmental risk factors play a role. Therefore, the gut microbiome, potentially vulnerable to these risk factors, could represent an integrator of overall environmental contribution to neurodegeneration.

Rationale and Feasibility: The human microbiome is highly individualized and inseparable from the human ecosystem. The gut microbiome affects our immune system, fitness, nutrition, and susceptibility and resilience to disease. Specifically, we hypothesize that an individual's gut microbiome affects their susceptibility to neurodegenerative diseases, including ALS. Early studies using limited numbers of ALS patients and controls have indicated potential differences in microbiome profiles. Using a large cohort of ALS patients with well-characterized demographic and clinical history, coupled with state-of-the-art molecular and advanced computational analyses, our study will potentially identify significant biomarkers predictive of ALS risk and progression. Of note, given the microbiome's highly individualized nature, it is perfectly positioned for future applications of precision medicine, i.e., personalized treatment based on an individual's unique biology and situational needs. Thus, microbiome intervention holds tremendous promise for precisely tuning and improving human health.

Scientific Objective: Our overall objective is to identify microbial biomarkers that are predictive of ALS disease risk and progression. In this study, to deeply characterize the microbiome features that are unique to ALS patients, we will use a large number of ALS patients -- including Veterans -- from Northern California, hailing from both metropolitan and rural areas, alongside controls matched for environment, diet, sex, and age. These ALS patients will all be tracked with diagnostic data and measures of disease progression, such as the ALS Functional Rating Scale and breathing capacity. We will use advanced computational and statistical methods to identify the microbial biomarkers that are most predictive of ALS disease risk and progression.

Applicability of Research:

Targeted Patient Populations: Successful microbiome therapy would help both civilian and military ALS patients, hopefully preventing disease onset and slowing progression for patients with ALS. Identifying the key components of the microbiome associated with ALS will enable development of therapeutics to help repair the dysbiosis caused by an imbalanced microbiome and create a healthier gut microbiome that is more resilient to ALS risk and progression.

Clinical Applications, Benefits, and Risks: Targeting the key microbiome biomarkers that are more predictive of ALS disease could serve as a low-cost therapy for ALS patients. These bacteria could also be further used to develop targeted antibiotics, microbe-derived metabolites, and/or probiotics accessible to groups at high risk of ALS and to potentially begin protecting our military personnel from this fatal disease before deployment.

Projected Timeline to Patient-Related Outcome: Therapeutics targeting key identified microbial biomarkers of ALS can be developed into simple oral capsules to treat gut dysbiosis. Currently, various ongoing microbiome therapy-based clinical trials are exploring treatments such as sodium phenylbutyrate-tauroursodeoxycholic acid. Thus, leveraging prior microbiome therapy success, outcomes from our study can be used to develop treatments in 3 to 5 years to slow disease progression.

Contributions to Accelerating ALS Research: Microbiome intervention holds tremendous promise for precisely tuning and improving human health. Microbiomes, with their highly individualized nature, are perfectly positioned for future applications of precision medicine. Given that ALS development is also highly variable and influenced by environmental factors, microbiome therapy could potentially be the solution to slowing disease onset and progression.

Proposal Title:	Identifying RNA Processing Factors That Restore Expression of Motor Neuron-Enriched Genes in ALS
Log Number:	AL220085
Current PI Name:	Daniel Mordes
Award Number:	HT9425-23-1-0197
Current Contracting Organization:	California, University of, San Francisco
Current Performing Organization:	California, University of, San Francisco
Web Approval Date:	08-31-2023

Amyotrophic lateral sclerosis (ALS), also called Lou Gehrig's disease, is characterized by the rapid and progressive loss of motor nerve cells that control muscle contraction. Detailed examination of patient spinal cords under the microscope has consistently demonstrated that changes in the location of RNA binding proteins occur in sick or dying nerve cells in nearly all cases of ALS. Additionally, in several families with inherited forms of this disease, mutations in RNA binding proteins that reside in the nucleus of nerve cells are sufficient to cause disease. However, it is not clear how changes in RNA processing cause nerve cells to stop functioning properly in this devastating disease.

Several advances in stem cell technology have enabled skin cells to be converted into proliferating stem cells that our lab then uses to generate millions of motor nerve cells in a dish, enabling studies of the cell type that is most affected in ALS. By directly studying human motor nerve cells, we can study causes of ALS and test potential treatments with a higher likelihood of success. We have previously used this method to identify molecular signs that indicate when neurons are stressed in disease. In this research proposal, we will investigate the relationship between RNA binding proteins and RNA-associated cellular pathways previously linked to ALS to better understand how this disease progresses. Specifically, we will examine how these proteins affect the expression of genes that are required to keep neurons healthy. Based on advances in gene-specific therapies, we will develop candidate therapeutics using stable nucleic acids called antisense oligonucleotides (ASOs) and validate their potential beneficial effects on nerve cell survival and regeneration. ASO-based therapies are advantageous because they can be designed to precisely alter the expression of a specific gene and can reach new drug targets that were previously thought to be undruggable. Recent clinical trials have demonstrated that delivering ASOs into the nervous system of ALS patients is safe and that ASOs likely function as expected upon reaching their targets in neurons. Upon the completion of this study, we expect to have candidate therapies that can be further modified and tested in relevant ALS animal models. By combining these technologies, our novel experimental approach will provide clear routes for the creation of safe and effective treatments for ALS.

Proposal Title:	Using CSF1R Suppression to Reprogram Microglia to a Neuroprotective State in ALS
Log Number:	AL220086
Current PI Name:	Justin Ichida
Award Number:	HT9425-23-1-0370
Current Contracting Organization:	University of Southern California
Current Performing Organization:	University of Southern California
Web Approval Date:	09-01-2023

Ultimate applicability of the research: There is an urgent need for therapeutics that slow disease progression in amyotrophic lateral sclerosis (ALS) patients. Recent ALS clinical trials targeting well-validated disease mechanisms in nerve cells have yielded disappointing results. This underscores a longstanding notion that disease processes in the cells surrounding the nerve cells may actually determine the rate of disease progression in mid-late stage ALS patients. Studies suggest that one of the non-nerve cell types that plays a key role in disease progression is the immune cell of the nervous system, known as the microglial cell. Thus, there is a pressing need for therapeutic strategies that mitigate microglial disease processes in ALS.

By studying microglia and nerve cells derived from ALS patients, we have identified a new potential therapeutic approach that targets microglia and may work for both familial and sporadic ALS. In the proposed study, we will use patient-derived microglia and nerve cells, as well as ALS mouse models, to test the efficacy of using antisense oligonucleotides (ASOs) to silence a gene called CSF1R in microglia. We predict that silencing CSF1R in microglia will "reprogram" them, or stop them from being toxic to ALS nerve cells and cause them to be protective of ALS nerve cells. We anticipate that our findings will facilitate the development of an intrathecally-administered CSF1R ASO for the treatment of both familial and sporadic ALS.

Types of ALS patients it will help and how: Our data suggest this therapeutic strategy would slow disease progression in patients with C9ORF72 ALS and we anticipate that it could also be effective in sporadic ALS patients. It is unclear if it would be effective in patients with mutations in SOD1, TARDBP, or FUS, but it is possible. If it were feasible to begin treatment before disease onset, it is possible this approach could significantly delay disease onset.

Potential clinical applications, benefits, and risks: If our study validates CSF1R suppression and reprogramming microglia as a new therapeutic strategy for ALS, we anticipate this will lead to the development of ASOs or other drugs targeting CSF1R and related proteins. These treatments could benefit ALS patients by slowing disease progression and extending survival. As with any new therapeutic approach, there is a risk of side effects or an acceleration of disease progression.

Projected time to achieve a patient-related outcome: We anticipate that if our study validates CSF1R suppression as a potential therapeutic strategy for ALS, it could lead to the development of ASO or small molecule drugs that could test this in the clinic within about 3 to 5 years from the end of our study. Before then, we anticipate interim outcomes. At the completion of our study, we will publish our results to disseminate the information to the public to accelerate new mechanistic and therapeutic studies based on our results. If possible, we will also outlicense our intellectual property covering the use of CSF1R suppression to treat ALS and composition of matter on CSF1R ASOs to a commercial entity interested in developing ALS therapeutics targeting CSF1R. If no commercial entity takes this approach forward immediately, we will pursue further funding to develop a CSF1R ASO-based therapeutic.

Likely contributions in advancing the development of therapeutics for ALS: Our study will likely advance the development of therapeutics for ALS in the following ways:

(1) We anticipate our study will validate CSF1R suppression as a new therapeutic approach for multiple forms of ALS, including C9ORF72 and sporadic ALS. This should cause ALS drug development groups to consider developing ASO or small molecule drugs targeting CSF1R.

(2) We anticipate our study will validate microglial reprogramming as a new method for rescuing ALS disease processes, which could help uncover additional therapeutic targets beyond CSF1R.

(3) The patient-derived ALS motor neuron/microglia co-culture assays optimized in our study could be used by other groups to test other therapeutic approaches for ALS that target microglia.

Proposal Title:	Safety of Metformin in C9orf72 ALS: Effects on RAN Proteins, Breathing, Imaging, and Metabolomic Outcome Measures
Log Number:	AL220089
Current PI Name:	Laura Ranum
Award Number:	HT9425-23-1-0065
Current Contracting Organization:	Florida, University of
Current Performing Organization:	Florida, University of
Web Approval Date:	09-01-2023

An abnormality in the human genetic system known as a repeat expansion that is located in a certain genetic location identified as C9orf72, is the most common cause of both inherited (transmitted between family members) and sporadic (no known family history) forms of amyotrophic lateral sclerosis and frontotemporal dementia (C9 ALS/FTD). There are currently no effective treatments available for C9 ALS/FTD, or for the more than 50 other diseases that share this type of genetic abnormality. In the brain and spinal cord of many patients with these repeat expansion mutations, a recently identified process called repeat associated non-AUG (RAN) translation has been shown to cause the production of toxic proteins (RAN proteins) and the accumulation of these proteins as aggregates that contribute to the disease. Metformin is a widely used, well-tolerated type-2 diabetes drug that was recently shown to block an important process that allows formation of toxic RAN protein aggregates in the brain. In a mouse model of C9 ALS/FTD, metformin treatment decrease RAN protein aggregates in the brain and improved the behavior of the mice. These data suggest metformin may also benefit patients C9 ALS/FTD. The purpose of this project is to complete a small-scale research study in human patients to test the safety and possible effectiveness of metformin for the treatment of C9 ALS and if the drug shows promise, to prepare for a large multi-site placebo-controlled follow-up trial.

This study will (1) determine the safety of using metformin for the treatment of C9-ALS/FTD;

(2) test if metformin reduces the RAN protein levels in patient body fluids; (3) identify substances in body fluids that can help to diagnose the disease earlier and track disease progression more effectively, and (4) if the current project is successful, a larger study will compare how different people respond to the use of this medication and whether it can improve their condition. This study has the possibility of rapidly moving this well-tolerated U.S. Food and Drug Administration (FDA)-approved drug into the clinic as a safe, low-cost treatment for the most common genetic cause of both ALS and FTD. Due to metformin's very strong safety profile, RAN protein lowering potential and strong anti-inflammatory properties, the time and work that it takes to complete most drug studies may be significantly shortened. This may allow faster FDA approval for use of metformin in C9 ALS patients. Importantly, if successful, metformin would be a very inexpensive treatment for C9orf72 ALS patients. Given that toxic (RAN) protein build up has also been identified in other repeat expansion diseases, results from this study may be able to help people with other diseases as well.

This study will be important to this area of science because it will go beyond finding out whether metformin can help in C9 ALS patients. We also collect a lot of other important information from other tests that will be performed during the trial, which will help in future studies of this kind. For example, despite a lot of progress in understanding C9-ALS, very little is known about the hidden systems that cause the disease, and that affect patients and their quality of life. The scientific testing of other data collected in this study from procedures like MRIs, breathing and swallowing studies, and repeated collection of body fluids (blood, etc.) will help identify new information to focus on in future studies. The resulting information will be useful in planning and assessing if drugs for future trials of ALS, FTD, and other neurological diseases are working.

All of these results will allow physicians and scientists to have better and easier ways to watch over patients affected by this disease by means of blood test results and more that will not only improve clinical trial design and outcomes, but will also have a positive impact on patient care, treatment, quality of life, and management of ALS.

Proposal Title:	Event-Triggered Gene Therapy for ALS: Smart Release of Therapeutics in the Brain
Log Number:	AL220095
Current PI Name:	Max Cynader
Award Number:	HT9425-23-1-0181
Current Contracting Organization:	British Columbia, University of
Current Performing Organization:	British Columbia, University of
Web Approval Date:	08-31-2023

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of unknown cause. Clinically, it is characterized by a rapid and progressive loss of motor neurons in the brainstem, spinal cord, and motor cortex resulting in muscle atrophy, weight loss, and paralysis leading to respiratory failure and death within 3 years of onset. It is believed that the activation of the immune system in ALS has significant contribution to the harmful processes that lead to the loss of muscle innervation and body paralysis. This is a complex process initiated by harmful processes such as the inflammation initiated by the proliferation and migration of inflammatory cells in the spinal cord and the brain, that release harmful proteins that damage the motor neurons that control the muscle contraction.

Our proposal will focus on special enzymes called proteases that digest proteins and change the cellular environment surrounding the motor neurons in the spinal cord and the brain. Many of the pathological events in the spinal cord and the brain can be traced to the local pro-inflammatory processes that trigger several downstream mechanisms including the activation of proteases, enzymes that cleave proteins in and around the cells of the brain. We propose a strategy to develop transmembrane proteins that can be delivered to the spinal cord and the brain, specifically designed to release therapeutic proteins at the sites affected by the disease, after being cleaved by proteases that are activated during ALS. Our team has developed a way to change these proteases so that instead of being harmful they can now release therapeutic proteins. Once inserted into the cell membrane of spinal cord and brain cells, these sensors can be activated by the injury, to release therapeutics in the spinal cord and the brain to avert loss of motor neurons and thus to slow or even prevent the progression of ALS.

Considering the significant personal, medical, and socio-economic impact of neurodegenerative diseases such as ALS, there is a pressing need for novel treatments. Our goal is to develop a powerful, prophylactic and locally acting, treatment to enable cells to release their own anti-inflammatory proteins to prevent the damage and loss of motor neurons. It is important that the therapeutic proteins are only released when and where they are needed to minimize any systemic side effects. We plan to develop ways to make these proteases to release therapeutic "agents" only at the "hot-spots" -- when and where a damaging inflammatory event occurs in the brain and spinal cord.

This strategy can be further applied as a prophylactic treatment for patients with other neurodegenerative diseases including: Frontotemporal Dementia, Alzheimer's, and Parkinson's disease, Multiple Sclerosis, traumatic brain and spinal cord injury, stroke, and Age-related Macular degeneration.

Proposal Title:	Combined Respiratory Training to Improve Pulmonary and Cough Function in Persons with ALS
Log Number:	AL220099
Current PI Name:	Lauren Tabor Gray
Award Number:	HT9425-23-1-0194
Current Contracting Organization:	Nova Southeastern University
Current Performing Organization:	Nova Southeastern University
Web Approval Date:	08-31-2023

All persons with amyotrophic lateral sclerosis (pALS) experience worsening of breathing and cough function as the disease progresses. Impairments in breathing and the inability to clear the airway contribute to respiratory infections, pneumonia, hospitalizations, and reduced quality of life. Currently, the use of prescription respiratory devices is recommended to maintain pulmonary health and reduce the risk of lung infections in pALS. However, these respiratory devices are typically prescribed reactively once significant impairment in function has already occurred. There are currently no effective, proactive interventions to help prevent deterioration of cough and breathing function in pALS. Therefore, identification of simple, accessible breathing exercises that can be performed immediately following ALS diagnosis is a priority to improve current clinical care for all pALS.

We propose that the use of a combined respiratory strength training exercise program will improve breathing and cough function in pALS. Thirty-nine pALS with mild disease severity will complete lung volume recruitment and expiratory muscle strength training exercises daily for 5 weeks. Both lung volume recruitment and expiratory muscle strength training have been studied independently in pALS and shown to be safe, feasible, and effective in improving respiratory and cough measurements. The goal of this study is to combine these exercise regimens to improve two very important measures of respiratory function and airway clearance: Forced vital capacity and peak cough flow. We will also assess how the exercise regimen impacts pALS-reported shortness of breath, exercise therapy burden, and quality of life.

The proposed combined respiratory training regimen represents an improvement from the current standard of care for the following reasons: (1) Training will be implemented early in the disease to capitalize on and maintain respiratory function and cough strength; (2) Training equipment is accessible, inexpensive, and easy-to-use; and (3) Training initiation does not require insurance approval or shipment of disposable replacement parts. The proposed study represents an opportunity to intervene proactively and empowers pALS with a tool to combat the loss of function early in the disease process. If successful, this combined training has the potential to improve the trajectory of decline in breathing and cough functions, ultimately prolonging survival in pALS.

Proposal Title:	CNS-Specific Disruption of Neuregulin Signaling to Stop Disease Progression in ALS
Log Number:	AL220100
Current PI Name:	Fei Song
Award Number:	HT9425-23-1-0315
Current Contracting Organization:	Illinois, University of, at Chicago
Current Performing Organization:	Illinois, University of, at Chicago
Web Approval Date:	08-31-2023

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is one of the most devastating and deadly neurological disorders for which we have no effective treatment. By the time a patient is diagnosed, the weakness is often localized to one region of the body, such as an arm or a leg. The disease then spreads relentlessly and often sequentially throughout the nervous system until the respiratory muscles are too weak to support life. While significant strides have been made in uncovering genes in a subset of patients with inherited forms of ALS, there are no genes or pathways that have been identified to block disease progression once it starts that could be used for all ALS patients, both familial (genetic) and sporadic.

Our effort here is to advance a novel drug treatment that focuses on disease progression for all ALS patients. We have strong evidence in both human tissues from a wide variety of patients and one genetic animal model called SOD1 that the growth factor neuregulin1 promotes disease progression through activation of inflammatory and pathogenic microglial cells. We also have exciting results that we can slow or even stop disease progression by blocking neuregulin1 and this inflammation either through a genetic change in the animals or with a novel drug we developed called GlyB4.

Here, we propose studies needed to advance GlyB4 toward clinical trials in patients with ALS. We will test whether blocking neuregulin1 in a second animal model will also slow disease progression, and prove that GlyB4 can block neureguiln1 in many types of human ALS stem cells. Since many drugs have failed clinical trials due to side effects, we will be giving GlyB4 directly into the brain cerebrospinal fluid (CSF) of mice and test its effects at different clinical stages of the disease: (1) At the beginning of the disease (onset, just after ALS is diagnosed in a patient) and (2) At the progressive stages of the disease. At each stage, we will show that the drug blocks neuregulin1 signaling, reduces microglial inflammation, and improves motor neuron structure and function.

As part of this project, we will measure the activation of inflammatory microglial cells as a function of drug treatment. Based on many of our observations in human ALS tissues and animal models, microglial activation is an excellent surrogate biomarker of disease progression and reducing this inflammation will be a good biomarker of GlyB4 drug treatment. Based on the results here, we can then justify the use of a number of existing brain and spinal cord imaging methods (PET imaging) that specifically can measure microglial activation as an important biomarker that will help us run clinical trials of GlyB4.

With these proof-of-concept studies, biomarkers studies, and other studies advancing GlyB4 drug development underway, we plan to launch phase I clinical trials in ALS patients within 5 years. The effects we have seen in ALS animal models with GlyB4 also occur in other animal disease models. After we advance GlyB4 for clinical use in ALS, we plan to target other diseases we have seen these similar effects including Alzheimer's, multiple sclerosis, and chronic pain.

Proposal Title:	Retinoid-Activating Gene Therapy for the Treatment of Amyotrophic Lateral Sclerosis
Log Number:	AL220103
Current PI Name:	David Medina
Award Number:	HT9425-23-1-0206
Current Contracting Organization:	St. Joseph's Hospital and Medical Center
Current Performing Organization:	St. Joseph's Hospital and Medical Center
Web Approval Date:	08-31-2023

This proposal seeks to develop a novel gene based therapy for the treatment of amyotrophic lateral sclerosis (ALS), the most common motor neuron disease for which no cures currently exist. Here, we will target the retinoic acid (RA) signaling pathway as a means of neuroprotection. RA is an important signaling molecule involved in a host of vital functions in the central nervous system (CNS). Vitamin A is converted into RA which then acts on retinoic acid receptors to control the transcription of a numerous genes. RA levels are controlled mainly by its degradation via cytochrome p450 family 26 (CYP26) enzymes (CYP26A1, CYP26B1, and CYP26C1). Genetic and protein studies have shown that members of the retinoid signaling pathway are alternatively expressed in neurodegenerative conditions including in post-mortem tissues of ALS patients and animal models of ALS. Our previous work demonstrated that activation of the RA signaling pathway was neuroprotective in preclinical models of ALS. However, our pharmaceutical approaches still had high obstacles to overcome for clinical development. We seek to build on previous work to address our overarching hypothesis: Increased retinoid signaling activity in the central nervous system is neuroprotective and can reduce ALS symptoms and disease progression.

We will use an adeno-associated virus (AAV) technology in order to deliver short hairpin RNA as a way to reduce the expression of retinoic acid degrading enzymes CYP26A1, CYP26B1, and CYP26C1. By utilizing a genetic approach we will overcome disadvantages of a traditional pharmaceutical approach such as limited blood-brain barrier penetration, rapid clearance of drugs, and off-target effects. Employing a genetic approach will allow us to produce a robust, site-specific, and long-lasting effect. We will reduce CYP26 enzyme levels specifically in the spinal cord and motor cortex, the sites most affected by ALS progression.

This proposal will generate preclinical data to demonstrate proof-of-concept of targeting CYP26 enzymes as an ALS intervention. Further, it will demonstrate the advantages of utilizing a genetic approach to activate neuroprotective pathways.

Our aims will be to (1) Establish proper dosing paradigms of viral vectors to achieve optimal RA signaling. (2) Measure the neuroprotective effects of CYP26 reduction in an ALS mouse model. (3) Validate biomarkers that are responsive to retinoid activation to demonstrate the therapeutic action of our treatment.

Successful completion of this project will enable us to progress our retinoid activating therapy towards use in clinical trials. As retinoid signaling has been demonstrated to modulate a host of pathogenic mechanisms such as response to oxidative stress, neuroinflammation, and synaptic function, its potential has wide applicability to different forms of ALS, either sporadic or familial.

Together, these studies will help simultaneously validate the use of RA activating strategies for the treatment of ALS and the use of AAV technology for the delivery of novel therapeutic approaches.

Proposal Title:	A Novel Therapeutic Strategy to Reduce TDP-43 Expression in Amyotrophic Lateral Sclerosis (ALS)
Log Number:	AL220105
Current PI Name:	Alison Axtman
Award Number:	HT9425-23-1-0286
Current Contracting Organization:	North Carolina at Chapel Hill, University of
Current Performing Organization:	North Carolina at Chapel Hill, University of
Web Approval Date:	08-31-2023

Kinases are an important class of proteins that are currently targeted by 71 small molecule U.S. Food and Drug Administration (FDA)-approved drugs. More than 85% of these drugs have been designed for oncological indications. Despite this incredible drug development success and their playing essential roles in the brain, not a single kinase drug has been approved to treat a neurological disorder. A lack of focus on targeting kinases in the brain to augment key disease-propagating pathways has left their potential to alter or halt neurological disease progression largely uncharacterized. There is a need for new targets and new approaches to treat amyotrophic lateral sclerosis (ALS). We propose that kinases represent a target class that can be modified using small molecules to develop new ALS drugs. Kinases regulate proteins and pathways that contribute to ALS pathology. The buildup of a specific protein (TDP-43) in the brains of ALS patients, for example, is proposed to be regulated by a few kinases. This particular protein accumulates in patients that suffer from both familial and sporadic ALS. We can use small molecules to specifically inhibit or degrade those kinases that enhance TDP-43 buildup, which will reduce or prevent this harmful process. These small molecules could be developed into drugs and offer new treatment options to ALS patients that currently do not have efficacious drug options available to them. Our early-stage drug candidates would not just address symptoms like the two currently available FDA-approved ALS drugs. Instead, these drugs would eliminate one major cause of patient decline in ALS and thus alter the course of the disease, with the potential to offer more long-term benefits to patients. Since protein buildup is common to nearly all individuals with the disease, our strategy is widely applicable and impacts nearly all ALS patients.

Proposal Title:	Establishing the Neuroprotective Potential of C9orf72 Hypermethylation in Repeat Expansion-Caused ALS
Log Number:	AL220106
Current PI Name:	Qiang Zhu
Award Number:	HT9425-23-1-0196
Current Contracting Organization:	Van Andel Research Institute
Current Performing Organization:	Van Andel Research Institute
Web Approval Date:	08-31-2023

ALS is a complex and devasting neurodegenerative disease, which origin has not been fully elucidated yet. Even in the case of ALS patients with known genetic causes, there is a lot of variability in both disease progression and symptom severity. This indicates that even if subjects carry genetic mutations that have been shown to cause ALS, there are other mechanisms that dictate how severely the disease will affect them. In addition to the genetic mutations, some patients, in which disease is significantly less severe, show a substantial increase in a type of epigenetic modifications that affect how the mutated gene manifests; this type of modification is called DNA methylation and the gene of interest in this proposal is C9orf72. Up to today, a large repeat expansion in this C9orf72 gene is the most common genetic cause for both ALS and frontotemporal dementia (FTD), known as C9ALS/FTD. A few years ago, the investigator leading this proposal generated multiple mouse models aiming to mimic what happens in human patients with this type of C9orf72 mutation. One of the transgenic animal models, which contains large human C9orf72 repeats, showed high resemblance to C9ALS/FTD. Interestingly, not all mice developed the same degree of deficits (cellular and behavioral) and, similarly to what has been documented in C9ALS/FTD patients, mice also displayed different levels of C9orf72 methylation. Importantly, the mice with higher methylation were the ones showing less prominent signs of the disease. These observations led us to propose that the increase in methylation can be the reason behind a milder C9ALS/FTD. Thus, this project aims to determine whether we can use editing tools to precisely change the level of C9orf72 methylation and test the corresponding effects using cellular and mouse models of ALS.

If successful, the ultimate goal of this project is to design a therapeutic strategy that would increase the methylation of the C9orf72 in order to prevent severe ALS and FTD. Since currently there is no treatment that can prevent disease initiation and progression, such therapeutic strategy will definitely improve the quality of life of individuals with C9ALS/FTD as well as their families and caregivers. This proposal delineates the first several steps required to achieve this goal. Moreover, the influence of epigenetic factors (e.g., DNA methylation) in ALS etiology are far from being completely understood. It is therefore possible that besides C9ALS, other forms of ALS are also affected by changes in their methylation status. Thus, they could also benefit from the findings of this project as the tools being developed in this proposal can easily be applied to other ALS-relevant genes. In addition, this project will establish whether the opposite strategy, namely, decreasing methylation, leads to worsening of the studied outcomes; there is added value in this experiment as it can lead to the generation of better ALS models with robust phenotypes. Such models could be used to facilitate and promote drug screenings and development of new therapies, by us and other researchers in the field.

Being an innovative project, it is difficult to foresee what risks will associate with such a therapeutic strategy. The experiments, to be performed in mice, will provide clues on its safety in addition to effectivity. Although this project is at a very early stage, we are using patient-derived cellular models, a "humanized" mouse model as well as tools that can easily be translated to accelerate its clinical development if successful. Also, the lead investigator has years of experience in therapeutic development and has already been involved in clinical trials for ALS in collaboration with biotech companies and clinicians, aiming to test his early

work on other approaches like antisense oligonucleotide treatment. In conclusion, we are pursuing a completely new line of work in the design of therapies that can stop or even prevent the development of C9ALS/FTD. The approach used includes some of the latest technologies and has taken into account steps that can accelerate their applicability in the clinic.

Proposal Title:	Restriction Spectrum Imaging as a Biomarker for Amyotrophic Lateral Sclerosis
Log Number:	AL220108
Current PI Name:	Iris Broce-Diaz
Award Number:	HT9425-23-1-0353
Current Contracting Organization:	California, University of, San Diego
Current Performing Organization:	California, University of, San Diego
Web Approval Date:	09-01-2023

Amyotrophic lateral sclerosis (ALS) is the most common form of adult-onset motor neuron disease and is characterized by progressive degeneration of both upper motor neurons of the motor cortex and lower motor neurons of the brainstem and spinal cord at disease onset. A small proportion of ALS results from established genetic causes inherited in a familial pattern. However, for most sporadic ALS cases, no single test can predict disease risk or provide an early definitive diagnosis. Also, given the extensive phenotypic variation in disease presentation, rate of progression, and the number of conditions that resemble ALS, many patients experience delays in diagnosis that prevents the testing and initiation of disease modifying treatments early in the course of disease when potentially most impactful. Thus, there is an urgent need for novel clinical biomarkers to improve early clinical diagnosis and prognosis in ALS, identify biologically meaningful patient subgroups, and provide surrogate endpoints in clinical trials.

The goal of the proposed project is to evaluate the diagnostic and prognostic value of a novel brain imaging technique called restriction spectrum imaging (RSI) in ALS patients. RSI is an advanced diffusion-weighted imaging technique that allows detection of disease-specific changes in the brain with greater sensitivity and specificity compared to other imaging techniques, such as diffusion tensor imaging, making RSI suitable for clinical applications. We will recruit a total of 50 ALS patients (30 sporadic patients) across two institutions, UC San Diego, and UC San Francisco. All patients will receive genetic testing through an ALS sponsored genetic testing program. Longitudinal RSI and clinical data will be collected in all patients, every 4 months for a year. Cerebrospinal fluid (CSF) biomarkers (e.g., neurofilaments and inflammatory markers) will be collected in a subset of patients, also every 4 months for a year. Imaging data will be collected at a single time point from 50 age- and sex-matched healthy individuals.

Our ALS clinics at UC San Diego and UC San Francisco are multi-disciplinary clinics. Combined they follow roughly 625 patients per year, of which 150 patients are new. Also, the imaging protocol we use can be incorporated into any facility with 3T General Electric and Siemens MRI technology. Therefore, our clinical biomarker has the potential of benefiting a large cohort of patients. Further, RSI has shown promise as an in-vivo biomarker of neuroinflammation. Therefore, once validated, our imaging biomarker will be clinically applicable to facilitate cohort stratification in therapeutic clinical trials targeting specific biological pathways, such as neuroinflammation, and may serve to predict therapeutic response and as an endpoint in clinical trials.

Risks of the proposed study include discomfort and possible patient burden from longitudinal imaging, neuropsychological testing, and CSF data collection. The study team has plans in place to minimize any risk should they arise. Further, while there will be no direct immediate benefit to the patients from taking part in this study, the novel clinical biomarker we develop will help in the treatment of future patients with ALS.

The minimum projected time it may take to achieve a patient-related outcome is 2 years. Data collection will occur the first year of funding. Data analysis and dissemination of research findings will occur over the course of 2 years and beyond.

Our clinical biomarker will result in major advancements in ALS treatment and help better define subsets for clinical treatment. Specifically, our imaging biomarker will improve early clinical diagnosis, help physicians track disease progression more effectively, and accelerate the testing and initiation of disease modifying treatments early in the course of disease when potentially most impactful. Ultimately, these efforts will ensure progress toward eradicating deaths and suffering from ALS.

Proposal Title:	Delivery of Mitochondria Using Extracellular Vesicles as a Novel Therapy for ALS
Log Number:	AL220116
Current PI Name:	Devika Soundara Manickam
Award Number:	HT9425-23-1-0218
Current Contracting Organization:	Duquesne University
Current Performing Organization:	Duquesne University
Web Approval Date:	08-31-2023

Ultimate applicability of the research: In ALS, the cells that control movement, motor neurons, break down and stop working. No matter the cause of ALS, familial or sporadic, one reason these cells stop working is that their power generators, the mitochondria, become damaged. Research over the past 3 decades has demonstrated this and yet clinical trials that try to repair the mitochondria have not been effective. This research will provide foundational results to advance a novel therapeutic approach: the delivery of new, healthy mitochondria to promote the health and function of the motor neurons. Mitochondria are the powerhouses of cells and produce most of the cell's energy to allow the cell to perform multiple complex functions. Mitochondria even regulate whether the cell lives or dies in diseases such as ALS. It is known that motor neurons contain damaged mitochondria during the very early stages of disease onset in ALS. The damaged mitochondria are known to cause multiple problems resulting in progression of the disease to a point of no return where the patient's cells cannot effectively respond to ALS drugs. Indeed, the current ALS drugs are only modestly effective at extending patient life span with the standard-of-care drugs allowing patients to survive for only as long as 3 to 6 months! Many different types of cells in the human body, including healthy neurons, naturally release tiny vesicles/buds, called microvesicles. A very interesting feature of the microvesicle is that they contain material such as healthy mitochondria. We are proposing to collect these mitochondria-containing vesicles from cell models of motor neurons to treat the experimental mouse model of ALS. The results from this study will establish the foundation to further advance microvesicles as promising therapy to decrease mitochondrial damage, increase motor neuron function, and therefore prolong survival of ALS patients.

What type of ALS patients will microvesicles help, and how will this help them? Clinical studies in ALS patients have shown that mitochondrial abnormalities/damaged mitochondria are present both in the "familial /genetic" as well as the "sporadic/non-genetic" forms of ALS. Therefore, delivery of healthy mitochondria can be expected to decrease mitochondrial damage in all types of ALS patients -- regardless of how they acquire the disease. By decreasing the mitochondrial damage in the motor neurons that connect with the muscles, these microvesicles can promote healthy survival of the motor neurons and therefore allow normal functioning of the muscles connected to the now healthier neurons. Overall, this approach of delivering mitochondria using microvesicles will decrease muscle weakness and loss of muscle functions.

What are the potential clinical applications, benefits, and risks of microvesicles? The most important advantage of delivering mitochondria using microvesicles lies in the fact that these microvesicles are very safe and will have minimal side effects/risks. Microvesicles are naturally derived from cells grown experimentally in a plastic dish, and ultimately may be derived from a sample from the individual patient, such as motor neurons derived from the patient's IPSCs collected from a skin sample. It is important to note that the project will determine that delivery of microvesicles containing mitochondria is safe AND effective in a limited motor neuron population in a mouse model of ALS. This is the critical first step for this development of this therapeutic idea. We are encouraged by our preliminary studies in a model of stroke that demonstrates effectiveness of this approach. From here, our future studies will determine the best route of delivery for an overall effect to delay disease progression and significantly extend survival.

The projected time it may take to achieve a patient-related outcome and description of the interim outcomes. We will carry out the proposed studies in the next 2 years and these results will generate the feasibility of increasing mitochondrial function and, therefore, the survival of motor neurons in ALS. After completing the current study in 2 years, we will proceed to complete additional preclinical studies (for 3 years) to determine the best routes of injection and also an effective dose of the microvesicles that can ultimately prevent progression of the disease into advanced stages of muscle loss and extensive damage. During this time, we will continue to work with our collaborator at a local start-up interested in commercialization of this mitochondria therapy for central nervous system and other disorders so we will be primed to begin Phase 1/2 clinical trials.

The likely contributions of this study in advancing the development of therapeutics for ALS. This study will test an entirely new approach to deliver mitochondria to decrease damage and death of motor neurons. Although prior studies have tested different approaches to improve individual functions of mitochondria -- but not deliver intact/healthy mitochondria as a whole. Therefore, this study is expected to make important contributions to further develop microvesicle mitochondria as a novel ALS therapeutic.

Proposal Title:	Precision Medicine Immunotherapy for the Treatment of Neurodegeneration in HERV-K-Positive Patients with Amyotrophic Lateral Sclerosis
Log Number:	AL220123
Current PI Name:	Ajay Verma
Award Number:	HT9425-23-1-0564
Current Contracting Organization:	FORMATION VENTURE ENGINEERING FOUNDRY INC
Current Performing Organization:	FORMATION VENTURE ENGINEERING FOUNDRY INC
Web Approval Date:	09-01-2023

Since 1980, more than 80 randomized controlled trials (RCTs) on amyotrophic lateral sclerosis (ALS) have been published, and just two drugs, riluzole and edaravone, have emerged as U.S. Food and Drug Administration-approved therapies for the disease. Reasons for the negative results of RCTs may include an incomplete understanding of ALS disease mechanisms, clinical diversity of ALS progression, shortcomings of study design, and how certain genetic profiles influence drug effectivity.

About 8% of the human genome has been found to consist of "endogenous" retroviruses such as human endogenous retrovirus-K (HERV-K), which are believed to be remnants of ancient viral infections that invaded our genetic code during evolution. These retroviruses initially infected a human egg or sperm cell and entrenched themselves in the human genetic code, allowing them to spread from one generation to the next. Over many millenia, the HERV genomic sequences have largely become mutated or otherwise rendered unable to produce gene products by protective mechanisms guarding against their expression. HERV-K are the most recent retrovirus to invade our genome and one of their subclasses, called HML2, can still activate gene expression, particularly when the protective defenses become weakened. Aberrant expression of HERV-K messenger RNA and proteins has been shown to be particularly toxic to certain cells like the large motor neurons that degenerate in ALS. In particular, the envelope (Env) protein expressed by HERV-K HML2 causes cell death when exposed to laboratory cultured human brain cells or when experimentally expressed in the nervous system of mice.

In recent years, mechanisms that guard against inappropriate gene expression have been shown to become weakened in the motor neurons of patients with ALS. A master regulator of gene expression called TDP-43 is commonly mutated or becomes dysfunctional in ALS. The discovery that TDP-43 normally governs the expression of genomic HERV-K sequences along with the discovery of HERK-V Env protein expression in degenerating ALS motor neurons has pointed to a novel disease mechanism and therapeutic opportunity for some forms of ALS. If one could readily identify patients with ALS, in which brain cell HERV-K activation is prominent, then a vaccine could be developed against the toxic Env protein to protect neurons in ALS. Just as vaccination against other viral maladies such Shingles can provide disease protection, this approach brings a well-proven therapeutic concept to the treatment of ALS.

To test this exciting and innovative hypothesis, we have assembled a collaboration between the world's foremost knowledgeable lab on HERV-K biology in ALS with two biotech companies that are developing cutting edge diagnostic and therapeutic technologies. This collaboration will allow the development of biomarkers to conveniently track nervous system activation of HERV-K and vaccines to target the most promising portions of the HERV-K Env protein as a novel precision medicine approach for ALS. The project will test whether active vaccination against the Env protein of HERV-K will safely generate immunity and provide clinical benefit in a mouse model that simulates HERV-K induced ALS. Results from this study will bring us a step further in the development of precision medicine for this subset of ALS patients.

Given the prior investment in the required technologies by the collaborating partners, we envision this project to identify a lead therapeutic candidate with animal model proof-of-concept and the requisite biomarkers for clinical development with approximately 2 years.

Proposal Title:	Peptide Inhibitors Targeting Sodium Channels to Treat Amyotrophic Lateral Sclerosis
Log Number:	AL220125
Current PI Name:	Fernanda Cardoso
Award Number:	HT9425-23-1-0146
Current Contracting Organization:	University of Queensland
Current Performing Organization:	University of Queensland
Web Approval Date:	08-31-2023

Novel peptidic ion channel inhibitors derived from spider venoms are an emerging class of drugs to treat complex neurological disorders. Their unique ion channel subtype selectivity and high potency make superior drugs compared to commonly used small molecule drugs, including Riluzole. Riluzole is currently used in the treatment of Amyotrophic Lateral Sclerosis (ALS) and is the only drug capable of extending the lives of people affected by this devastating disease. Because our novel NaV inhibitors can selectively target sodium channels (NaV) subtypes in motor neurons, they are able to reverse motor neuron hyperexcitability linked to the early onset and progression of ALS and common pathological mechanism in all types of ALS while maintaining the normal function of vital organs such as the heart and the skeletal muscles. This way, these spider venom-derived NaV inhibitors are safer drugs that can be administrated at doses to rich their maximum benefit to ALS patients. Riluzole, conversely, is also an ion channel inhibitor targeting a broader range of ion channels, and, therefore, its therapeutic efficacy is impeded by poor therapeutic index and high risks of serious side effects.

Besides ALS, these novel NaV inhibitors can treat a range complex neurological disorders where hyperexcitability of neurons is associated to initiation and progression of disease. Beneficial therapeutic effects with low to nil side effects are an important aspect of our drug development strategy. Therefore, we have opted to initially administrate our ALS selective peptide NaV inhibitors via injection in the spinal canal hoping to accelerate its transition into clinical trials. Further research is being undertaken to develop small molecule derivatives displaying the same therapeutic benefits which will be available for oral administration in the future.

Our research is developing drugs that will benefit both types of ALS patients, familial and sporadic, as the disease mechanisms targeted by our drugs are common in both types of ALS. More specifically, the peptide inhibitors we are designing can reverse the hyperexcitability of motoneurons, which is characteristic of the early onset of ALS and occurs before the neurodegeneration is established, which persists during the progression of this disease. Such motor neuron hyperexcitability is also observed in preclinical models of ALS, which facilitates the preclinical relevance and development of useful leads for clinical trials. Furthermore, we are focusing on biomarkers demonstrating the reduction of hyperexcitability in neurons, which is well established by members of our proposed research team.

If funded, this research proposal will expedite the transition of our novel ALS selective peptide NaV inhibitors from preclinical to clinical trials, as we have already identified a peptide lead with proved therapeutic efficacy in a preclinical model of motor neurodegeneration in zebrafish, and in rodent motor neurons ex vivo preparations in which our drug lead was able to reverse hyperexcitability to normal neuronal function. We project that at the end of this proposed research project in 2025, we will have lead candidates available to initiate clinical trials. Additionally, this proposal will fill a major gap in applying naturally occurring NaV inhibitors and optimized derivatives in research for novel drugs to treat ALS. If funded, this proposal will provide a pathway for the development of other related drugs using a similar strategy by targeting ion channels in motor neurons to reverse ALS.

Research in novel ion channels inhibitors derived from spider venoms is rapidly expanding and benefiting from modern scientific methods to investigate and develop such peptide inhibitors into useful drugs. Our research team is composed of experts in the scientific methods required for this research, and we have direct access to the instrumentation required to execute this state-of-the-art research proposal. In addition to this, our research team is associated with world leading experts in preclinical models of ALS, which positioned us with a unique opportunity to develop novel ALS drugs, and to expedite the clinical trials of these novel drugs, which will enormously benefit ALS patients.

Proposal Title:	Beyond CuATSM: Novel Copper Complexes for Improved ALS Therapy
Log Number:	AL220129
Current PI Name:	Levi Beverly
Award Number:	HT9425-23-1-0773
Current Contracting Organization:	University of Louisville Research Foundation, Inc.
Current Performing Organization:	University of Louisville Research Foundation, Inc.
Web Approval Date:	09-01-2023

A small copper-containing molecule known as copper-ATSM or CuATSM is one of the most promising potential amyotrophic lateral sclerosis (ALS) treatments to emerge in recent years. Scientists have shown that CuATSM protects vulnerable neurons like those that are affected by ALS from being damaged by a variety of toxic processes that are thought to play a role in ALS. There are at least eight reports that CuATSM can improve outcomes in mouse models of ALS. Based on these studies, CuATSM is now being tested in human clinical trials and early results are encouraging. So far, most studies of CuATSM have focused on mutant SOD1 models but there is growing evidence that this complex has activity in other forms of ALS, including sporadic ALS.

However, CuATSM has some drawbacks. The main problem is that it has a small therapeutic window -- this means there is only a small difference between the dose that is high enough to be effective in ALS and the dose that is low enough to not have toxic side effects. Consequently, some patients on the trial may not be getting a sufficiently high dose of CuATSM for it to be effective, but it is not possible to raise the dose further without causing side effects. Another problem is that researchers do not understand exactly how CuATSM works in ALS and that makes it difficult to know which individual patients or which types of ALS will respond to treatment. It also makes it hard to design a "better CuATSM" and so there has not been much research in this area to date.

The idea for this project stems from our work in the cancer field where we have been evaluating copper complexes as potential anticancer agents. Using new approaches that our group designed, we have synthesized and characterized a large number of copper complexes similar to CuATSM. We have found some complexes that have a much higher activity against cancer cells than non-cancer cells (i.e., a large therapeutic window). It is believed that this activity stems from the ability of copper complexes to enter cells and release copper inside them. Copper complexes are designed so that they release copper in stressed cells (like cancer cells or ALS affected cells) but not in normal healthy cells. Thus, they selectively target copper delivery to either cancer cells or ALS-affected cells, but the outcomes are opposite. Cancer cells have plenty of copper to start with and die due to excess copper, whereas ALS neurons appear to be copper-deficient and have many proteins inside them that need copper to function properly, so they are rescued and protected by the delivery of copper.

Our vision for this project is to develop new copper complexes that greatly improve upon CuATSM and are optimized for the following features: (1) highly selective delivery of copper to ALS-affected cells, which should provide improved activity with minimal side effects; (2) ability to reach the brain and spinal cord where they need to be; (3) oral bioavailability so they can be taken as a daily pill; (4) new complexes that can be patented, so that pharmaceutical companies will want to license them or partner with us to pursue clinical trials; and (5) a well-defined mechanism of action to help move them quickly through regulatory approvals. We propose to also develop a version of this complex made with a radioactive form of copper (64Cu). This can be used as an imaging agent and biomarker that might predict which persons with ALS are most likely to respond to this therapy. Those patients with uptake of the radiolabeled copper complex in

certain regions of their brain (which can be detected by standard PET scanning) would be good candidates for treatment with the (non-radioactive) daily pill version of the copper complex. Those patients who did not show brain uptake in the biomarker scan would avoid undergoing a therapy unlikely to benefit them and could seek alternative treatment options.

Our project is at an early stage of development, but we want to find out as quickly as possible if it is likely to work or not, and then pursue human clinical trials if warranted. We plan to use the mutant SOD1 mouse model of ALS in this study to test if our idea works (because this is a well-known and reliable model), but we expect that our strategy will be suitable for treating many other subtypes of ALS, including cases of sporadic ALS. We can confirm this in future studies by using alternative animal models, or cells derived from ALS patients with various forms of the disease, or in humans via PET imaging with the 64Cu complex. If awarded, this ALSRP Therapeutic Idea grant would allow us to rigorously test our hypothesis. If all goes well, the experimental new drug we hope to develop could begin testing in human trials as soon as a year or two after completing this project and would take several more years to become an approved therapy. The PI for this project has been involved in the bench-to-bedside translation of a drug candidate she co-discovered in the past and has served as a consultant for pharmaceutical companies developing new drugs. As such, she is well-positioned to ensure that the focus for this project remains on translating the discoveries we make into patient impact as rapidly and safely as possible.

Proposal Title:	Preclinical Studies for the Therapeutic Development of a Novel Neuroprotectant for ALS
Log Number:	AL220134
Current PI Name:	Corinne Lasmezas
Award Number:	HT9425-23-1-0080
Current Contracting Organization:	Florida, University of
Current Performing Organization:	Florida, University of
Web Approval Date:	08-31-2023

Amyotrophic lateral sclerosis (ALS) is a devastating degenerative disorder of the motor neurons for which there is no cure. Since 2007, over 80 phase 2 or 3 clinical trials have been conducted leading to U.S. Food and Drug Administration (FDA) approval of only one drug substance with limited efficacy, in addition to Riluzole, which was approved in the 1990s. There is an urgent need for a treatment able to slow down disease progression, also called a disease-modifying treatment.

We have discovered that depletion in a metabolite called nicotinamide dinucleotide (NAD) induces a major energetic deficiency and neuronal demise in ALS. Most importantly, we have shown that restoring healthy NAD levels protects neurons against the mechanism of toxicity operating in ALS. Based on these findings, we initiated and implemented a large drug discovery effort that led to identification of a neuroprotective molecule called VITNP1. When administered to ALS mice in drinking water, VITNP1 delayed motor impairment and loss of muscle strength and increased brain NAD levels. By performing iterative rounds of chemical modifications and testing of biological properties, we have further improved the compound's safety, stability, and brain penetration, leading to the drug candidate VITNP1.2.

Herein, we propose to perform studies that will support the transition of VITNP1.2 to clinical trials in ALS patients. We will determine the optimal doses needed to safely restore NAD levels and provide therapeutic benefit. Since ALS is a heterogenous disease, with patients having different genetic make-up, clinical presentation, and disease progression rate, we will test VITNP1.2 in two different mouse models of ALS to demonstrate that our drug candidate will help a large proportion, and maybe all, ALS patients. We will test VITNP1.2's therapeutic efficiency when administered after disease onset in mice, to show its potential as a treatment for ALS, as opposed to a prophylaxis. Our aim is to develop an oral treatment improving the patient condition and slowing disease progression in familial and sporadic ALS. Our data in mice show that VITNP1.2 allows for NAD restoration without the side effects observed with high doses of NAD precursors, so-called "NAD boosters", and that it can be administered safely to mice over several months.

VITNP1.2 dosage, safety, and therapeutic efficacy data will be important to support the FDA application for an investigational new drug by the small company Vova Ida Therapeutics, Inc. (VIT) with which we are partnering. VIT will perform regulatory studies and is planning to initiate a first-in-human clinical trial within 24 months.

We will also measure NAD in blood and cerebrospinal fluid of VITNP1.2 and placebo-treated mice to show that the treatment efficacy can be monitored by using NAD as a biomarker. This will be important to provide a way to quickly evaluate if VITNP1.2 is biologically active in humans in the phase IIa clinical trial, and to be able to start a larger clinical trial enrolling more ALS patients as soon as possible. In this project, we will also evaluate if NAD levels measured in blood and CSF at various stages of disease reflect disease progression, such that NAD could be used as a novel biomarker supporting clinical trials for other candidate therapeutics.

Therefore, our project will accelerate the development of new therapeutics for ALS by providing a novel drug candidate with disease-modifying properties to be tested in clinical trials, and by providing a new tool to accelerate clinical trials for drugs developed by other investigators.

Proposal Title:	Anti-RAN Targeting Immunotherapy for C9orf72 ALS and Genetically Unknown RAN-Positive Sporadic ALS
Log Number:	AL220147
Current PI Name:	Laura Ranum
Award Number:	HT9425-23-1-0287
Current Contracting Organization:	Florida, University of
Current Performing Organization:	Florida, University of
Web Approval Date:	09-01-2023

Background: Amyotrophic lateral sclerosis (ALS) is a complex set of neurological diseases that kills motor neurons and results in a devastating loss of motor function and death. Approximately 90% of ALS cases do not have a family history of the disease and are referred to as having sporadic ALS (sALS). The lack of knowledge of the underlying causes of sALS presents a huge barrier to finding effective therapies. The most common genetic cause of sALS is too many copies, referred to as an expansion, of a GGGGCC sequence in a gene located on chromosome 9 called the chromosome 9 open reading frame 72 (C9orf72) or C9-ALS. In 2011, the Ranum lab made the surprising discovery that these repeat expansions produce unexpected toxic repetitive proteins without the normal signals previously thought to be required for cells in the body to produce proteins. These repetitive proteins are called repeat-associated non-AUG (RAN) proteins because they are made without the normal "AUG" protein start signal. RAN proteins have been shown to be toxic to cells and to play an important role in C9-ALS. Treating mice with antibodies against the C9 RAN proteins improve the disease features in mice that mimic the disease (C9-BAC mice). While this approach is promising, it is costly, requires frequent injections, and does not penetrate the brain, which justifies the need for a second-generation antibody approach that targets RAN proteins.

Patient Population: This anti-RAN antibody approach is primarily targeted at ALS patients with the C90orf72 mutation. However, the Ranum lab has recently shown that these RAN proteins also accumulate in a large number of sALS autopsy brains that are curiously negative for C9 and SCA36. This data suggests this anti-RAN antibody approach may also be effective in the much larger group of sALS cases. This proposal will test if this antibody approach works with both C9-ALS mice and sALS cells, which will ultimately lead to potentially providing a therapeutic option for large portion of the existing ALS population.

Research and Clinical Application: The ultimate application of this research is the generation of a therapeutic treatment for ALS patients in addition to increasing our knowledge about how ALS develops and how RAN proteins contribute to the disease. In the future this research will provide supporting evidence for potential future clinical trials that will determine how effective the approach will be for reducing symptoms in ALS patient. The benefits of this approach will be that it targets the cause of the disease, RAN proteins, at a molecular level rather than the symptoms of the disease. Additionally in the case of sALS patients, this approach does not require knowledge of the disease-causing mutation just simply the fact that the patient's cells express a RAN protein that already has an antibody against it. The risk of this approach is that it requires expression of the antibody using a viral vector, which can cause inflammation, although this virus approach is already used in a number of existing therapies. Since the underlying genetic mutation for the sALS cell models used in this proposal has not been identified nor have animal models generated, this avenue of research will not progress at the same rate as the C9- based work. However, the demonstration that this AAV-alpha-GA immunotherapy approach works in a large portion of sALS cases will provide strong impetus for future clinical trials.

Time for Patient-Related Outcomes: The current research application is in the preclinical stage and could provide, at least for C9-ALS, efficacy data in support of a future clinical trial in the 2-5 year post-study time

frame. While the AAV-based approach to face more regulatory hurdles than already approved Food and Drug Administration drugs, such as metformin, there are over 250+ current clinical trials on clinicaltrial.gov that utilize AAV. By the end of the proposed study, if sufficient preclinical efficacy data is generated from the C9-BAC mice, it is possible that plans and regulatory paperwork could be filed for the initiation of early phase AAV-alpha-GA antibodies safety clinical trials. The sALS work as outlined above have a long-time frame to patient-related outcomes but successful demonstration that an AAV-alpha-GA immunotherapy approach works in for sALS cases will provide supporting data for future clinical trials targeted at broader group of ALS cases.

Advancing the ALS therapeutic development: This study will contribute across multiple levels to advancing development of therapeutics for ALS. First and foremost, it will provide an AAV-based antibody drug that potentially works for C9 and genetically unknown GA(+) sALS cases. Second, it will contribute to the field of knowledge that support RAN proteins as a driver of disease and a leading therapeutic target. Third, the development and utilization of cell culture models for genetically unknown sALS will provide additional model system to test future therapeutic approaches. Combined this findings and tools from this study will help advance ALS therapeutic development and push forward much needed data for future clinical trial.

Proposal Title:	TDP43 in Circulating Neuron-Derived Extracellular Vesicles as Prognostic and Diagnostic ALS Biomarker
Log Number:	AL220151
Current PI Name:	Erez Eitan
Award Number:	HT9425-23-1-0077
Current Contracting Organization:	NEURODEX INC
Current Performing Organization:	NEURODEX INC
Web Approval Date:	08-31-2023

Ultimate applicability of the research: This project is designed to advance the development of a new blood-based biomarker that can predict whether a patient with ALS will progress rapidly or slowly. Such a marker can help patients plan for care and is needed to help drug developers narrow the pool of patients who participate in clinical trials to (a) increase the likelihood of successfully identifying drugs that work and (b) reduce the number of patients required for each trial. It is expected that such biomarker would accelerate drug development and increase the success rate of ALS clinical trials.

Type(s) of ALS patients the project is designed to help and how it will help them: This tool is currently intended for patients with sporadic ALS, of which, more than 90% have the marker of interest. Faster, less expensive drug development would benefit patients in this population by getting drugs to market faster and allowing drug developers to spread their investments across more drug candidates. The tool could also potentially help doctors decide which patients are likely to respond to specific drugs, and it could help patients and their families make better plans based on the patient’s likelihood of progressing slowly or quickly.

Potential clinical applications, benefits, and risks: The initial application of this biomarker is for improving clinical trial design and recruitment, but it has the potential to also be used to help doctors determine which patients will progress slowly to inform care planning. The primary clinical risk of the tool would be incorrectly classifying a patient by their likely progression speed, which may result in care planning being misaligned with actual outcomes. However, there currently is no test available to predict progression, so the tool would be a net improvement. In addition, up to 15% of patients are misdiagnosed, and the tool could eventually be used to correct this and redirect doctors to test for other conditions that likely have a different prognosis and treatment plan.

Projected time it may take to achieve a patient-related outcome? With adequate funding, this tool could be applied to clinical trial recruitment within 1 year for evaluating treatment response and within 2 years to predict rate of progression. Use in clinical care as a CLIA test could also be achieved within 2 years, though U.S. Food and Drug Administration (FDA) approval and insurance coverage would be achieved in 3 to 6 years depending on the type of additional testing the FDA requests.

Likely contributions of this study to accelerating progress toward eradicating deaths and suffering from ALS: This project has the potential to aid in conducting more efficient clinical trials, which allow testing more treatments and eventually increase the chance of finding an effective treatment for at least a subgroup of ALS.

Proposal Title:	Development of an UNC13A Antisense Oligonucleotide Treatment for ALS and FTD
Log Number:	AL220161
Current PI Name:	Zhihua Feng
Award Number:	HT9425-23-1-0469
Current Contracting Organization:	AcuraStem Incorporated
Current Performing Organization:	AcuraStem Incorporated
Web Approval Date:	09-01-2023

Background: Amyotrophic lateral sclerosis (ALS) is a complex disease with diverse genetic causes. While a few genes are known to cause ALS (i.e., SOD1, C9ORF72, etc), these only account for around 15% of patients. We know that most of the other 85% of ALS patients also have genetic causes, because twin studies have shown that ALS is highly heritable, but those causes are unknown (these patients are referred to as sporadic ALS). A major focus of AcuraStem is to develop therapeutic treatments that will work for most ALS patients, both sporadic and genetically defined.

Recent research has identified an event that appears to be early in ALS disease progression and central to ALS pathology. In >95% of ALS cases a protein TDP-43 is inexplicably lost from the nucleus of neurons and forms into toxic cytoplasmic aggregates. While most treatments in development focus on pathology resulting from the aggregates, the new research focuses on pathology resulting from the loss of TDP-43 in the nucleus. TDP-43 is involved in RNA processing -- the act of turning DNA into messenger RNA and thus into proteins. The researchers identified that, when TDP-43 is absent from the nucleus, a piece of intronic DNA (i.e., the non-coding region) from the UNC13A gene, is mistakenly included into the mRNA and less UNC13A protein is made. This mistaken piece of mRNA is referred to as a cryptic exon. This UNC13A cryptic exon has now been identified in post-mortem brain samples from many ALS and FTD patients that have the TDP-43 pathology.

This is exciting because a genetic mutation in UNC13A has been known for many years as a major risk factor for ALS. Now we know why. The mutation is in the same region where the cryptic exon gets included into the mRNA and it makes it worse. If you have the mutation on both alleles you get more cryptic exon than if you have the mutation on one allele, which provides more cryptic exon than if you are an ALS patient but don’t have the mutation. Similarly, patients who have the mutation have a dose-dependent reduction in survival as compared to other patients that don’t have the mutation. This strongly suggests that the disruption to the UNC13A protein is central to disease pathology, and that fixing this could have substantial therapeutic benefit. Furthermore the treatment would be relevant to all patients who have TDP-43 pathology (not just the UNC13A mutation carriers), since they all have the cryptic exon which is caused by the loss of TDP-43 from the nucleus.

Project Objectives: AcuraStem has a proven platform comprising cellular models of ALS derived from ALS patients. We were able to recapitulate the cryptic exon event on our platform and design drugs that suppress the cryptic exon and restore normal UNC13A protein levels. In this project we will develop functional experiments that will help us understand exactly how the cryptic exon disrupts UNC13A and its impact on neurotransmission. This will give greater confidence that targeting the cryptic exon will make a big impact for patients. In parallel, we will make a mouse model of the human cryptic exon event to ensure our drugs suppress the cryptic exon in a living animal. Lastly we will identify potential target engagement biomarkers -- proteins or mRNA that we can measure in patient biofluids to estimate the potency of a given dose level in

the clinic. At the end of this project we will have the assays established that we can use to optimize our drug candidates and identify a bona fide development candidate for advancement into toxicity studies -- a major milestone.

Clinical Application and Timelines: The genetic studies show a 3- to 4-year survival benefit for patients without the UNC13A mutation versus mutation carriers, and provides an estimate of potential clinical benefit. No other drugs in the ALS pipeline have such compelling genetic data. No animal model correctly recapitulates the cryptic exon pathology and it will take years to develop. Therefore, we are planning to test our drug in a small cohort of ALS patents with the UNC13A mutation following the precision medicine regulatory pathway, and in parallel develop an ALS animal model that recapitulates cryptic exon pathology. Thus, we could be testing in patients within one year of the conclusion of this project (2025).

Contribution to the Field: We have a best-in-class human platform for accurately determining the impact of the cryptic exon on the UNC13A protein and neurotransmission. This will be a key step for the field in understanding how the cryptic exon event triggers downstream ALS pathology.

Proposal Title:	Longitudinal Neuroimaging and Molecular Biomarkers of Cerebrovascular Health in ALS
Log Number:	AL220164
Current PI Name:	Nadine Bakkar
Award Number:	HT9425-23-1-0352
Current Contracting Organization:	St. Joseph's Hospital and Medical Center
Current Performing Organization:	St. Joseph's Hospital and Medical Center
Web Approval Date:	09-01-2023

Vascular alterations have recently emerged as a common feature of many neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). These alterations are thought to occur prior to neuronal degeneration, at least in ALS mouse models. Yet, the role of the cerebrovasculature in human ALS pathobiology as it correlates to disease progression is largely unknown. Since the neurovasculature is both a gate for drug delivery into the brain and a gateway for peripheral immune infiltration into the central nervous system, there is a critical need for accurate minimally invasive biomarkers of its health.

The ongoing Target ALS longitudinal biofluids clinical research study is a longitudinal study occurring at the Barrow Neurological Institute in Phoenix, AZ, along with other sites across the nation. It collects clinical information, blood, CSF, urine, RNA, DNA for whole genome sequencing, and at-home measures of speech and respiratory function. All samples, genetic and clinical information are de-identified and made available to the research community. We propose to use Department of Defense funding to add longitudinal neuroimaging measures of cerebrovascular function and blood flow, along with biofluid biomarkers of vascular injury. In addition, we will use samples from this study to investigate molecular biofluid markers of vascular injury in the CSF and blood and explore the immune cell composition of these biofluids by single cell RNA-sequencing (scRNA-seq). Finally, we will correlate neuroimaging data of vascular health with markers of vascular injury and peripheral neuroimmune infiltration to identify quantitative biomarkers of vascular health and dysfunction in ALS. This approach leverages existing infrastructure and patient samples thus reducing redundant efforts and costs to patients and researchers, while maximizing the data collected. Linking this information to the Target ALS datasets will provide an excellent means to maximize sample and data utilization and enable future studies that combine all these data types with clinical information.

This comprehensive multidisciplinary study will provide a novel understanding of cerebrovascular structure and function in ALS patients, as well as the timing of vascular alterations with respect to various clinical measures of disease. Since disruption of vascular physiology is thought to occur early in the neurodegenerative disease process, this knowledge can provide a potential target to prevent or delay disease progression, but also prove essential for discovery and development of prognostic biomarkers of disease progression and pharmacodynamic biomarkers for therapies that target the BBB or BCSFB.

Proposal Title:	Testing RNA Aptamers in SOD1 G93A ALS Mouse Model
Log Number:	AL220167
Current PI Name:	Li Niu
Award Number:	HT9425-23-1-0374
Current Contracting Organization:	New York, State University of, Albany
Current Performing Organization:	New York, State University of, Albany
Web Approval Date:	09-01-2023

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that results in the death of motor neurons in the brain and spinal cord. Excitotoxicity is a leading pathogenic mechanism for the selective death of motor neurons. Because excitotoxicity is mainly mediated by Ca2+ influx to motor neurons via abnormally expressed, Ca2+-permeable AMPA receptors, developing inhibitors to block excessive AMPA receptor activity is a promising ALS drug discovery strategy. In fact, riluzole and edaravone, the two ALS drugs, both act to reduce glutamate-induced excitotoxicity, among their other activities. Here we combine two approaches for developing a new class of AMPA receptor inhibitors or RNA aptamers that are different from traditional, small-molecule compounds.

First, using an in vitro evolution approach called SELEX, we have previously identified a group of potent RNA aptamers selectively targeting AMPA receptors. These RNA aptamers are more potent and selective towards AMPA receptors, as compared with existing AMPA receptor inhibitors, and they are water soluble by nature. These superior properties would enable us to use aptamers at the lowest dose possible to achieve therapeutic efficacy by more tightly and selectively blocking AMPA receptor activities in vivo, but with minimal or no side effects.

Next, we propose to test these aptamers in SOD1 G93A mice. Transgenic mice overexpressing human Cu /Zn superoxide dismutase type-1 with a glycine to alanine mutation in position 93 (SOD1 G93A) is a well-established rodent ALS model since these mice develop a phenotype similar to ALS in humans. The SOD1 G93A mouse model is the most widely used one for ALS research and drug development. Earlier studies have demonstrated that glutamate-induced toxicity has a contributing role in the neurodegeneration in the SOD1 G93A model, and glutamate receptor blockade leads to neuroprotection.

This proposal has two major objectives: (a) making a group of highly potent, selective, water-soluble RNA-based aptamers that inhibit AMPA receptors, and (b) testing these aptamers for their safety and efficacy in rescuing the ALS phenotype in SOD1 G93A mouse model. The series of tests we propose to conduct include (i) behavioral assays, such as grip strength and rotarod assay to measure motor activity, body weight, (ii) tissue assays, such as the count of the number of motor neurons and the size of these motor neurons, and (iii) survival study. We hope to identify a set of aptamers that are efficacious and without any significant side effects. If successful, this research will lay critical groundwork for clinical testing of several most efficacious, safe and long-lasting RNA aptamers as a new ALS therapy.

Proposal Title:	Validating tRNA Viruses to Target SCN2A-Related Autism Phenotypes
Log Number:	AR220030
Current PI Name:	Aislinn Williams
Award Number:	HT9425-23-1-0279
Current Contracting Organization:	Iowa, University of
Current Performing Organization:	Iowa, University of
Web Approval Date:	07-10-2023

The SCN2A gene encodes a protein called a sodium channel that regulates the balance of excitatory activity in the human brain. Mutations in SCN2A are associated with autism spectrum disorders (ASD). SCN2A was one of the earliest genes associated with ASD, and up to half of patients with SCN2A mutations are autistic.

Mutations in the SCN2A gene can put in the wrong type of amino acid, resulting in dysfunctional sodium channel protein, or they can put in a “stop” signal that introduces an abnormal premature termination codon (PTC) within the channel’s reading frame, much like a misplaced period in the middle of a sentence. Making less than the entire protein (due to the “stop” signal) reduces the amount of functional protein so much that it causes widespread abnormal brain activity. This results in abnormal brain development, autism, and seizures. Unfortunately, there are no available mouse models of SCN2A PTC mutations that could help spur a better understanding of autism and the development of therapeutic strategies for clinical management. Furthermore, existing small molecule (daily pill) therapies for PTC-associated diseases replace the “stop signal” with a wrong amino acid (which is not tolerated by SCN2A) and are clinically ineffective. Gene editing approaches like CRISPR can target unintended genes, and any such effort would require specific validation for each of the many individual PTC mutations, which is an onerous task. New tools and therapeutic strategies are needed for the treatment of autism-associated SCN2A PTCs.

Our group at the University of Iowa has developed a novel gene-correction approach which uses a genetically engineered transfer RNA (ACE tRNA) to repair PTCs. Our highly innovative method works at the source of the mutation to correct the mistaken genetic code and put the correct amino acid in the correct place in the protein. We have identified ACE tRNA sequences for the repair of every known human PTC, a potentially game-changing discovery. We have made two new mouse models of SCN2A PTCs, Y84X and R1626X, which match mutations found in autistic SCN2A patients. We will analyze the sodium channel activity and behaviors of these mice, including both male and female mice, since there are known sex differences in autism symptoms and in co-occurring conditions. We will then determine whether we can correct SCN2A PTCs in neurons from these mice. We will also test whether we can correct PTCs in human neurons generated from autistic SCN2A patients. This project addresses three FY22 ARP Idea Development Award Area(s) of Interest: (1) mechanisms underlying sex differences in ASD; (2) mechanisms underlying conditions co-occurring with ASD; and (3) assessment of novel therapeutics using valid preclinical models.

This project will have multiple high-impact outcomes for ASD research and, hopefully, for the lives of autistic individuals and their families. For basic scientists, our SCN2A PTC mice will be an invaluable resource for the ASD research community. These models will advance our understanding of the molecules and brain circuits that underlie ASD and will serve as a preclinical testbed for future treatments.

In the short term, our studies of mice carrying human SCN2A patient mutations will help us understand how ASD develops in the context of these mutations and may help reveal some of molecular basis for sex differences in ASD manifestation, as well as some of the co-occurring conditions frequently observed alongside ASD, specifically sleep disturbances and seizures. Similarly, our studies in human-derived

neurons will help us understand on a basic level what goes wrong when these mutations occur, and develop a novel method to fix them.

-In the long term, our experiments will provide the proof-of-concept data necessary to move forward with clinical trials using ACE tRNA as a gene therapy. This work will enable the development of delivery modes for the correction of autism-associated PTC mutations for SCN2A syndromes, other genetic forms of ASD, and the diverse array of devastating PTC-related diseases. We have worked closely with SCN2A patients and their families to develop the models and experimental plans we have proposed in the hope of moving forward with work that they believe will be of highest impact for them.

Proposal Title:	Assessing the Molecular Basis of Microbiome-Gut-Brain Signaling in Autism Spectrum Disorder Using a Humanized Zonulin Transgenic Mouse and Human Intestinal Organoids
Log Number:	AR220042
Current PI Name:	Marcy Kingsbury
Award Number:	HT9425-23-1-0702
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	07-24-2023

Autism spectrum disorder (ASD) is behaviorally defined by deficits in communication and social reciprocity along with repetitive stereotypic behaviors. Data released by the U.S. Centers for Disease Control and Prevention (CDC) in 2021 revealed that ASD now affects 1 in 44 school-age children and is 4.2 times more common among boys than girls. Furthermore, a recent 2021 study from the CDC now estimates that 5.4 million adult individuals aged 18 and older are currently living with ASD in the U.S. The social and financial burdens imposed by this debilitating disease include poor quality of life, high health care costs, and substantial loss of productivity.

Gastrointestinal (GI) symptoms are frequently experienced by individuals with ASD, but their prevalence and nature remain elusive, thus hindering treatment. Increased intestinal permeability (“a leaky gut”) and an altered composition of the gut microflora and microbiota (gut dysbiosis) have been reported in ASD, suggesting a possible role for GI abnormalities in the clinical manifestations of the disease. Evidence is now accumulating that gut microbiota dysbiosis may cause functional changes in intestinal permeability, facilitating the passage of bacteria and/or food particles into the bloodstream, leading to inflammation and immune dysfunction in genetically susceptible individuals.

Tight junctions tightly regulate paracellular intestinal permeability and control the passage of microorganisms and food-derived molecules through the gut epithelial barrier. Zonulin, a member of the family of zonulin related peptides, has been identified in humans as the precursor form of haptoglobin 2 (pre-HP2), the only human protein discovered to date that is known to reversibly regulate intestinal permeability by modulating tight junctions. Thus, individuals carrying two HP2 alleles (HP2-2 genotype) may have the greatest predisposition for leaky barriers due to enhanced zonulin production and more significant GI symptoms. Intriguingly, haptoglobin genotype analysis reveals a trend for over-representation of the HP2 allele in ASD GI patients compared to the HP1 allele, and elevated zonulin serum levels also corelate with disease severity in ASD children.

We hypothesize that a genetic predisposition for increased barrier permeability (an HP2-2 genotype) synergizes with dysbiotic gut microbiota from ASD patients to induce a “leaky” gut and blood-brain barrier, producing neuroinflammation and behavioral deficits. To test our hypothesis, we will use three innovative approaches: (1) a humanized zonulin transgenic mouse (Ztm) model in which Ztm mice genetically predisposed to increased intestinal permeability (HP2-2 genotype) will be engrafted with stools from ASD children or from neurotypical healthy (NT) children; (2) human intestinal organoids derived from biopsied tissue taken from ASD patients with an HP1-1 or HP2-2 genotype during a clinically indicated biopsy; and (3) the zonulin inhibitor AT1001 as an interventional tool to mechanistically link brain function and

behavior to the synergy between zonulin and the gut microbiota in ASD. Our proposal addresses multiple FY22 ARP Idea Development Award Areas of Interest including the assessment of a novel therapeutic for ASD, mechanisms underlying sex differences in ASD, mechanisms underlying conditions co-occurring with ASD and long-term treatment outcomes to alleviate co-occurring conditions.

The outcomes of the proposed study will have a tremendous clinical impact on the ASD population, including possible long-lasting benefits for those individuals particularly afflicted with co-morbid GI symptoms. First, we will validate zonulin and the HP2-2 genotype as biomarkers of enhanced gut/blood-brain barrier permeability to allow stratification of the ASD affected population to identify children that will better benefit from targeted interventions aimed at correcting gut permeability (i.e., specific anti-zonulin molecules such as AT1001). Second, we hope to identify specific ASD gut microbiota signatures and/or microbial-associated pathways mechanistically linked to ASD to help define targeted therapeutic interventions to modify and rebalance the intestinal microbiota composition (i.e., the use of well-defined probiotics). Moreover, results from our study will significantly advance our understanding of microbiota-gut-brain signaling in ASD pathogenesis and validate the zonulin inhibitor AT1001 (already showing robust safety profiles in human trials) as a possible targeted treatment to ameliorate the core symptoms of ASD and therefore improve the quality of life of ASD-affected children and their families.

Proposal Title:	Biomarkers of Hyperacusis in Autism Spectrum Disorder
Log Number:	AR220048
Current PI Name:	Kelly Jahn
Award Number:	HT9425-23-1-0326
Current Contracting Organization:	Texas, University of, at Dallas
Current Performing Organization:	Texas, University of, at Dallas
Web Approval Date:	07-12-2023

Background: Autism spectrum disorder (ASD) is one of the most common chronic conditions in the United States (U.S.), affecting at least 3.5 million Americans and 34,361 U.S. military dependents (Tricare 2019). In addition to social and behavioral symptoms of ASD (e.g., communication difficulties and restricted /repetitive behaviors), most autistic people experience sensory sensitivities that diminish their quality of life.

Sensitivity to sound, also known as hyperacusis, occurs in 50-70% of autistic people and frequently limits their social interactions, health care access, education, and employment opportunities. Hyperacusis is a hearing problem that involves difficulty tolerating everyday sounds that do not bother most people (e.g., traffic sounds or dogs barking). Even though we know that hyperacusis is prevalent among autistic people and that it can profoundly affect one's quality of life, we do not know why so many autistic people experience hyperacusis. Unfortunately, because of this major gap in knowledge, health care providers do not have reliable tools to diagnose hyperacusis in ASD or any effective treatments for the condition. Therefore, through this study, we intend to test a potential mechanism of hyperacusis in ASD that will enable the development of sensitive tools that can be adapted to help monitor hyperacusis symptoms in clinical settings. Our proposed work will address the urgent need to identify "Mechanisms of underlying conditions co-occurring with ASD" and "Mechanisms of heterogeneous clinical expression of ASD" based on the FY22 ARP Career Development Award Areas of Interest.

We believe that hyperacusis in ASD is associated with abnormally large responses to sound in one's brain. This assumption is based on many years of animal research. Animals with hyperacusis show unusually large responses to sound in areas of the brain that are responsible for processing aversive (or "bad") sensory stimuli. Animals who respond in this way become hyperactive and/or distressed when they hear ordinary tones and noises. Likewise, being overly sensitive and experiencing distress caused by ordinary sounds, such as dishes clashing or cars honking, are two key symptoms of hyperacusis in ASD. These experiences suggest that autistic people with hyperacusis may have abnormally large brain responses to sound, just like the animals. However, even though hyperacusis has been linked to enhanced brain responses in animals, this same connection has not been made in people with ASD. Therefore, we wish to collect data on behavior and sound processing in autistic and non-autistic young adults (ages 18-35) with hyperacusis who are matched to autistic and non-autistic young adults without hyperacusis. We will record their brain waves (also called electroencephalography [EEG]), changes in their pupil size (or the dark circular area of one's eye), and changes in their skin conductance (or sweat secretion from one's skin) as we play different sounds. We will also record how they perceive the loudness of the sounds, their emotional reactions to the sounds, and how well they can hear the sounds.

Impact: Through this work, we expect to improve our understanding of hyperacusis in ASD and generate tools to measure hyperacusis symptoms in autistic people. The data we collect will lay the foundation for future long-term and intervention studies to address the current lack of treatments and management strategies for hyperacusis in ASD. Although this initial study will be conducted with autistic adults, we wish to do similar work with children in future studies. Ultimately, by dedicating effort to understanding this critical condition, we can make a profound impact on the autistic community by improving social participation, increasing employment and educational gains, and reducing the psychological and physical distress associated with hyperacusis in ASD.

Career Development: Dr. Kelly Jahn will lead this project. She is an audiologist and an auditory neuroscientist with an extensive track record studying the effects of sound and hearing loss. This project will allow Dr. Jahn to apply her audiology clinical and research knowledge to establish a line of independent autism research that can offer meaningful outcomes to autistic individuals, military and civilians alike, including a better quality of life for themselves and their loved ones.

Proposal Title:	Defining the Developmental Time Course and Therapeutic Window for Sensory Circuit Impairments in a Rat Model of Fragile X Syndrome
Log Number:	AR220055
Current PI Name:	Benjamin Auerbach
Award Number:	HT9425-23-1-0395
Current Contracting Organization:	Illinois, University of, Champaign/Urbana
Current Performing Organization:	Illinois, University of, Champaign/Urbana
Web Approval Date:	07-18-2023

Central auditory processing disruptions are some of the most common and debilitating features of autism spectrum disorders (ASD) and related neurodevelopmental disorders like Fragile X Syndrome (FXS). Despite the prevalence and severity of auditory disruptions in FXS and ASD, we are only beginning to understand their underlying brain mechanisms. A major unresolved question is how sensory disturbances in FXS and ASD emerge during development. Intriguingly, early-life sensory deficits in FXS and ASD have been shown to be predictive of the development and severity of behavioral and cognitive symptoms in adulthood. Thus, understanding the developmental mechanisms of auditory processing deficits in FXS will not only directly impact this clinically important sensory symptom, but may extend to other behavioral and cognitive domains as well. The goal of the current project is to identify when and where auditory dysfunction emerges in FXS. Identifying the sequence of events that leads to central auditory processing deficits in FXS is critical for determining optimal windows for initiation of treatments in FXS and related neurodevelopmental disorders. This proposal will address this need by determining the developmental time-course of central auditory processing deficits a Fmr1KO rat model of FX, which we have recently shown to exhibit sound hypersensitivity and central auditory hyperactivity in adulthood. In addition, we will systematically investigate the effectiveness of pharmacological, environmental, and combined intervention during critical developmental time windows using the robust auditory behavioral and neurophysiological phenotypes that we have previously characterized in adult Fmr1KO rats. This work has the potential to provide important insight into the development of central auditory processing disruptions in FXS and other autism-related disorders, which will be crucial for guiding future clinical treatment studies and pre-clinical screening of novel therapies.

Proposal Title:	Randomized, Controlled Trial of a Novel, Mechanistic Treatment for Anxiety in Young Children with ASD
Log Number:	AR220066
Current PI Name:	Amy Keefer
Award Number:	HT9425-23-1-0587
Current Contracting Organization:	Hugo W. Moser Research Institute at Kennedy Krieger, Inc.
Current Performing Organization:	Hugo W. Moser Research Institute at Kennedy Krieger, Inc.
Web Approval Date:	07-24-2023

Study Rationale: Anxiety disorders affect 40% of young children, under 7 years of age, with autism. These disorders negatively affect autistic children because they may interfere with the child's ability to participate in developmental treatments and increase the risk that a child will have anxiety or depression in adulthood. However, few studies have examined anxiety treatment for autistic children in this age range, and young autistic children with cognitive and language delays have been largely excluded from these trials. In school-age children with autism, cognitive-behavioral therapy (CBT) has been used successfully to reduce anxiety. CBT approaches may also reduce intolerance of uncertainty (IU), which is a specific type of fear in which children respond negatively to situations in which they do not know what is going to happen. In autistic children, IU is hypothesized to contribute to the emergence and maintenance of anxiety. This study seeks to address this gap in anxiety treatment by examining if a new, adapted CBT intervention, DINO Strategies for Anxiety and intolerance of Uncertainty Reduction (DINOSAUR), which targets both anxiety and IU, reduces anxiety in young autistic children.

Objectives: Our study addresses three FY22 ARP Career Development Award Interest Areas:

1. Mechanisms underlying conditions co-occurring with autism: Our study targets IU, an underlying mechanism of anxiety in children with autism.

2. Improve diagnosis and access to services across the life span: We examine the efficacy of a novel intervention for a neglected age group in whom anxiety disorders are prevalent, young autistic children.

3. Understanding heterogeneity in treatment response: We include children with developmental delays (cognitive and language) and assess the association of these variables with treatment response. The study's main goal is to examine the efficacy of a new treatment that targets both anxiety and IU, i.e., DINOSAUR, in young autistic children with varying cognitive and language levels. To accomplish this goal, we will investigate whether DINOSAUR is superior to an active control condition (parents only receive information about anxiety but are not taught specific anxiety management strategies) in reducing anxiety. We will also investigate whether DINOSAUR is superior to an active control in reducing IU. A secondary, exploratory goal is to understand how children's language and cognitive level affect their response to anxiety treatment. We will also explore whether high IU levels before treatment contribute to higher anxiety after treatment. Seventy children, 4-6 years of age with autism and clinically significant anxiety and their parents will be randomly assigned to receive either the DINOSAUR or the active control (35 in each group) over 14 weeks via telehealth. Anxiety and IU will be assessed following treatment and at 4-month follow-up.

Applicability and Impact: If, at the conclusion of our 3-year study, we show that DINOSAUR is better than the active control in reducing anxiety, it would provide the most compelling support to date regarding the potential efficacy of adapted CBT in reducing anxiety in young autistic children. It would also set the stage for a large-scale efficacy trial and the potential that DINOSAUR could become a viable anxiety treatment model disseminated to the larger autism community. Treating anxiety when autistic children are young may

lead to lower anxiety levels across the child's development and reduce the chance they will develop other psychiatric disorders across the lifespan. Having an effective anxiety treatment for young children may also encourage autism providers to screen for anxiety when children are young. Positive findings would also suggest that targeting IU in addition to anxiety may be helpful for children with autism and that future studies should study whether treating IU together with anxiety produces lower anxiety levels than treating anxiety alone. If we show that children's language and cognitive skills predict how well they respond to anxiety treatment, this information will help clinicians decide whether adapted CBT should be considered to treat anxiety in autistic children with developmental delays.

Military Relevance: Young autistic children with high anxiety and IU often become highly distressed by major transitions associated with military life, e.g., parent deployment and family relocation. Reducing anxiety and IU may significantly improve the child's response to these changes and decrease family stress. DINOSAUR's telehealth model will also allow families who live in remote locations to receive high-quality anxiety treatment.

Career Development: The proposed trial will provide the principal investigator (PI) with the necessary experience and empirical data to advance her career as a clinical trialist in the field of autism and anxiety. Through the support of her mentorship team, the proposed study will provide the PI with invaluable training in the design, research methods, and data analytic strategies of a clinical trial. Furthermore, positive findings signaling a treatment effect will support an application for a large-scale efficacy study of the DINOSAUR intervention funded by federal and/or foundational grants and, potentially, future studies investigating how DINOSAUR can best be shared with the autism community.

shared with the autism community.

Proposal Title:	Neuromorphogenesis and Neural Circuit Development: The Pedf/Plxdc1 /Adnp Signaling Pathways
Log Number:	AR220067
Current PI Name:	Kazuhito Toyo-Oka
Award Number:	HT9425-23-1-0190
Current Contracting Organization:	Drexel University
Current Performing Organization:	Drexel University
Web Approval Date:	07-24-2023

Topic Area of the Research: This project addresses one of the Fiscal Year 2022 Autism Research Program Idea Development Award Areas of Interest: Assessment of novel therapeutics using valid preclinical models. Our research will assess the efficiency of a novel therapeutic medication using well-established autism mouse models. We will focus on early stages of brain development, where defects can have profound effects on subsequent brain activity and behavior. We believe that correcting defects in early brain development with our proposed medication would prevent autism from developing or reduce symptom severity. This results in the improvement of the patients' quality of life and their families by reducing symptom severity without the need for lifelong daily medication. In addition, our research will provide insights into the mechanisms underlying the differences seen in autism between men and women by separately analyzing the responses in male and female mice.

Central Critical Problems: In April 2021, the Center for Disease Control and Prevention announced that the prevalence of autism spectrum disorder in the United States is 1 in 44 children. More than 5.4 million (more than 2%) of Americans and 7.5 million (about 1%) of the world population have autism spectrum disorder. Also, it is reported that more than 20,000 military dependents have autism spectrum disorder. Autism spectrum disorder is characterized by four core symptoms: (1) impaired communication and social interaction, (2) restrictive interests, (3) repetitive behaviors, and (4) irritability. Many patients with autism spectrum disorder experience daily pain and frustration, as many cannot make friends, engage in social activities, or find comfort in most environments. Autism spectrum disorder is a huge burden on not only patients but also their families and local and federal health systems. More importantly, this creates huge challenges both for the soldiers with autism spectrum disorder and their family due to the long separation and less access to proper treatments during their active service. There is an urgent need to alleviate these burdens, but to date, no effective treatments addressing the cause of autism spectrum disorder exist. Current medications are limited only to reducing irritability. To relieve the severe burdens of this disease, developing improved and novel medications that can address the other core symptoms of autism spectrum disorder are essential. The creation of a new effective medication will improve patients' physical and mental health, by increasing their ability to communicate and learn, as well as reduce the strain on families and institutions such as health care and mental and social services. By providing treatment for the children of active Soldiers and Veterans, the strain on their families at home would be reduced.

Innovation: The proposed studies are conceptionally innovative as we are the first to validate the possibility of protein-derived peptides, pigment epithelium-derived factor and activity-dependent neuroprotective protein, as potential therapeutic medications to reduce autism spectrum disorder. Studies have indicated the possibility of the peptides derived from pigment epithelium-derived factor as a potential medication for tumors and retinopathy. However, little is known about its functions in brain development and its effects on neurodevelopmental disorders like ASD. In addition, our approach is unique in terms of targeting early developmental stages. Many previous studies have focused on the functions of mature neurons. While this is important, recent studies revealed the importance of early developmental stages, including the brain and neuron formation, that may affect later developmental stages, including neural activity and behavior. The

mechanisms behind the flaws in early development in autism spectrum disorder have not been thoroughly investigated, and the potential medications targeting defects in early developmental stages have not been considered.

Impact of the Research: Our project could identify a new effective therapy for autism spectrum disorder, which would have tremendous short-term and long-term treatment implications. The success of our proposal will enable the patients to be treated at the early developmental stages, thus reducing the period of therapies and the dose of medications in their later life. This could also greatly ease the strain on their families and the healthcare system. Especially for Soldiers, new medications specifically improving communication and sociability are directly beneficial for them during active service. Also, this is greatly beneficial for a team working with Soldiers with autism spectrum disorder because it could reduce the risk of life-threatening miscommunication during the execution of military operations. For their families, the availability of medications effective for autism core symptoms could relieve their negative emotions and apprehension when their offspring serve on active duty away from them.

Proposal Title:	Biomarkers for Nocturnal Epileptiform Discharges in Children with Autism
Log Number:	AR220077
Current PI Name:	William Bosl
Award Number:	HT9425-23-1-0333
Current Contracting Organization:	University of San Francisco
Current Performing Organization:	University of San Francisco
Web Approval Date:	07-14-2023

Background and Focus Areas: In the absence of seizures, interictal epileptiform discharges (IED) have been found in up to 60% of children diagnosed with ASD. There is evidence that IEDs can cause lasting language, cognitive, motor, and behavioral impairments, as well as an increased risk to develop epilepsy. But the risks of IEDs are understudied because we lack easy techniques to screen children with ASD for IEDs. Our preliminary research suggests that that IEDs may result from abnormal brain dynamics that can be measured and characterized by analysis of short segments of electroencephalograms (EEG) taken while a child is awake using advanced computer algorithms.

Three areas of particular interest to the FY22 ARP Idea Development Award program are addressed in this project:

1. Mechanisms of heterogeneous clinical expression of ASD. We believe that a subgroup of ASD will have IEDs as predicted by low complexity wake EEG signals. This may present a profile of an ASD subgroup helpful for future development of personalized treatment.

2. Mechanisms underlying conditions co-occurring with ASD: seizures. The centrality of seizure or abnormal electrical activity in ASD remains unknown. What is known is that a large number of people with ASD suffer from comorbid epilepsy. This project will contribute to that understanding by computing dynamical parameters and comparing ASD patients with and without IEDs.

3. Create tools to increase the speed with which evidence-based practices are deployed in community-based settings. If successful, our screening method could be deployed in primary care settings, convenient to the families, and analyzed automatically with our algorithms, providing a universal screening tool that will enable timely, personalized care.

Impact: The result of this project could immediately provide methods to test personalized treatment approaches that improve cognitive or behavioral symptoms and reduce epilepsy risk in children with ASD. It is likely that treatment of IEDs, which can also be monitored with our methods, will lead to new research opportunities and therapies. EEG screening could ascertain a subgroup of ASD which could lead to personalized treatment and prevention analogous to a “lab test” to guide treatment decisions.

Innovation: This project is based on a conception of the brain as a complex dynamical system. Our approach to analyzing this system uses a novel integration of multiscale, nonlinear signal analysis, together with novel machine learning based on supervised tensor factorization. The result is a computational framework for personalized therapeutic monitoring of children with ASD. Together, these advanced computational tools promise to detect IEDs, provide insight into the neurophysiology of these abnormal brain electrical activities, and provide biomarkers to monitor therapy or developmental trajectories.

Proposal Title:	Codesigning and Evaluating a Novel Tele-Assessment Tool for Autistic Adults
Log Number:	AR220096
Current PI Name:	Zachary Warren
Award Number:	HT9425-23-1-0778
Current Contracting Organization:	Vanderbilt University Medical Center
Current Performing Organization:	Vanderbilt University Medical Center
Web Approval Date:	07-24-2023

Considerable attention has been paid to the diagnosis of young autistic children, including recent use of innovative telemedicine assessment tools whose use and benefit has been dramatically accelerated during the COVID-19 pandemic. However, it is increasingly recognized that (1) many autistic individuals are not identified as young children, and (2) accurate identification of autistic adults can help provide meaningful understanding and supports that may ultimately promote autonomy, inclusion, happiness, and improved quality of life across the life span. Local expertise and diagnostic pathways are often extremely limited for undiagnosed adults. Telemedicine has the potential to address many traditional barriers to care access related to geography and availability of providers; however, there are no available psychometrically sound, validated tools for remote assessment of adults with autism-related concerns. The explicit goal of this project is to develop and evaluate a tele-assessment tool and procedure that supports diagnostic decision-making for adults. The objective of this proposal specifically aligns with the FY22 ARP Idea Development Award Area of Interest related to “Improving the diagnosis across the life span.”

In this study, we will co-produce of a telemedicine tool for assessment of autism in adults. We will use our established innovative methodology for fusing advanced data analyses with shareholder expertise by (1) using a clinical research registry to identify key behavior targets and (2) translating these key behaviors to explicit assessment techniques for use via telehealth. We will do this in full partnership with autistic adults and international experts in autistic adult assessment. We will then deploy and evaluate the performance and potential clinical value of the novel telemedicine tool in relation to traditional in-person assessment methods. By co-producing a tele-assessment tool for autistic adults via fusion of computational, clinical, and shareholder expertise and directly evaluating its performance relative to traditional assessment measures, we will gather crucial data for further deployment, investigation, and understanding of its true impact on better supporting and engaging undiagnosed adults across diverse communities of care.

We expect this work to yield a tele-assessment tool (1) targeted to key areas of service access and health care disparity for autistic adults that (2) can be rigorously evaluated to accelerate appropriate, meaningful use across settings. We will make the developed tool freely available across the life span of its development. Beyond simply creating and validating a new tool for the potential direct benefit to autistic adults, we seek to understand and transform the landscape of diagnostic service delivery using community-informed and co-designed processes, maximizing the potential for uptake and equity in care access. We view this work as a part of a larger, scientific movement in which diverse voices are included in the creation and study of novel tools and paradigms to address and overcome current disparities in autism-related care. It is also likely this tool could be used across research and other assessment contexts to better engage and support autistic adults.

The current proposal is innovative in a significant number of ways, among which are principally (1) emphasis on stakeholder co-production to create meaningful approaches for a traditionally underserved population of autistic adults; (2) measure development fusing computational strategies, clinical expertise, and stakeholder partnerships; (3) use of telehealth platforms for evaluation and diagnosis; (4) rapid response to ongoing limitations to service access amplified due to COVID-19; (5) refinement of new measures that

will increase access to underserved and marginalized populations; (6) focus on adulthood as critical point for diagnostic improvement; (7) methodology that partners with self-advocates and advocates across all points of research development; and (8) use of ubiquitous technology (smartphones, tablets) to address diagnostic access challenges.

Proposal Title:	Evaluation of ASD Tele-Assessment in Underserved Communities
Log Number:	AR220098
Current PI Name:	Zachary Warren
Award Number:	HT9425-23-1-0460
Current Contracting Organization:	Vanderbilt University Medical Center
Current Performing Organization:	Vanderbilt University Medical Center
Web Approval Date:	09-14-2023

Although there is evidence that autism spectrum disorder (ASD) can be accurately identified during the second year of life and that early intervention can improve developmental outcomes, many children in the United States are not diagnosed with ASD until much later. Families seeking ASD evaluation often face barriers such as low availability of specialists, lengthy waitlists, and long distances to tertiary care diagnostic centers. This is especially true for children from underserved groups and rural communities. Without an innovative approach for prompt identification of ASD in young children, families, and clinicians will continue to struggle with accessing and providing care. Telemedicine offers tremendous potential for addressing this need. In prior work, we have shown that many children with ASD can be accurately identified using tele-assessment procedures (both in Part C centers and home settings), that participating families report high levels of satisfaction with tele-assessment services, and that deploying such systems in rural settings has the potential to dramatically reduce wait times for identification and service. Moreover, subsequent unanticipated broad dissemination of our telemedicine measure (the TAP) during COVID-19 revealed potential widespread value to providers and families. Although extremely promising, this work has not yet demonstrated the value and impact of ASD tele-assessment for those underserved and under-resourced families most likely affected by existing barriers to traditional evaluation, nor have we established a replicable, sustainable strategy for implementation in real-world settings with limited resources.

In the current study, we will partner our state Part C early intervention (EI) system to deploy a tele-assessment program, inclusive of the TAP, across four of the most rural and medically underserved regions of Tennessee. This project is aligned with the FY22 ARP Clinical Trial Award Area of Interest: “Improve diagnosis and access to services across the life span.” We will study the gradual rollout of this telemedicine paradigm examining data prior to and after rollout. We will study the child, family, provider, and system-level impact of use across the entire population served by these districts over several years. Using input from key stakeholders and characteristics of individual sites, we will present guidelines for implementing a model of ASD tele-assessment that is responsive to the needs and limitations of service providers and then deploy and evaluate its performance, usability, and cost-effectiveness relative to traditional systems of care. We will gather critical information regarding systems-level referral data, stakeholder input, model implementation, and model acceptance to understand and optimize uptake within real-world settings. We will also assess clinical outcomes associated with the implementation model to better understand benefits for underserved families. This work will provide a critical test of the potential true impact of tele-assessment on care for toddlers with ASD in underserved, rural settings. If successful, the components of this model could be rapidly disseminated nationally across Part C early intervention programs and, potentially, military early assessment programs.

Proposal Title:	Oxytocin Effects on Bone Metabolism in Children with Autism Spectrum Disorder
Log Number:	AR220116
Current PI Name:	Madhusmita Misra
Award Number:	HT9425-23-1-0219
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	07-03-2023

Autism spectrum disorders (ASD) are increasingly prevalent disorders affecting 1 in 44 American children. Children with ASD have lower bone mineral density (BMD), lower cortical and trabecular bone integrity, and a higher risk for hip fracture than controls. Potential factors causing bone loss in children with ASD include increased cortisol secretion, low levels of physical activity, and restricted diets and medication use, factors that are not easily modifiable. Since childhood and adolescence are a critical time for bone accrual towards attainment of peak bone mass, it is crucial to determine therapeutic strategies to optimize BMD during childhood in these children. Oxytocin (OXT), a hormone produced in the hypothalamus in the brain, is an important mediator of bone turnover, promoting bone formation over bone loss, based on preclinical studies. In addition to direct effects on bone, OXT may indirectly improve bone health by reducing the stress response, leading to lower cortisol levels. Finally, OXT increases muscle mass in rodents and humans, and lean mass is a critical determinant of bone health. Thus far, intranasal OXT has not yet been investigated as a therapeutic strategy to optimize bone mass acquisition. We propose a randomized, placebo-controlled investigation of intranasal OXT for bone health in children with ASD by studying in parallel the longitudinal effects of OXT vs. placebo on bone turnover markers, BMD, bone structure, and estimated bone strength. We hypothesize that, in children with ASD receiving OXT, bone density, structure, and strength estimates will improve over time. Further, OXT will result in an increase in bone formation and a decrease in bone loss (as indicated by surrogate bone turnover markers), consequent to both direct OXT effects and through reductions in cortisol and increases in lean mass (assessed by DXA and high resolution peripheral quantitative computed tomography [HRpQCT]). In this innovative proposal we will use cutting-edge tools (HRpQCT and microfinite element analysis) to determine changes in not only BMD, but also bone structure and estimated bone strength in children with ASD randomized to OXT vs. placebo, and mechanisms that may mediate the impact of OXT on bone turnover. This study will be the first to systematically investigate the efficacy and safety of OXT as a novel therapeutic agent to improve bone outcomes in children and adolescents with ASD. The potential impact of this trial is an increase in bone accrual and bone density in children with ASD with improved bone structure, increased bone strength and a reduction in fracture risk, both immediate and also long-term, given the expected impact on peak bone mass, a key determinant of future bone health.

Proposal Title:	Training the Future Pediatric Workforce to Implement Best Practices for ASD Within Primary Care
Log Number:	AR220172
Current PI Name:	Jeffrey Hine
Award Number:	HT9425-23-1-0668
Current Contracting Organization:	Vanderbilt University Medical Center
Current Performing Organization:	Vanderbilt University Medical Center
Web Approval Date:	09-14-2023

This proposal is entitled, “Training the Future Pediatric Workforce to Implement Best Practices for ASD within Primary Care” and will focus on training medical residents to better support patients with autism spectrum disorder (ASD) and their families. Our team has designed an innovative training system that links and measures asynchronous training to practice change within a resident’s continuity clinic. This system—ASD in Primary Care Education (ASD-PRIME)—was designed to meet established historical training needs of pediatric residency programs, which are often severely lacking in appropriate training for future primary care providers (PCPs), especially surrounding appropriate care coordination and transition-age guidance and resources. This project addresses multiple FY22 ARP Idea Development Award Areas of Interest including (1) development of healthcare provider-focused training/tools to improve healthcare delivery for individuals with ASD across the lifespan and continuum of care, (2) creating tools/strategies to increase the speed with which evidence-based practices are deployed in community-based settings, and 3) factors promoting success in key transitions to independence for individuals living with ASD.

This project is innovative because it provides a novel system to both guide and measure practice change. This includes focal training for residents during rotations focused on developmental/behavioral concerns, as well as practice guides embedded within the electronic health record (EHR). The asynchronous modules include targeted practice behavior targets that are linked to clinical decision-making guides available for residents during their appointments with patients with ASD. These practice behavior targets and clinical guides (checklists) will be designed using stakeholder-informed focus groups. By leveraging the EHR, we are also able to track specific practice behaviors and documentation related to ASD care. We will then be able to specifically link these expected changes to family outcomes such as service access and an ability to navigate and access resources.

Approximately one in five children living in poverty in the U.S. receives care in a resident continuity clinic. As such, deploying novel programs to promote provider competence and ASD-related practice ownership for residents could dramatically and directly enhance care for families disproportionately affected by service delays, while creating a workforce capable of carrying best practices forward to other community systems of care. At minimum, an embedded, dynamic, training system can support pediatric physicians leaving their residencies with an enhanced competency in effectively communicating with families and other providers and implementing best practices for ASD throughout the continuum of care. Once we establish this impact within our own training program, we intend to package, disseminate, and test the system across other training programs. Widescale examination of this program’s impact on future and long-term practice change for PCPs is critically important to the long-term success, happiness, and wellbeing of children and adults with ASD and their families.

Proposal Title:	The Role of Environmental Stressors on Veteran's Neurocognitive Aging
Log Number:	AZ220042
Current PI Name:	Sharlene Newman
Award Number:	HT9425-23-1-0224
Current Contracting Organization:	Alabama, University of, Tuscaloosa
Current Performing Organization:	Alabama, University of, Tuscaloosa
Web Approval Date:	05-01-2023

Background: Dementia is a progressive decline in neurocognitive function that is deeply distressing for patients and caregivers. The prevalence of Alzheimer's disease (AD) and other dementias in U.S. military Veterans is expected to escalate in the coming year. Greater risk of developing dementia has been associated with increased exposure to environmental stressors before, during, and after time in service. Military personnel may incur a multitude of additional risks including traumatic brain injury (TBI), which in conjunction with exposure to environmental stressors may make them more vulnerable to developing AD. This is important as the majority of TBI in the military are mild to moderate with many going undiagnosed. The goal of this proposal is to investigate the relationship between TBI, exposure to environmental stressors, and neurocognitive aging.

Objective: Neurocognitive functioning is the result of a number of genetic and experiential factors. Each individual has an accumulation of a unique set of interactions with the world. It is that accumulation of interactions that may lead a person down one of potentially multiple paths to dementia. It is critical that we characterize these paths so that we can develop effective interventions to either stop individuals from taking them or slow their progression once on the path to dementia. The objective of the work proposed is to extend previous work in important ways by developing a better understanding of how different interactions with the environment via different exposures to stressors contribute to the development of AD. We will also develop a rich dataset because of the innovation in the combination of data collected from toxin levels, AD blood biomarkers, magnetic resonance imaging (MRI) measures, the type and timing of exposure to stressors and a behavioral/cognitive assessment. The dataset will be made open access to further not only our work but the work of the research community.

During the 3 years of the project, our goal is to recruit 120 Veterans aged 55 years and over who have been identified with potential cognitive decline or mild cognitive impairment (MCI) within the past 5 years through a collaborative effort between The University of Alabama (UA) and the Tuscaloosa VA Medical Center. It is important to note that many military Veterans will not have a formal diagnosis of TBI even though they may have experienced one. A focus on MCI and not individuals with dementia may be expected to aid in identification of these individuals and help to better explain the relationship between TBI and AD risk. We will assess history of exposure to: (1) environmental toxins, (2) chronic psychosocial stress including post-traumatic stress disorder (PTSD), and (3) history of TBI. Biospecimens will be collected to measure inflammatory, toxins, and AD biomarkers as well as MRI scans on a subset of participants to examine brain structure and function. Finally, we will administer a battery of neuropsychological assessments to determine cognitive status.

Innovation and Impact: The near-term scientific impact of the proposed research is to improve our understanding of whether and how exposure to environmental stressors – chronic psychosocial stress and toxin exposure – contribute to the development of AD in individuals with TBI. We anticipate the longer-term impact of creating a more complete characterization of the role that environmental stressors play in the development of AD will be the development of interventions that prevent exposure to and that reduce the

impact of those stressors. The outcomes of this work will impact both the research community and military communities (Veterans, active-duty personnel, and military families). Reducing exposure and/or addressing previous exposures to stressors may significantly reduce dementia rates in military Veterans.

Proposal Title:	Harnessing the Power of VHA EHR Data to Examine Synergistic Associations Between TBI History and Other Risk Factors on AD/ADRD in Military Veterans
Log Number:	AZ220049
Current PI Name:	Victoria Merritt
Award Number:	HT9425-23-1-0230
Current Contracting Organization:	Veterans Medical Research Foundation of San Diego
Current Performing Organization:	Veterans Medical Research Foundation of San Diego
Web Approval Date:	04-04-2023

Study Rationale: Traumatic brain injury (TBI) has been identified as a risk factor for dementia— specifically Alzheimer's disease (AD) and AD-related dementias (ADRD)—by several research groups. However, other investigators have determined that TBI history is not strongly associated with AD/ADRD. Notwithstanding these conflicting findings, we know that there is not a one-to-one relationship between TBI and AD/ADRD—that is, not everyone with a history of TBI develops AD/ADRD and not everyone with AD/ADRD has a history of TBI, suggesting that the relationship between TBI and AD/ADRD is complex, nuanced, and influenced by a multitude of factors. Beyond TBI, several other risk factors have been associated with AD /ADRD including sociodemographic characteristics (e.g., age, sex, race/ethnicity), lifestyle and environmental factors (e.g., smoking and alcohol use, degree of social connectedness), and other medical conditions (e.g., high blood pressure, high cholesterol, diabetes, heart conditions). However, the extent to which TBI history interacts with these other variables to increase AD/ADRD risk is not well understand. More research is therefore needed to improve understanding of the complex relationship between TBI, AD /ADRD, and other risk variables so that more timely and targeted prevention and intervention strategies can be offered.

Objective and Focus Area: The overall objective of our study is to determine the combination of factors that increase risk for AD/ADRD, with the ultimate goal being to develop a risk score calculator that can be disseminated to clinicians to assist with determining an individual's risk for AD/ADRD. As such, our proposal addresses two of the Fiscal Year 2022 Peer Reviewed Alzheimer's Research Program Investigator-Initiated Research Award focus areas: (1) diagnostic, environmental, and prognostic factors and (2) foundational research. To accomplish our overall study objective, we will utilize pre-existing data routinely collected during clinical care (i.e., electronic health record data) from across the Department of Veterans Affairs (VA) Health System, which has data for over 14 million Veterans. The specific patient population will be military Veterans enrolled in the VA Health System with a documented TBI diagnosis (cases) and a comparison sample of military Veterans without a TBI diagnosis (controls). After determining the cohort of eligible Veterans, we will review medical record data and extract the following variables of interest: AD /ADRD diagnostic status; cognitive screening and neuropsychological assessment scores; and other risk variables (i.e., sociodemographics, lifestyle and environmental factors, medical comorbidities, lab values). After extracting all relevant data, we will examine associations between TBI history, other risk variables, and AD/ADRD diagnostic status. Finally, we will develop and validate an AD/ADRD risk score calculation tool.

Clinical Application, Scientific Contribution, and Timeline: This research is novel in that we will utilize multiple sources of routinely collected clinical data to more carefully define TBI and AD/ADRD diagnoses, an advancement from existing research. Moreover, unlike previous studies, we will examine numerous risk factors simultaneously to understand the combination of factors (in the presence and absence of TBI) that

increase AD/ADRD risk. Another strength of our proposal is having access to a large and diverse sample of Veterans from across the nation; this will result in greater generalizability of findings. Finally, we will translate our results into a usable tool for clinicians both within and outside of the VA through the development and validation of an AD/ADRD risk score calculator. As such, data from this large-scale investigation of TBI, AD/ADRD, and other risk factors can be used to better tailor treatments to the individual, potentially altering the recovery process following TBI and mitigating negative health consequences following injury. This would result in both improved quality of life for Service Members and Veterans and health care cost savings. Results from this study also have great potential to inform clinical practice guidelines for both TBI and AD/ADRD, with particular sensitivity to whether current guidelines would benefit from revision when much larger and diverse samples are used to inform care. Finally, it is our goal that the risk tool that will be developed can be easily implemented across health care settings within 1-2 years of its validation to aid in clinical decision-making.

Benefit to Military Service Members and the Public: Given the high numbers of Veterans with a history of TBI, there is an immediate need for research to improve understanding of the link between TBI and AD /ADRD. Furthermore, military Service Members and Veterans may be particularly susceptible to developing AD/ADRD given the unique environmental conditions they face during deployment. Notably, a dramatic increase in AD/ADRD incidence has been predicted within the VA in the coming years as the Iraq /Afghanistan-era Veteran population ages. Thus, there is an urgent need for this research so that our health care systems are best equipped to effectively and efficiently meet the needs of this growing and vulnerable population.

Proposal Title:	Highly Scalable and Cost-Effective Home-Based Sampling and Testing Solution for Glymphatic Clearance/Impairment in TBI Veterans at Risk for Alzheimer's Disease
Log Number:	AZ220073
Current PI Name:	William Haskins
Award Number:	HT9425-23-1-0392
Current Contracting Organization:	Gryphon Bio, Inc.
Current Performing Organization:	Gryphon Bio, Inc.
Web Approval Date:	05-03-2023

Focus Area: Military Service Members are at particular risk of traumatic brain injury (TBI) because of sudden trauma, which causes damage to the brain, though TBI occurs in civilians too. Evidence suggests that brain injury triggers pathological outcomes that could manifest into Alzheimer’s disease (AD) and AD-related dementias over time. This project addresses the Fiscal Year 2022 Peer Reviewed Alzheimer’s Research Program Accelerating Diagnostics Research Award Focus Area “Diagnostic, environmental, and prognostic factors” by performing research that will enable better detection, diagnosis, and treatment of AD following military Service and/or TBI.

Objectives and Rationale: Decades of unsuccessful attempts to cure AD and late-stage dementia have resulted in a paradigm shift to focus on the more challenging aspect of preventing dementia. AD is thought to begin in asymptomatic individuals with no clinical evidence of disease activity; however, detection of such individuals is currently challenging and cost prohibitive. We have identified blood biomarkers, i.e., biological molecules in the blood that can be used in blood tests to diagnose AD at a very early stage and/or to monitor disease progression. Developing home-based blood tests for AD may therefore be feasible and is crucial to identify at-risk individuals and to define optimal treatment windows for early interventions. The objective of this study is to develop a highly scalable and cost-effective home-based sampling and testing solution to detect AD blood biomarkers for early detection and monitoring of subclinical evidence of disease activity, i.e., detection of AD before clinical evidence or symptoms occurs. This solution, known as Gryphon’ s Out of Hospital Sampling and Testing (GOHST) solution, will use plasma samples that are separated from whole blood and dried on filter paper spots after blood is drawn from a finger prick as well as high-sensitivity immunoassays of the spots at a central laboratory to assess blood biomarkers for two frequently co-occurring AD risk factors: sleep-wake disruption and TBI.

Clinical Applications and Patients: This project will be of benefit to patients who have suffered a TBI and are therefore at increased risk of developing AD. It will also benefit patients who suffer from sleep disruption, which is common after TBI and similarly increases the risk of AD. The GOHST solution to be developed in this project will provide a way to identify patients with AD before symptoms occur by performing finger-prick blood collection, which can be performed in the comfort of a patient’s home. This will eliminate the need for standard AD diagnostic tests which are invasive (like lumbar puncture for cerebral spinal fluid), and expensive and time-consuming (like magnetic resonance imaging). These tests are also used for monitoring progress of patients with AD, so our GOHST solution will support patient monitoring over time from the comfort of their own homes. In addition, identifying a set of validated blood biomarkers for AD will allow early diagnosis, which will ultimately support early treatment to improve patient outcomes and quality of life. Ongoing patient monitoring using this approach will also support improved clinical decision-making and patient care. Furthermore, our GOHST solution can be used to assess the impact of new therapies for AD, which will ultimately improve clinical utility and care of these patients.

The risks of the GOHST solution are minimal – akin to finger stick blood collection for glucose testing of diabetes patients.

Timeline: Short-term outcomes that will benefit patients, such as verifying a home-based sampling device for finger-prick blood collection of a set of top-ranked blood biomarkers for early diagnosis of AD should be available at the end of the 3-year project. Longer term outcomes such as improved patient care through early treatment after early diagnosis would likely only be available at a later time following large therapeutic clinical trials.

Broader Impact: The GOHST solution will improve knowledge related to AD by validating a set of blood biomarkers for AD. These biomarkers will ultimately improve patient care by enabling: (1) early AD diagnosis, before symptoms occur, particularly in high-risk individuals such as those who have had a TBI, and (2) monitoring of patient progress over time. The benefits are enhanced, as blood biomarkers can be sampled and tested using the GOHST solution from the comfort of patients' own homes. This solution is inexpensive and non-invasive and will thus ease the burden of the families and caregivers of AD patients, both from a financial perspective and in terms of ease-of-access. In addition, the GOHST solution will ultimately support improved patient care by allowing early therapeutic interventions to maximize patient benefits and improve quality of life.

Military Impact: TBI is a signature injury of military personnel, and nearly half a million American Veterans live with AD, highlighting the need for improved identification and care of military personnel and Veterans with TBI and/or AD. The convenient, home-based sampling of the GOHST solution to identify blood biomarkers for AD will support improved care for Veterans who have had a TBI and are at risk of developing AD, as well as those who have AD who need ongoing monitoring.

Proposal Title:	Do Microbiome-Derived Cell Fragments in the Brain Accelerate Transition from TBI to AD/ADRD?
Log Number:	AZ220077
Current PI Name:	Colette Cywes Bentley
Award Number:	HT9425-23-1-0172
Current Contracting Organization:	Brigham and Women's Hospital, Inc.
Current Performing Organization:	Brigham and Women's Hospital, Inc.
Web Approval Date:	03-27-2023

Traumatic brain injury (TBI) frequently precedes the onset of Alzheimer's disease (AD) or AD-related dementia (ADRD), sometimes by decades. Multiple animal and human studies have linked microbiota composition and blood-brain barrier disruption with TBI, which would allow microbial material seeded into the bloodstream to gain access to the brain. Our project and its scientific rationale are based on evidence that microbial cells and fragments accumulate in the brain and contribute to neuropathology, leading to the behavioral and cognitive declines observed in humans with neurological diseases, including AD and ADRD. Our initial data demonstrate time-dependent accumulation of microbial fragments expressing the highly conserved microbial surface polysaccharide, poly-N-acetyl D-glucosamine (PNAG) in the brains of mice within days of receiving a closed head injury. Detection is accomplished with a fully human monoclonal antibody specific to microbial PNAG, which is not expressed by mammals, to detect microbial fragments in the brain. Immunizing AD mice with a PNAG-targeting vaccine led to prevention of cognitive deficits and removal of microbial PNAG associated with beta amyloid. The molecular and cellular basis for microbial material mediating neuronal dysfunction, and behavioral and cognitive decline in TBI and AD/ADRD pathology is unknown. Based on these data, we hypothesize that neuropathology is a result of inflammation driven by the persistence of undegraded microbiota-derived cells or cell fragments in the brains of affected humans and mice. Microbial fragments may gain access to the brain via disrupted mucosal and blood-brain barriers, or possibly via direct access from the oropharyngeal tract to the olfactory bulbs after TBI. These undegraded microbial remnants act as a focus leading to chronic inflammation. Preventing further accumulation of microbial factors with systemic antibody to PNAG, and/or removal and effective degradation of these microbial fragments in the brain, would reduce inflammation and tissue pathology resulting in spared cognitive and behavioral decline associated with TBI and AD/ADRD.

Focus Area: Using these highly specific antibodies to microbial material, we will improve the understanding of the mechanisms, etiology, and potential therapeutic/treatment for symptoms of AD/ADRD after TBI by addressing the following questions:

Specific Aim 1: Does TBI increase microbiota-derived microbial material access to brains in mice?

Specific Aim 2: Can PNAG-specific antibodies impact TBI-mediated onset of AD/ADRD? Specific Aim 3: Do PNAG-specific antibodies enhance glial cell phagocytosis of PNAG and PNAG-Abeta or PNAG-tau composites?

AD/ADRD affects ~6.2 million Americans aged 65 and older, resulting in 121,499 deaths from AD in 2019. AD is the sixth-leading cause of death in the United States and the fifth-leading cause of death among Americans aged 65 and older. Health care, long-term care, and hospice cost are estimated to be $355 billion for 2021. As yet there is no treatment for TBI or AD/ADRD and associated behavioral and cognitive decline. The onset of cognitive decline frequently follows a fall or infection, events that are linked with disruption of microbiota and increases in the mucosal and blood-brain barrier permeability. This would facilitate microbial cells and fragments gaining access to the brain. Being able to prevent these factors from accessing the brain

could potentially decrease inflammation and behavioral and cognitive decline associated with AD/ADRD and TBI.

Relevance to Military Health: The median age of U.S. male Veterans currently is 65, with the Veterans Health Administration (VHA) estimating dementia prevalence at 9.6%, like the 10.5% observed in the general population. Numerous studies have linked mild (2.3 times greater risk) and severe (4.5 times greater risk) TBI to the risk of developing dementias in later life and also as a precipitating factor in early onset dementia. Understanding the link between TBI and AD/ADRD, and possible strategies to treat or ameliorate the consequences of TBI, is a matter of urgency.

The vaccine and antibodies have already been in early clinical trials in humans and shown to be safe and to lead to sustained levels of functional antibody. Thus, at this point the risks are fairly small, and if effective, the benefits large. Because the vaccine and antibody to PNAG have already been in humans, the path to getting a signal to support a trial in TBI and or AD/ADRD patients is shorter than for most other novel therapies. The key determinant now is demonstrating the presence of PNAG-containing fragments in the affected areas of the brains from TBI individuals that are not seen in uninjured controls, and showing that the vaccine and antibody to PNAG have significant impact on cognitive and behavioral decline in mouse models of TBI and AD. These are the usual studies to support human trials for any antimicrobial vaccine or antibody. We anticipate such preclinical studies could be completed in 2 years, and, if positive, support the design and implementation of a trial in humans comparable to others investigating drugs for prevention of progression of TBI and AD.

Proposal Title:	Do Microbiome-Derived Cell Fragments in the Brain Accelerate Transition from TBI to AD/ADRD?
Log Number:	AZ220077P1
Current PI Name:	Michael Whalen
Award Number:	HT9425-23-1-0261
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	03-27-2023

Traumatic brain injury (TBI) frequently precedes the onset of Alzheimer's disease (AD) or AD-related dementia (ADRD), sometimes by decades. Multiple animal and human studies have linked microbiota composition and blood-brain barrier disruption with TBI, which would allow microbial material seeded into the bloodstream to gain access to the brain. Our project and its scientific rationale are based on evidence that microbial cells and fragments accumulate in the brain and contribute to neuropathology, leading to the behavioral and cognitive declines observed in humans with neurological diseases, including AD and ADRD. Our initial data demonstrate time-dependent accumulation of microbial fragments expressing the highly conserved microbial surface polysaccharide, poly-N-acetyl D-glucosamine (PNAG) in the brains of mice within days of receiving a closed head injury. Detection is accomplished with a fully human monoclonal antibody specific to microbial PNAG, which is not expressed by mammals, to detect microbial fragments in the brain. Immunizing AD mice with a PNAG-targeting vaccine led to prevention of cognitive deficits and removal of microbial PNAG associated with beta amyloid. The molecular and cellular basis for microbial material mediating neuronal dysfunction, and behavioral and cognitive decline in TBI and AD/ADRD pathology is unknown. Based on these data, we hypothesize that neuropathology is a result of inflammation driven by the persistence of undegraded microbiota-derived cells or cell fragments in the brains of affected humans and mice. Microbial fragments may gain access to the brain via disrupted mucosal and blood-brain barriers, or possibly via direct access from the oropharyngeal tract to the olfactory bulbs after TBI. These undegraded microbial remnants act as a focus leading to chronic inflammation. Preventing further accumulation of microbial factors with systemic antibody to PNAG, and/or removal and effective degradation of these microbial fragments in the brain, would reduce inflammation and tissue pathology resulting in spared cognitive and behavioral decline associated with TBI and AD/ADRD.

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could potentially decrease inflammation and behavioral and cognitive decline associated with AD/ADRD and TBI.

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Proposal Title:	Novel solTNF Inhibitor Improves Outcomes in a Mouse Model of TBI-Induced AD
Log Number:	AZ220080
Current PI Name:	Kirsty Dixon
Award Number:	HT9425-23-1-0188
Current Contracting Organization:	Virginia Commonwealth University
Current Performing Organization:	Virginia Commonwealth University
Web Approval Date:	03-27-2023

Fiscal Year 2022 (FY22) Peer Reviewed Alzheimer's Research Program (PRMRP) Investigator-Initiated Research Award (IIRA) Focus Area: These studies will take advantage of a biologic known as XPro1595 that selectively neutralizes the soluble form of tumor necrosis factor (TNF), preventing it from activating its receptor TNFR1. XPro1595 has shown success in a phase 1b clinical trial of elderly Alzheimer's patients, and a phase 2 clinical trial has just commenced. Given the selective nature of XPro1595, we will use this biologic to improve our understanding of the relationship (underlying mechanisms of action) between traumatic brain injury (TBI) and Alzheimer's disease (AD), while simultaneously assessing its efficacy to minimize TBI as a risk factor for the development of AD. Therefore, these studies speak directly to the intent of the FY22 PRARP IIRA focusing on Foundational Research, which seeks to "improve understanding of the mechanisms and develop therapeutics/treatments for AD/AD-related dementias after TBI."

Rationale: TBI is a risk factor for the development of AD, and preclinical rodent studies suggest that TBI accelerates the development of AD brain pathology and cognitive impairment. Another risk factor for AD is the gene known as ApoE4 that also increases AD brain pathology. Both TBI and ApoE4 regulate an aspect of the brains inflammatory system: a specific cell signaling pathway, known as TNFR1 cell signaling (or Tumor Necrosis Factor Receptor 1), that may be responsible for causing underlying changes to the brains neural circuitry, which ultimately impacts cognitive abilities (learning and memory). Recently a biologic was developed that is a specific inhibitor of TNFR1 signaling, known as XPro1595, with no known side effects, and has been trialed in elderly Alzheimer patients. Interim data from the clinical trials reveals that XPro1595 can reverse (cf. slow progress of) brain and serum inflammatory levels. Given that both TBI and ApoE4 regulate TNFR1 signaling and XPro1595 is proving to improve outcomes in elderly Alzheimer patients, this prompted our interest to investigate the use of XPro1595 following TBI to prevent the development of AD pathology. Our preliminary data reveal that XPro1595 treatment following TBI in mice can almost completely prevent glial reactivity and AD pathology (amyloid beta and tau immunoreactivity), which is a precursor to cognitive impairment. These provocative new findings cannot be overstated.

Who Will Be Helped by This Research: TBI affects 1.5 million people annually in the United States, including 20,000-30,000 new military personnel, costing $30,000 and $400,000 for each new mild and severe case, respectively. Although it is difficult to determine the exact percentage of individuals with a TBI who may develop AD, it is estimated that 5% to 15% of severe TBI patients may develop dementia, and the presence of the ApoE4 gene increases chances by 10-fold, compared to non-ApoE4 gene carriers. Therefore, we can postulate that at least 15% of the individuals who suffer a TBI may develop late-onset dementia and may be helped with this biologic.

Potential Clinical Applications, Benefits and Risks: At present, XPro1595 has been used in trials for cancer patients, COVID patients, and Alzheimer's patients, with no known side effects, and the biologic appears safe and well tolerated. Therefore, there are no known risks and only benefits. XPro1595 has also shown successful in numerous preclinical animal models of inflammatory-based injury and disease, including our own TBI, pancreatitis, and cardiac arrest studies (all peer-reviewed published), and in general the biologic

appears to attenuate the inflammatory response to improve outcomes; therefore, these are all potential clinical applications of XPro1595.

Projected Timeline to Achieve Patient Related Outcomes: As indicated, XPro1595 is already in clinical trials for various inflammatory indications, and we have published our preclinical studies using XPro1595 in a mouse model of TBI. We are currently collecting a team to submit a grant application in 2022 to the Congressionally Directed Medical Research Programs Traumatic Brain Injury and Psychological Health Research Program for the use of XPro1595 in patients with TBI. Although AD will not be a focus of these TBI clinical studies, the data from these trials will add to our knowledge base of the efficacy of XPro1595 to improve outcomes following TBI, including those that overlap with AD. Therefore, based on successful funding, we would expect to see patient-related outcomes within 5 years.

Contribution of Proposed Research to Advance Knowledge, Treatments, or Quality of Life of Individuals with TBI and/or AD: These studies will advance the knowledge of the relationship between TBI and AD but will also deliver a promising therapy for TBI-induced AD, which will improve the quality of life of Service Members with TBI.

How Will the Proposed Research Impact the Health of Service Members: Completion of these studies would show that blocking of solTNF using XPro1595 will improve the quality of life of Service Members with TBI by preventing the development of dementia symptoms. Our studies also have direct relevance to military health by addressing a major health problem faced by military personnel, which is TBI, leading to the long-term development of dementia.

Proposal Title:	Culprit Burning Pollutant Associated with Alpha-Synuclein-Related ADRD
Log Number:	AZ220086
Current PI Name:	Xiaobo Mao
Award Number:	HT9425-23-1-0346
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	04-04-2023

Military Service Members may expose to hazardous air pollutants, such as particulate matter (PM) in fire smokes from burn pits, wildfires, and prescribe fires. The exposure of PM has been strongly associated with dementia, and thus can serve as an important but modifiable risk factor of dementia in Veterans. Increased fire emissions of PM are expected under global warming and subsequent drought, therefore also influence the public health of the U.S. general population. However, it remains largely unknown whether and what components of fire smoke PM led to dementia via what specific mechanisms. This project will determine using animal models if whole-body exposure of fire smoke PM (mainly black carbon and organic matter) from well-controlled flaming and smoldering biomass and waste burning can induce -synuclein-related dementia via prion-like -synuclein pathology propagation in mice experiments. This study will identify the types and pathogenic fractions of fire smoke PM that can cause dementia in military-related environment, which can inform the Department of Defense for targeted pollution control and health policymaking.

Proposal Title:	Culprit Burning Pollutant Associated with Alpha-Synuclein-Related ADRD
Log Number:	AZ220086P1
Current PI Name:	Pengfei Liu
Award Number:	HT9425-23-1-0347
Current Contracting Organization:	Georgia Tech Research Corporation
Current Performing Organization:	Georgia Tech Research Corporation
Web Approval Date:	04-04-2023

Military Service Members may expose to hazardous air pollutants, such as particulate matter (PM) in fire smokes from burn pits, wildfires, and prescribe fires. The exposure of PM has been strongly associated with dementia, and thus can serve as an important but modifiable risk factor of dementia in Veterans. Increased fire emissions of PM are expected under global warming and subsequent drought, therefore also influence the public health of the U.S. general population. However, it remains largely unknown whether and what components of fire smoke PM led to dementia via what specific mechanisms. This project will determine using animal models if whole-body exposure of fire smoke PM (mainly black carbon and organic matter) from well-controlled flaming and smoldering biomass and waste burning can induce -synuclein-related dementia via prion-like -synuclein pathology propagation in mice experiments. This study will identify the types and pathogenic fractions of fire smoke PM that can cause dementia in military-related environment, which can inform the Department of Defense for targeted pollution control and health policymaking.

Proposal Title:	Microglial NOX2/NLRP3 Inflammasome Activation Drives Tau Pathology and Cognitive Impairments in Chronic Traumatic Brain Injury
Log Number:	AZ220143
Current PI Name:	Joseph Ojo
Award Number:	HT9425-23-1-0201
Current Contracting Organization:	The Roskamp Institute Inc
Current Performing Organization:	The Roskamp Institute Inc
Web Approval Date:	03-15-2023

This proposal involves a highly synergistic collaboration between Dr. Joseph Ojo (Roskamp Institute, Florida) and Dr. David Loane (Trinity College Dublin, Ireland). We will conduct impactful preclinical research to directly addresses the Fiscal Year 2022 Peer Reviewed Alzheimer's Research Program Investigator-Initiated Research Award focus on Foundational Research understanding of the mechanisms for Alzheimer's disease (AD) and AD-related dementias (ADRD) after traumatic brain injury (TBI). Specifically, we will interrogate pathogenic mechanisms of chronic neuroinflammation following exposure to single severe TBI (ssTBI) or repetitive mild TBI (r-mTBI), how they can contribute to the development of tau seeding and chronic neurodegenerative diseases, and whether selectively targeting these mechanisms can improve long-term recovery.

TBI is the leading cause of death and morbidity in the under-45 age group in industrialized countries. In the U.S., over five million Americans are estimated to be living with disabilities resulting from TBI. Among the Soldiers who survived Iraq/Afghanistan conflicts, TBI accounts for a larger proportion of their casualties than in any other war in recent U.S. history.

TBI can be classified as severe, moderate, or mild. Severe TBI is the least frequently occurring type of TBI but represents the patient population with the highest rate of mortality and morbidity. Patients who suffer from a severe TBI (e.g., penetrating injury, cerebral lacerations, or contusions) display macroscopic changes to the brain typified by bleeding, bruising, or swelling. These patients also exhibit a prolonged length of altered consciousness and memory loss, and those who survive show a plethora of neurological and psychological symptoms akin to neurodegenerative disease that can last for a lifetime. Many young adults never regain premorbid skills or responsibilities despite intensive and comprehensive rehabilitation efforts on their behalf. There is also a significantly increased risk of suicide (by 2.45-fold) compared to those with no TBI diagnosis.

Mild TBI (mTBI) or closed head injuries significantly outnumber severe TBI and other penetrating injuries. Eight-four percent of TBIs among patients evaluated at the Department of Veterans Affairs (VA) occurs in a non-deployed setting involving vehicle crashes, falls, sports, and military training activities (Defense and Veterans Brain Injury Center statistics). In particular, the deleterious effects of repetitive mTBI (r-mTBI) in the last decade has received more attention because of the link between exposures to repeated concussions in contact sports and the development of chronic traumatic encephalopathy. r-mTBI is now recognized to be a major health concern for civilian and military populations, and mounting evidence suggests that r-mTBI may increase the risk for developing dementia-like symptoms where patients experience memory loss, executive dysfunction, and depression.

To date, there are no disease modifying therapies for TBI of any severity. Current treatments for TBI focus on the primary consequences of the injury, such as brain swelling, because the chronic biological consequences of TBI are still mostly unknown. There are very few current clinical trials in the U.S. or worldwide which are specifically addressing mTBI, and typically the drugs under investigation were

developed for other conditions and have shown very little success thus far, presumably due to the lack of knowledge of the molecular mechanisms that constitute the brain's secondary response to TBI. These numerous and complex mechanisms may persist for months and even years after the event, and naturally are significant contributors to the patient's overall outcome.

To address this problem, we have developed and characterized mouse models of single severe TBI (ssTBI) and r-mTBI, which recapitulate many features of human TBI pathology and the heterogeneity of TBI in humans. In our models, we have shown the critical role of an inflammatory cell type – microglia – which remain chronically activated in the brain many months after injury and promote inflammation and cognitive deficits. Importantly, we have confirmed a critical molecule and novel target that appears to regulate the maladaptive transformation of these cells in the chronic stages of TBI from a neurorestorative to a dysfunctional pathogenic phenotype. In this proposal, we plan to use our heterogenous mouse models of ssTBI and r-mTBI to further evaluate the role of this novel mechanism as a target for TBI therapeutics, and its contribution to the long-term neurological and functional outcomes of TBI. Our studies will utilize a genetic model that allow us to manipulate the expression levels of our target in the specific inflammatory cell of interest in the brain at different timepoints relative to the injury, to demonstrate whether we are able to modulate chronic brain inflammation profiles towards recovery of the "normal" profile of an uninjured animal, rescue chronic TBI mediated neurodegeneration, and improve long-term behavioral outcomes. This work will, in 3 years, provide critical information on a potential therapeutic target to mitigate the chronic neurodegenerative consequences of TBI and, importantly, provide preliminary data on possible treatment strategies that may be effective in that regard. This will provide a laboratory-based platform for future studies to further evaluate these targets and explore repurposed drugs or new compounds that modulate our target, with experiments designed to translate the basic science into clinical practice – namely studies of how different doses or administration paradigms influence the outcome after TBI; when the optimum treatment window occurs; how the compounds are distributed and metabolized in the body. This project will thus be of considerable significance in identifying novel approaches and effective treatments for TBI and will contribute to the much-needed increase in flow of viable, TBI-focused treatments. In future work, rigorous preclinical testing of candidate therapeutics against our validated target, particularly if repurposed, could result in novel, well rationalized clinical trials and validated therapeutics for both ssTBI and r-mTBI (a large proportion of our military TBI population) in the next 5-10 years.

Proposal Title:	Dual Threat Combat Injury: Optimizing Combat Casualty Care for Combined Trauma /Shock and Chemical Attack (C4TraCe): A Translational Combat-Relevant Model
Log Number:	BA220027
Current PI Name:	Vikhyat Bebarta
Award Number:	HT9425-23-2-0008
Current Contracting Organization:	Colorado, University of, at Denver
Current Performing Organization:	Colorado, University of, at Denver
Web Approval Date:	07-18-2023

Objectives and Reasoning: Traumatic injuries account for 150,000 civilian deaths in the U.S. each year and half of all combat-related deaths. On the battlefield, many of these traumatic injuries are a result of explosions causing injuries including broken bones, traumatic brain injury, and major blood loss. In the past 20 years, highly toxic chemicals have also been used to harm military and civilian populations. Recently, the invasion of Ukraine by Russian forces has renewed concerns in the international community of the potential deployment of chemical weapons. The Department of Homeland Security has also become increasingly aware of the potential for the weaponization of manmade opioids and, in 2002, Russian forces demonstrated this ability when they used such opioids against Chechen rebels, killing 125 people. The United States military reports chemical injuries often occur combined with trauma (e.g., bone fractures and blood loss), highlighting the need for the development of medical treatments for combined injury (chemical exposure and trauma) as a priority. Additionally, over 56,000 opioid overdose deaths were reported in 2020, with driving under the influence increasing the likelihood of experiencing a motor vehicle accident, resulting in a combined traumatic injury. Yet, there is no good experimental model for the research and development of medical treatments.

The objective of this proposal is to optimize a pig model of combined chemical (cyanide, VX, fentanyl, or carfentanil) and traumatic injury (bone fracture and hemorrhage) and to identify laboratory markers that can be used to measure the degree of injury to help guide care. The optimized pig model will help increase the understanding of the pathophysiology of combined chemical and traumatic injury and can be used in future studies to develop medical treatments for military and civilian use.

Project Timeframe: The objectives of this proposal will be achieved in 3 years. Following ACUROS approval, optimization of chemical agent exposures (VX, carfentanil, fentanyl) will be accomplished. Since our research group has extensive experience with the swine model of cyanide poisoning, we will first optimize the cyanide and traumatic injury model, followed by the VX and trauma model in year 2. In year 3, we will optimize the fentanyl and carfentanil combined injury models. Throughout the entire study period, we will evaluate laboratory indicators of injury. In the final year, we will disseminate results in the form of peer-reviewed publications and conference presentations and work with operational commands and military research directorates to study new or repurposed therapies in this model.

Proposal Title:	Advancing Development of Lyophilized Platelet-Derived Extracellular Vesicles (LEVs) as a Hemostatic Agent for Hemorrhagic Shock and Polytrauma
Log Number:	BA220178
Current PI Name:	Shibani Pati
Award Number:	HT9425-23-1-0489
Current Contracting Organization:	California, University of, San Francisco
Current Performing Organization:	California, University of, San Francisco
Web Approval Date:	09-26-2023

Topic Areas: This proposal is submitted to the FY22 the Broad Agency Announcement for the U.S. Army. The topic areas that are relevant to this proposal are: 1) Developing life-saving resuscitation interventions for TBI and polytrauma/complex injury with the LPEVs; 2) Fielding point-of-injury therapeutic interventions to mitigate secondary effects of brain injury, such as neuroinflammation and intracranial hemorrhage, which are all addressable with LPEVs; 3) Research and development into the delivery of care during transport. LPEVs can be utilized during enroute care or prolonged field care of wounded Warfighters to stop bleeding and organ failure; 4) Regenerative medicine involves the use of innovative technologies such as cell-based treatments to restore Service Members who have suffered combat-related injuries. LPEVs are a regenerative treatment that can help improve the secondary consequences of acute injury to the body or brain.

Objectives and Rationale Behind Proposed Work: Uncontrolled hemorrhage is the leading cause of preventable death worldwide and polytrauma is the leading cause of death worldwide in all individuals between the ages of 1-46. In this proposal, we will explore how a dried product derived from human platelets (LPEVs) can stop uncontrolled bleeding and protect organs after polytrauma with traumatic brain injury (TBI). LPEVs stands for lyophilized (dried) platelet extracellular vesicles. We will measure the effects of LPEVs on improving outcomes in models of severe trauma including TBI. This proposal will aim to support the development of a product (LPEVs) in in three ways: first, by optimizing the production and drying process with a focus on product development; second, by demonstrating that optimized LPEVs stop blood loss in a model of trauma and hemorrhage; third, by testing the ability of LPEVs to reduce brain swelling, hemorrhage, and inflammation in the brain using a polytrauma model (TBI+torso trauma+ hemorrhage) in mice.

Problem to Be Addressed: Platelets are a critical component in stopping bleeding and forming clot formation in bleeding patients. Preventing blood loss and mitigating TBI and polytrauma can save lives. A variety of blood products can help stop bleeding but are not always available especially in battlefield or austere settings. This proposal will develop a dried product from platelets called lyophilized platelet extracellular vesicles (LPEVs) that we and others have shown can stop bleeding and help improve injury to organs in animal models of trauma. The advantage of a dried product is that it has a longer shelf life (approximately 2 years at room temperature) that can be an adjuvant for pre-hospital care and stop bleeding and protect organs when blood products are not readily available. Platelets can only be stored for 5-7 days at room temperature so they are not a great product for remote locations like the battlefield.

Clinical Applications/Benefits and Risks: This research will develop a novel product, LPEVs, that can be used to treat patients who are bleeding especially in remote settings where fully supported medical treatment is not available. This can be applicable not only to military personnel deployed in the field but also to individuals injured in major metropolitan areas where transport time can be lengthy or in remote settings

which are not close to hospitals. In addition to blood loss from the body, we plan to investigate hemorrhage in the brain which is the major complication of TBI. We believe this product can reduce hemorrhage in the brain and decrease brain swelling, to improve survival and overall outcomes for patients who suffer traumatic brain injury (TBI) and polytrauma.

Benefit to Service members, Veterans, and Their Families: The limited shelf life and refrigeration requirement of standard blood products, such as plasma and platelets, makes it difficult to provide plasma on the battlefield for immediate treatment of bleeding combat casualties. LPEVs are predicted to be stable for as long as 2 years at a wide range of temperature and are easy to use, small in size, and could be made available at all levels of care. This product may benefit Service Members who are acutely injured and, in the long run, these therapies may protect their organ function which can often be compromised years later, so the product can have immediate and long-term benefit with minimized risk to the health and safety of the Service Members.

Timeline: We hypothesize that the timeline for bringing this product to commercialization will highly depend on our development partner, Cellphire. Since they have previous expertise, we predict we can start phase 1 safety clinical trials in 3 years and phase 2 efficacy trials in 5 years. The results of those trials will determine whether the product will move to a phase 3 larger-scale trial, which could take another 5 years for full commercialization. So this could realistically be a 12-13-year project.

Proposal Title:	RNA Therapeutics for Infectious Diseases: Viral Hemorrhagic Fever
Log Number:	BA220275
Current PI Name:	Katherine Fitzgerald
Award Number:	HT9425-23-1-0546
Current Contracting Organization:	Massachusetts, University of, Medical School
Current Performing Organization:	Massachusetts, University of, Medical School
Web Approval Date:	07-24-2023

Overview: U.S. military members are frequently deployed to areas of the world with viral threats and emerging infectious diseases. Viruses that cause viral hemorrhagic fever (VHF) are a significant threat; well-known examples include Ebola, Marburg, Lassa, and yellow fever virus. VHF is characterized by fevers, sore throat, vomiting, rashes, abdominal pain, and joint aches and can have high mortality rates. Viruses that cause VHF are relatively understudied, and no U.S. FDA-approved vaccines and therapeutics for VHF are available. In the past, such viral pathogens have been infrequent in the U.S., but they are increasingly encroaching into new territories, placing U.S. citizens at increased risk for VHF. Some have pandemic potential and can be used for bioterrorism, and thus are considered a high priority. Oligonucleotide (oligo) drugs, namely small interfering RNAs and antisense oligos, are ideal for preventing and treating VHF. As international leaders in oligo drug development, we have platforms for designing and generating drugs that can be rapidly developed and dispensed to combat VHF worldwide. Target patient group: We will develop an oligo drug for treating VHF in U.S. military members and frontline workers. We also aim to use oligo drugs to prevent VHF in those who could be exposed to these viruses.

Objectives: Our main objectives are to:

1. Identify host and viral factors required for VHF. We will explore how each is involved in the disease process. Our strategy is to pinpoint key common factors needed for disease development so that we can design advanced oligo drugs to prevent and treat all VHFs.

2. Develop oligo drugs to target host and viral factors to prevent and treat VHF and examine their safety and efficacy in a small animal model of VHF.

3. Advance a top candidate to pre-Investigational New Drug (IND) discussions with the U.S. FDA and provide an oligo drug platform that can be easily adapted for other pathogens.

Potential Clinical Applications, Benefits, and Risks: The primary clinical application is the prevention and treatment of VHF. The benefits and risks are summarized below.

Benefits: Oligo drugs are injectable, provide long-lasting protection, can be rapidly produced at a low cost, are stable at tropical temperatures (where VHF infections originate), and do not require refrigeration for storage. Because oligo drugs can be chemically modified, they can be formulated to direct them to specific tissues or to multiple organs that are the primary sites of infection. Unlike vaccines or antivirals, oligo drugs can simultaneously target both the virus and human genes that the virus hijacks for its own purposes. A receptor that a virus uses to enter human cells would be one such target. Oligo drugs are ideal as a quick-acting treatment following virus exposure because they work rapidly (within 12 hours). Once the sequence of a virus is known, advanced mapping tools can be used to design a virus-specific therapeutic within 48 hours. Manufacturers can scale up oligo drug production to treat millions of people within months of identifying new threats. Our team has already successfully designed oligo drugs for other viruses and diseases such as COVID-19, amyotrophic lateral sclerosis, and Huntington’s disease. Oligo drugs have multiple advantages, including an unprecedented duration of effect (up to 6 months following a single injection), a clean safety profile, and high specificity.

Risks: A potential risk of oligo-based drugs is unwanted activation of the immune system. Our team is leading efforts on chemical modifications to oligo drugs to mitigate this risk.

Timeline: In this 4-year project, we will identify key factors required for VHF disease, develop oligo drugs targeting these factors, optimize delivery to relevant tissues and cell types, and test our candidate oligo drugs in a relevant small animal model of disease. At the end of the 4 years, we will have generated data to begin pre IND discussions with the FDA.

Benefits to Service Members, Veterans, and/or Their Family Members: Our goal is to generate oligo drugs to prevent and treat VHF for our Service Members and the public. The proposed research will result in a better understanding of how these viruses infect and cause disease in humans, enabling customized treatment strategies for service members and the public. Also, the oligo drug design platform is easily adaptable for other viral threats encountered by our service members, veterans, and family members.

Summary: Our program provides essential information on understudied viruses that will lead to better treatment and prevention of VHF. Our platform optimizes oligo delivery to specific tissues, organs, and cell types. The backbone and chemical modifications to oligos developed for VHF can be applied to other viruses simply by knowing their sequence. Proof of efficacy in animal models will help expedite pre-IND discussions with the FDA on the top-performing candidate and help lay the groundwork for future studies of oligo drugs to prevent and treat viral pathogens that threaten our U.S. military, Veterans, and the public.

Proposal Title:	VaxCelerate 3.0: A Scalable and Efficient Self-Assembling Vaccine Platform to Enable Rapid Development of US Military Vaccines
Log Number:	BA220292
Current PI Name:	Mark Poznansky
Award Number:	HT9425-23-1-0586
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	07-24-2023

Traditional development of a new vaccine against an infectious disease like influenza or Mpox is incredibly expensive (costing millions to billions of dollars) and takes a long time (5 to 10 years) from start to finish. This is to a large degree due to the fact that each vaccine is individually designed and tailor-made. The primary objective of our proposal is to optimize our new vaccine platform that has the potential to make many different vaccines for different infectious diseases or pathogens using the same design and manufacturing process. Our approach, if successful, would simplify vaccine development and could make new vaccines less expensive to develop and reduce the time it takes to do so. Our so-called self-assembling vaccine, or SAV for short, uses two chemical components or parts that can fully assemble spontaneously into a final vaccine. One component is a protein that helps deliver the vaccine to immune cells and enhance the vaccine response against the infectious agent. This protein is the same for all vaccines produced on our platform. The other component is a part of a protein that helps immune cells recognize an infecting agent. This component is different for each vaccine depending on the infectious agent. These two components snap together like “Lego bricks” when mixed together in a simple salt solution at room temperature. The product is also stable and can be stored in a simple refrigerator.

While this vaccine platform has shown the ability to stimulate immune responses against different kinds of infectious agents, there are specific things that can be done that would both speed up the time required to make a new vaccine and make the vaccine better. In this project, the scientists and physicians will do three things:

1. Show that the same SAV platform can make multiple different vaccines that can protect against two different types of infectious viruses (a virus that causes the respiratory disease called flu and a pox virus that causes skin lesions).

2. Make the necessary improvements to the way the vaccine is made to maximize efficiency and the amount of the vaccine that can be produced in a short period of time.

3. Show that these improvements in SAV production can allow investigators to make a completely new vaccine very quickly that is also protective in an animal model.

While the development of this vaccine platform is designed to make new vaccines specifically for the U.S. military, SAV would have general application in making all kinds of vaccines more quickly and less expensively. If this project is successful, the use of the same design for multiple vaccines would shorten the development time and lower the cost, especially in the early stages of vaccine development.

The purpose of the project is to make it easier to design new vaccines against infectious agents against which there is not yet a vaccine in order to provide additional protection to military personnel. At times, military

personnel require vaccines most civilians would not need to have because our troops travel and work all over the world. However, some vaccines developed for the military will also prove to be useful to Veterans and family members of military personnel as well as to the general public.

Proposal Title:	Extracellular Vesicles as Biomarkers, Mediators of Immune Dysfunction, and Universal Countermeasures Against Radiation Syndrome and Associated Polytrauma
Log Number:	BA220315
Current PI Name:	Robert Maile
Award Number:	HT9425-23-1-0913
Current Contracting Organization:	Florida, University of
Current Performing Organization:	Florida, University of
Web Approval Date:	09-26-2023

What Is the Problem: Radiological and nuclear threats are increasing rapidly. Examples can include detonation of a nuclear weapon, use of a dirty bomb, a stealth radiological dispersal device, deliberate attack on a nuclear power plant, and radiological/nuclear accidents. The number of people who will need medical attention after a large-scale event will be large. These threats have the ability to involve military and civilian populations. Radiation causes significant death as a result of tissue damage, with the degree of damage depending on the duration and dose. Radiation survivors have a significantly dysfunctional immune response causing damage to the victim's own tissue yet unable to control infection. In addition, when radiation injury is accompanied by additional traumas, such as burn injury, there is increased chance of acute death and even more altered effects on the immune system. After radiation injury and combined injuries, patients experience a range of short and long-term consequences such as increased rates of infection, cancer and many other diseases. To add to this, if you consider that at times it is not immediately obvious that people have been exposed to radiation, for example placement of a stealth radiological source in a public area or water source, we currently have no way to rapidly identify individuals exposed and the magnitude of exposure.

Our Solutions: Our group has spent 25 years investigating ways to explain and solve these problems, developing models of burn injury, radiation injury and combined injury models in clinically relevant mouse models. We also correlate all our findings in trauma patient samples, having developed a pipeline to collect immune cells from burn patients. We base this proposal on three key findings:

1. We have discovered that naturally occurring lipid-protein structures, called extracellular vesicles (EV), normally produced by cells as a means for cells to signal to each other, become "bad" and now able to cause the dysfunctional immune response associated with radiation and burn injuries. We believe that using a simple naturally-occurring molecule isolated from milk called lactadherin, we can deplete these EV from a patient and help return the immune system to normal.

2. In addition, we have shown that we can examine the specific cargo of the EV and the identity of the cargo can be used as a stable biomarker to detect and quantify radiation exposure in people. We can examine EV from blood or saliva of patients which allows us to examine large numbers of people quickly. Development of this biomarker and the calculations to relate EV cargo with degree of exposure is the first step to developing a point-of-care device that can be used in the field.

3. We have also discovered that EV produced by certain stem cells (MS-EV) cultured in the laboratory injected into radiation and/or burn injured mice prevented these mice from forming a dysfunctional immune responses. These MS-EV can be grown in large quantities, stored for very long periods of time (even at 4

degrees C refrigerator temperature), and can be used “universally” without having to be “tissue-matched” with recipients. We will test these ideas using our pre-clinical mouse models of injury, and with human samples collected from burn patients, as detailed in the Proposal.

Benefits: We propose that the main benefits of this study are that twofold: (1) EVs can be used early and non-invasively to detect and quantify radiation exposure and (2) removal of host pathogenic EV or administration of MSC-EV early and late after radiation injury or complex burn + radiation injuries will diminish dysfunctional immune responses and will, in turn, improve short and long-term clinical outcomes and prevent the devastating consequences of these injuries. Our goal is to convert these findings into tangible benefits, as it relates to Service Members and all our patients, to help address their immediate reduced immune function as quickly and effectively so that they can return to health without limitation at their highest capacity. This proposal fulfils the foci of multiple DOD research interests, including the stated research interest of the Combat Casualty Care Research Program (CCCRP) in researching “care of casualties that have sustained both polytrauma and or burn injuries in addition to injuries related to a radiological or nuclear scenario. Work is also considered related to medical countermeasures for acute ionizing radiation injury.” We anticipate completing animal validation, human outcome studies, and ex vivo work for this within 5 years of the project completion and clinical trials within 5 years.

Proposal Title:	Hormonal Biomarkers of Exertional Heat Stroke Susceptibility in Females
Log Number:	BA220322
Current PI Name:	Orlando Laitano
Award Number:	HT9425-23-1-0769
Current Contracting Organization:	Florida, University of
Current Performing Organization:	Florida, University of
Web Approval Date:	09-26-2023

What Is the Problem: Exertional heat stroke (EHS) is characterized by central nervous system dysfunction in hyperthermic subjects during physical activity. The condition can be lethal or result in long-term health complications later in life. The rate at which women will experience EHS is expected to increase markedly in the upcoming years. This prediction is supported by the increasing participation of women in military specialty roles previously accessible only to men. With the lift of the Combat Exclusion Rule on January 24, 2013, women are eligible to serve in front line combat and complete combat operations. From 2020 to 2021, the U.S. armed services reported an increase of ~12% in EHS cases among female Warfighters. This increasing occurrence of EHS in women is of particular concern to the U.S. armed services because EHS is the most severe manifestation of exertional heat illnesses and one of the main causes of death during physical activity. Environmental aspects such as climate change and increases in heat wave's frequency and duration are additional contributing factors to the increased occurrence of EHS in women. Females are underrepresented in exercise thermoregulation research. The lack of studies addressing physiological responses of females in extreme environments has generated a knowledge vacuum regarding their susceptibility to heat related illnesses. Understanding the fundamental factors associated with EHS response in women is necessary to develop preventative and treatment strategies tailored specifically to this cohort. Currently, there are no reliable biomarkers to predict susceptibility of EHS in females. Our preliminary observations indicate that the ovarian hormones (e.g., estrogen and progesterone) are strong candidates to predict susceptibility to EHS in this cohort. Whether the ovarian hormones affect the efficacy of heat acclimation (HA) and organ damage in response to EHS remains unknown and will be elucidated here. The studies herein proposed cannot be accomplished using human volunteers because it is unethical to experimentally induce EHS in humans. Thus, we will rely on our clinically relevant and highly reproducible mouse model of EHS to accomplish our experimental goals aimed at improving our ability to predict and prevent EHS in females.

Objective: We will determine whether ovarian hormones can be used as biomarkers to predict susceptibility to EHS in females. In addition, we will determine the role of ovarian hormones on HA in females. We will use our reliable mouse preclinical model of EHS to study female responses when the ovaries are intact (SHAM) or removed (OVX). In addition, we will use implanted osmotic pumps containing either estradiol or progesterone to determine which of the two hormones are involved in the heat tolerance phenotype observed in females. We will determine whether HA efficacy to protect against EHS is affected by ovarian hormones.

How We Address the FY23 Focus Area: The proposed work meets the intent of the award mechanism and focus area of Military Operational Medicine Research Program under the research area of Environmental Health and Protection. We propose to determine whether ovarian hormones can be used as hormonal biomarkers to predict susceptibility to EHS in females. We will also investigate the influence of ovary-related hormones on HA efficacy and subsequent response (e.g., organ damage and dysfunction) to EHS. We hypothesize that ovarian hormones delay EHS occurrence, attenuate organ damage during recovery from EHS, and facilitate HA in females.

Applicability: These preclinical studies will provide critical knowledge regarding the role of ovarian hormones in EHS susceptibility and HA efficacy and will provide foundational data that can be transitioned to human studies to develop more sex-specific HA and clinical treatment protocols to reduce lost duty days, decrease medical care costs and accelerate return to duty following an EHS event. Our goal is to begin transitioning the findings to humans. We will explore our long-term collaborative efforts with USARIEM and the Army Heat Center to adopt a “bench-to-bedside” approach. Since the ovarian hormones can be detected in blood and saliva samples, we foresee no difficulties in translating the science to predict the susceptibility of EHS and HA efficacy in the female Warfighter.

Proposal Title:	Epigenetic Signatures to Predict Pathological Nodal Stage for De-escalation of Axillary Surgery in Patients with Clinically Node-Positive Breast Cancer
Log Number:	BC220116
Current PI Name:	Maggie Dinome
Award Number:	HT9425-23-1-0030
Current Contracting Organization:	Duke University
Current Performing Organization:	Duke University
Web Approval Date:	02-08-2023

Clinical trials have shown that removing lymph nodes with surgery as part of breast cancer treatment does not improve cancer outcomes for many patients with breast cancer. The results of these practice-changing clinical trials have changed the way we treat breast cancer patients currently. However, these trials notably excluded patients who presented with cancer already spread to the lymph nodes, known as clinically positive nodes. Because of this, the standard of care for breast cancer patients who have lymph node disease at diagnosis and who do not receive chemotherapy drugs before surgery (neoadjuvant chemotherapy [NAC]) remains axillary lymph node dissection (ALND), whereby up to 20 lymph nodes in the underarm area are routinely removed with surgery. The majority of these patients have estrogen receptor positive (ER+) and human epidermal growth factor receptor 2 negative (HER2-) breast cancers, which accounts for more than two-thirds of all breast cancers. ALND carries a number of undesirable side effects for breast cancer patients, including fluid buildup in the arm and swelling (lymphedema) and impairment of limb function (functional morbidity), which can be disabling. Yet, recent work from our group and others has shown that more than half of breast cancer patients with ER+/HER2- breast cancer who undergo ALND for clinically positive nodes have only limited disease in their lymph nodes at surgery (< 3 positive lymph nodes). As a result, most of these patients have a significant number of normal, healthy lymph nodes removed from their bodies and only a small number of diseased lymph nodes. ALND, a highly invasive surgical procedure, is likely unnecessary for many patients with clinically node positive breast cancer and represents a glaring overtreatment for patients with only limited lymph node disease. However, a reliable method to identify which patients have limited lymph node disease from those who carry a higher disease burden (4 or more positive lymph nodes) without surgical removal of the nodes has remained elusive. There is a critical need to fill this gap, to avoid invasive ALND for patients where it is otherwise unnecessary and reduce overtreatment of breast cancer.

This proposal addresses the Fiscal Year 2022 Breast Cancer Research Program Overarching Challenge: surgical overtreatment. Our research group has identified three distinct epigenetic signatures (EpiSig) in patients with ER+/HER2- breast cancer that show high accuracy for distinguishing which patients have low lymph node burden from those who have higher lymph node burden. Indeed, our preliminary work shows that these epigenetic profiles, which we have termed EpiSig 10, EpiSig 13, and EpiSig 14, have high accuracy (AUC=0.98) in identifying patients with low nodal disease. We hypothesize that these EpiSig of primary invasive, ER+/HER2- breast cancers can effectively stratify patients into categories of low or high nodal disease burden. These EpiSig have great promise for providing a highly reliable, non-invasive method for determining a breast cancer patient’s lymph node stage that can reduce or prevent unnecessary surgical intervention, thereby providing individualized management of care and reducing the surgical overtreatment of breast cancer patients who are found to have limited nodal disease.

We will address the gap in knowledge by testing and validating our three EpiSig in cohorts of breast cancer patients who present with clinically positive lymph node disease. We will test the efficacy of the EpiSig in accurately identifying patients with low nodal disease from tumor DNA of an independent cohort of these patients. We will also develop and optimize a reliable assay that targets our EpiSig that will be clinically useful and easy to adopt for clinical translation. The assay will be developed based on the distinct genomic regions of the signature with the highest accuracy and optimized for use in patient biopsies taken before the patient has undergone surgery. Finally, we will test the accuracy of our EpiSig assay on an independent set of presurgical tissue samples in identifying patients with low nodal burden who could potentially forgo ALND.

Without an accurate, reliable, non-invasive method to identify breast cancer patients with low nodal disease, these patients will continue to undergo invasive surgical intervention. Our EpiSig represents a highly promising, practice-changing method for filling the gap of this urgent clinical need. The discovery and translation of molecular signatures have transformed breast cancer care. Genomic assays based on a patient's primary breast cancer have allowed for de-escalation of medical therapies and omission of chemotherapy in patients with favorable tumor profiles. Completion of the objectives of this proposal will provide a crucial breakthrough for identifying patients for whom ALND is unnecessary, thereby reducing the surgical overtreatment of breast cancer patients with low nodal disease and resulting in an accurate, predictive biomarker for lymph node staging.

Proposal Title:	Integrating Molecular Pathology, Radiology, and Genetics to Improve Breast Cancer Risk Prediction
Log Number:	BC220142
Current PI Name:	Mark Sherman
Award Number:	HT9425-23-1-0234
Current Contracting Organization:	Mayo Clinic and Foundation, Jacksonville
Current Performing Organization:	Mayo Clinic and Foundation, Jacksonville
Web Approval Date:	04-12-2023

Each year, over a million women in the U.S. undergo breast biopsies (i.e., tissue sampling) to determine whether a suspicious lesion found on a mammogram or on physical examination represents breast cancer (BC). Although 75% of breast biopsies show benign breast disease (BBD), which is reassuring, numerous studies have established that women diagnosed with BBD experience a 1.5- to 4-fold increased risk of developing BC in the future compared with women not diagnosed with BBD. Classification of BBD by pathologists as non-proliferative, proliferative without atypia or atypical hyperplasia identifies groups of patients at progressively increased BC risk; however, pathology alone is insufficient to provide accurate risk information for individual women. We propose to leverage our recent discovery of genetic, radiologic, and molecular risk factors for BC to develop improved approaches for individual risk prediction, enabling most women to receive reassurances of relative safety, while identifying high-risk women who might benefit from intensive screening or therapy that would prevent their cancers entirely or detect them at an early, curable stage. Further, our unique study will compare the molecular pathology of biopsies to the radiological lesions from which they were derived, thereby improving our ability to assess future risks posed by specific mammographic findings (e.g., microcalcifications) of radiologically similar appearing lesions that are not biopsied to rule out ductal carcinoma in situ or invasive BC but may represent markers of BC risk throughout the breasts.

Many factors impact BC risk, but few are strong individually; thus, a multi-modality approach that combines factors with additive impact is needed to achieve a breakthrough in BC risk prediction. We have identified several candidate markers that address this requirement. First, research has identified variation in genes, referred to as single nucleotide polymorphisms (SNPs), which individually are associated with small BC risks but which when combined into a polygenic risk score (PRS) are highly predictive. We analyzed a PRS developed, tested, and validated among over 200,000 women, in a subset of five studies, demonstrating that women with BBD and the highest 10% of PRSs experienced an 11-fold increase in BC risk compared with women without BBD in the lowest PRSs. Importantly, risks associated with PRS and BBD were additive and similar across studies. Also, we have demonstrated that mammographic density and certain diagnoses of BBD contribute additively to BC risk prediction, and more recently we and others have determined that women whose mammograms show multiple microcalcifications are at increased risk. In our data, BBD severity and microcalcifications are independent BC risk factors. Further, several novel histopathologic features that are not routinely assessed microscopically by pathologists and molecular markers in breast tissues are also linked to BC risk. While multi-modal risk prediction approaches have been proposed, we aim to integrate them into a single clinical approach to achieve a breakthrough and link risk to specific carcinogenic mechanisms (hormones, inflammation, growth factors, etc.) that may point to prevention strategies for future testing in clinical trials.

To achieve our major goals within the 4-year life cycle of this grant, we will identify women diagnosed with BBD who progressed to BC and women matched on age and follow-up time who remained cancer-free at Mayo Clinic, Rochester, and University of North Carolina, Chapel Hill. We will collect germline DNA for genetic testing, radiological images for analysis of mammographic density and additional features such as

artificial intelligence (AI) and perform microscopic, AI, and molecular analyses of biopsies. We will define features from these approaches that contribute additively to predict BC risk and re-evaluate the genetic and radiological markers among screened women at Mayo with available genetic data, radiological images and follow up for development of BC. We reported that providing novel risk information beyond that offered by risk prediction models in clinical use (e.g., PRS) impacts women's receptivity to using chemoprevention, and it is known that endocrine chemoprevention (e.g., tamoxifen) is highly effective in reducing BC risk among BBD patients, suggesting high potential for clinical translation. Improving BC risk prediction is timely, as increased knowledge of mechanisms of breast carcinogenesis and development of novel lifestyle and pharmacological preventive interventions offers the promise of achieving precision prevention in coming years. Further, because we will compare radiologic and molecular pathologic features, our results have important implications for characterizing radiological signs of BBD, which may themselves not require biopsy to rule out BC but represent important markers of BC risk.

Proposal Title:	Integrating Molecular Pathology, Radiology, and Genetics to Improve Breast Cancer Risk Prediction
Log Number:	BC220142P1
Current PI Name:	Melissa Troester
Award Number:	HT9425-23-1-0235
Current Contracting Organization:	North Carolina at Chapel Hill, University of
Current Performing Organization:	North Carolina at Chapel Hill, University of
Web Approval Date:	04-12-2023

Each year, over a million women in the U.S. undergo breast biopsies (i.e., tissue sampling) to determine whether a suspicious lesion found on a mammogram or on physical examination represents breast cancer (BC). Although 75% of breast biopsies show benign breast disease (BBD), which is reassuring, numerous studies have established that women diagnosed with BBD experience a 1.5- to 4-fold increased risk of developing BC in the future compared with women not diagnosed with BBD. Classification of BBD by pathologists as non-proliferative, proliferative without atypia or atypical hyperplasia identifies groups of patients at progressively increased BC risk; however, pathology alone is insufficient to provide accurate risk information for individual women. We propose to leverage our recent discovery of genetic, radiologic, and molecular risk factors for BC to develop improved approaches for individual risk prediction, enabling most women to receive reassurances of relative safety, while identifying high-risk women who might benefit from intensive screening or therapy that would prevent their cancers entirely or detect them at an early, curable stage. Further, our unique study will compare the molecular pathology of biopsies to the radiological lesions from which they were derived, thereby improving our ability to assess future risks posed by specific mammographic findings (e.g., microcalcifications) of radiologically similar appearing lesions that are not biopsied to rule out ductal carcinoma in situ or invasive BC but may represent markers of BC risk throughout the breasts.

Many factors impact BC risk, but few are strong individually; thus, a multi-modality approach that combines factors with additive impact is needed to achieve a breakthrough in BC risk prediction. We have identified several candidate markers that address this requirement. First, research has identified variation in genes, referred to as single nucleotide polymorphisms (SNPs), which individually are associated with small BC risks but which when combined into a polygenic risk score (PRS) are highly predictive. We analyzed a PRS developed, tested, and validated among over 200,000 women, in a subset of five studies, demonstrating that women with BBD and the highest 10% of PRSs experienced an 11-fold increase in BC risk compared with women without BBD in the lowest PRSs. Importantly, risks associated with PRS and BBD were additive and similar across studies. Also, we have demonstrated that mammographic density and certain diagnoses of BBD contribute additively to BC risk prediction, and more recently we and others have determined that women whose mammograms show multiple microcalcifications are at increased risk. In our data, BBD severity and microcalcifications are independent BC risk factors. Further, several novel histopathologic features that are not routinely assessed microscopically by pathologists and molecular markers in breast tissues are also linked to BC risk. While multi-modal risk prediction approaches have been proposed, we aim to integrate them into a single clinical approach to achieve a breakthrough and link risk to specific carcinogenic mechanisms (hormones, inflammation, growth factors, etc.) that may point to prevention strategies for future testing in clinical trials.

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Proposal Title:	Functional Identification of High-Dimensional Driver Combinations in Basal-Like Breast Cancer
Log Number:	BC220146
Current PI Name:	Zhe Ying
Award Number:	HT9425-23-1-0038
Current Contracting Organization:	Icahn School of Medicine at Mount Sinai
Current Performing Organization:	Icahn School of Medicine at Mount Sinai
Web Approval Date:	01-10-2023

Basal-like breast cancer (BLBC) is one of the most aggressive subtypes of breast cancer that does not harbor currently known druggable mutations. As a result, precise and efficient molecular targeted therapy is still not available for this disease, leaving chemotherapy as the main treatment option. Although chemotherapy can efficiently halt BLBC growth in 30% of patients, for the rest, rapid relapses will occur within 3 years of treatment.

One key to solve these major challenges facing survival of BLBC patients is to identify mutations that act as drivers of BLBC initiation, maintenance, and recurrence from a myriad of mutations found in patients. Identification of BLBC drivers can lead to two potential strategies for the successful treatment of BLBC: (1) develop molecular targeted therapies to block driver lesions that are required and essential to program basal-like fate and (2) improve the efficiency of existing chemotherapies by targeting recurrence drivers.

The mouse mammary gland is a powerful system to identify breast cancer drivers because it resembles major properties of human counterparts. However, modeling large amounts of patient-derived genetic lesions in mouse mammary glands and identifying cancer drivers from them is not easy. First, most traditional genomic engineered mouse models can only test one genetic condition in one animal, and the process of generating mouse models can take years. Moreover, in BLBC evidence from our previous tumor screens and published large-scale tumor-sequencing projects suggests that basal-like breast cancer requires a combination of multiple drivers to form. These are not modeled adequately in current mouse models – nowhere near to the degree of complexity and diversity required to understand what genetic lesions drive BLBC formation, drug resistance, and recurrence locally and at different metastatic sites.

To solve these problems, in the past few years, we have developed a novel strategy that allows us to deliver and test up to ~1200 patient-derived mutations in the mammary glands of a single mouse. In the current project, we will model and test highly complicated combinations of BLBC mutations in mouse mammary glands. Using these cutting-edge approaches, we will identify driver combinations that are required and essential to induce primary BLBCs, drive treatment resistance, and ultimately cause recurrence.

Successful completion of the current project will point out which drivers are the most efficient candidates for developing molecular targeted therapy of BLBC, will identify which drivers are associated with resistance to specific chemotherapy agents, and will determine whether inhibition of these drivers in combination with chemotherapy can achieve prevention of BLBC recurrence, as a temporary/alternative plan before molecular targeted therapy fully replace chemotherapy.

Proposal Title:	Revealing the Potential for mSWI/SNF as Biomarkers in Breast Cancers
Log Number:	BC220157
Current PI Name:	Capucine Van Rechem
Award Number:	HT9425-23-1-0131
Current Contracting Organization:	Leland Stanford Junior University, The
Current Performing Organization:	Leland Stanford Junior University, The
Web Approval Date:	03-08-2023

Decades of research have advanced our understanding of breast cancer and improved treatment options, but we are still far from living in a world where every breast cancer is curable. Cancers present genomic alterations that, ultimately, transform the cell and lead to the disease. Therefore, understanding the consequences of such alterations is of great help in the fight against cancer.

Epigenetic factors present high genomic alterations in all types of cancers. These factors have roles in the cell nucleus, where our DNA is stored. Among them, the SWI/SNF complexes are altered in 40% of breast cancers. While recent studies start to shed light on the therapeutic potential behind these complexes' functions in cancers, their normal and cancer-associated mechanisms are still not well understood. Advancing this scientific knowledge will help save lives and advance toward a world without breast cancers.

We uncovered that cancer cell lines harboring genomic alterations in SWI/SNF are more sensitive to specific drugs when compared to other cancer cell lines. All these drugs, directly or not, target protein synthesis. Protein synthesis takes place in the cell cytoplasm. This cellular mechanism allows rapid adaptation to various stimuli and is highly altered in cancers. For example, cancer cells adapt to the need of sustained proliferation by increasing global protein synthesis and/or synthetizing specific proteins essential to the development of the disease and to combat therapy. Furthermore, protein synthesis is key to a cell's response to stress. Within the tumor microenvironment, cancer cells are exposed to various stresses, such as a lack of oxygen and nutrients. These stresses, and, consequently, cancer cell's responses to these stresses, were shown to drive the disease by leading to tumorigenesis, metastasis, and resistance to therapy.

How could SWI/SNF complexes, epigenetic factors with known roles in the cell nucleus, be linked to protein synthesis that takes place in the cell cytoplasm? Our preliminary data demonstrate that these complexes are also in the cell cytoplasm and have roles in protein synthesis. While the current literature focuses on these complexes' roles in the nucleus, we pioneer the study of their roles in the cytoplasm.

We propose that SWI/SNF complexes have direct breast cancer-relevant roles in protein synthesis within the cytoplasm, that differ from their established roles in the nucleus, thus revealing new biomarkers for therapies impacting protein synthesis and greatly expanding the frontier of personalized therapy.

This proposal addresses two Fiscal Year 2022 Breast Cancer Research Program overarching challenges, "Identify what drives breast cancer growth; determine how to stop it" and "Identify why some breast cancers become metastatic," by focusing on two questions: What are the consequences of SWI/SNF's breast cancer alteration on protein synthesis under breast cancer-relevant stresses? Could these roles lead to the establishment of SWI/SNF as biomarkers in specific subtypes of breast cancers or during disease progression such as metastasis?

Under the first aim, we will modify normal breast epithelial cells to mimic the two main SWI/SNF alterations we observed in 95% of our breast tumor samples to characterize the consequences of these

alterations on protein synthesis under lack of oxygen or nutrients. Under the second aim, we will examine 500 tumors from 200 breast cancer patients to determine the correlation between protein synthesis, stresses, and expression and localization (nucleus or cytoplasm) of SWI/SNF.

Our preliminary data highlights the fact that SWI/SNF are altered in all breast cancer subtypes, including triple-negative, and across all disease stages (primary tumor, local recurrence, metastasis). Therefore, our proposal has great potential to impact many breast cancer patients.

Therapies targeting protein synthesis are already used in the clinic and could gain more effective stratification tools. Therefore, identifying new biomarkers for therapies impacting protein synthesis could achieve a patient-related outcome in a reasonable timeline.

The impact of this project is that the characterization of specific roles for SWI/SNF in the cell cytoplasm, even if not their principal roles, has great potential to unveil an Achilles' heel and be essential to the identification of new biomarkers and therapeutic strategies for the many breast cancers presenting alterations in these complexes. Ultimately, our approach could lead to personalized targeted therapy based on each patient's specific breast cancer alteration, which is the hope in today's fight against cancer.

Proposal Title:	Revealing the Potential for mSWI/SNF as Biomarkers in Breast Cancers
Log Number:	BC220157P1
Current PI Name:	Gregory Bean
Award Number:	HT9425-23-1-0129
Current Contracting Organization:	Leland Stanford Junior University, The
Current Performing Organization:	Leland Stanford Junior University, The
Web Approval Date:	03-08-2023

Decades of research have advanced our understanding of breast cancer and improved treatment options, but we are still far from living in a world where every breast cancer is curable. Cancers present genomic alterations that, ultimately, transform the cell and lead to the disease. Therefore, understanding the consequences of such alterations is of great help in the fight against cancer.

Epigenetic factors present high genomic alterations in all types of cancers. These factors have roles in the cell nucleus, where our DNA is stored. Among them, the SWI/SNF complexes are altered in 40% of breast cancers. While recent studies start to shed light on the therapeutic potential behind these complexes' functions in cancers, their normal and cancer-associated mechanisms are still not well understood. Advancing this scientific knowledge will help save lives and advance toward a world without breast cancers.

We uncovered that cancer cell lines harboring genomic alterations in SWI/SNF are more sensitive to specific drugs when compared to other cancer cell lines. All these drugs, directly or not, target protein synthesis. Protein synthesis takes place in the cell cytoplasm. This cellular mechanism allows rapid adaptation to various stimuli and is highly altered in cancers. For example, cancer cells adapt to the need of sustained proliferation by increasing global protein synthesis and/or synthetizing specific proteins essential to the development of the disease and to combat therapy. Furthermore, protein synthesis is key to a cell's response to stress. Within the tumor microenvironment, cancer cells are exposed to various stresses, such as a lack of oxygen and nutrients. These stresses, and, consequently, cancer cell's responses to these stresses, were shown to drive the disease by leading to tumorigenesis, metastasis, and resistance to therapy.

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We propose that SWI/SNF complexes have direct breast cancer-relevant roles in protein synthesis within the cytoplasm, that differ from their established roles in the nucleus, thus revealing new biomarkers for therapies impacting protein synthesis and greatly expanding the frontier of personalized therapy.

This proposal addresses two Fiscal Year 2022 Breast Cancer Research Program overarching challenges, "Identify what drives breast cancer growth; determine how to stop it" and "Identify why some breast cancers become metastatic," by focusing on two questions: What are the consequences of SWI/SNF's breast cancer alteration on protein synthesis under breast cancer-relevant stresses? Could these roles lead to the establishment of SWI/SNF as biomarkers in specific subtypes of breast cancers or during disease progression such as metastasis?

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alterations on protein synthesis under lack of oxygen or nutrients. Under the second aim, we will examine 500 tumors from 200 breast cancer patients to determine the correlation between protein synthesis, stresses, and expression and localization (nucleus or cytoplasm) of SWI/SNF.

Our preliminary data highlights the fact that SWI/SNF are altered in all breast cancer subtypes, including triple-negative, and across all disease stages (primary tumor, local recurrence, metastasis). Therefore, our proposal has great potential to impact many breast cancer patients.

Therapies targeting protein synthesis are already used in the clinic and could gain more effective stratification tools. Therefore, identifying new biomarkers for therapies impacting protein synthesis could achieve a patient-related outcome in a reasonable timeline.

The impact of this project is that the characterization of specific roles for SWI/SNF in the cell cytoplasm, even if not their principal roles, has great potential to unveil an Achilles' heel and be essential to the identification of new biomarkers and therapeutic strategies for the many breast cancers presenting alterations in these complexes. Ultimately, our approach could lead to personalized targeted therapy based on each patient's specific breast cancer alteration, which is the hope in today's fight against cancer.

Proposal Title:	Harnessing Host Innate Immunity with Innovative Therapeutic Strategies in Triple-Negative Breast Cancer
Log Number:	BC220241
Current PI Name:	Qiwei Wang
Award Number:	HT9425-23-1-0026
Current Contracting Organization:	Dana-Farber Cancer Institute
Current Performing Organization:	Dana-Farber Cancer Institute
Web Approval Date:	12-15-2022

Patients with triple-negative breast cancer (TNBC) are facing outsized challenges to recovery, largely due to lack of effective therapies and high risk of recurrence and metastasis. In recent years, there has been substantial interest and efforts in developing immunotherapy for cancers including TNBC. However, while immunotherapy is particularly effective in melanoma and lung cancer, it is relatively less effective in patients with TNBC. Therefore, it is very meaningful to explore a new target for immunotherapy to combat TNBC. In this project, we will focus on exploiting a type of immune cells called macrophages for treatment of TNBC. This is not only because those macrophages are highly enriched in TNBC tumors, but also because they strongly promote tumor growth. Here, we propose to re-educate these enemies (pro-tumor macrophages) to become our soldiers (anti-tumor macrophages) to combat drug resistance and prevent TNBC lung metastasis. Thus, this project is designed to address two overarching challenges: (1) revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival and (2) eliminate the mortality associated with metastatic breast cancer. Modulating macrophages for cancer treatment has proven extremely difficult. We found that activation of a protein called STING reduces pro-tumor activity of macrophages. However, STING agonism alone appears to be ineffective in eliciting sufficient anti-tumor macrophages to combat cancers. More recently, in a pilot study, we noticed that deletion of a gene called STAT3 in macrophages strongly increases anti-tumor activity of macrophages when combining with STING agonism. Therefore, we think optimal activation of anti-tumor macrophages requires both STING activation and STAT3 inhibition. In this project, we will combine a next-generation STING activator with a novel STAT3 inhibitor to harness anti-tumor potential of macrophages for treating drug-resistant models of TNBC. We will also evaluate whether this combination can harness tissue- resident macrophages in the lungs to protect lungs from breast cancer metastasis. Through this project, we will provide novel and crucial proof-of-concept strategies for exploiting macrophages to combat drug resistance and prevent lung metastasis. Our lab has a proven record of translating basic scientific discoveries into clinical trials. The preclinical information derived from this project will guide the rational design of breakthrough clinical trials and thus accelerate the progress in mitigating the burdens of advanced TNBC for our patients.

Proposal Title:	The Role of MELK in Stromal Reorganization of Aggressive Breast Cancers
Log Number:	BC220287
Current PI Name:	Chandra Bartholomeusz
Award Number:	HT9425-23-1-0087
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	02-06-2023

Triple-negative breast cancer (TNBC) and inflammatory breast cancer (IBC) are aggressive breast cancer subtypes with poor clinical outcomes due to the lack of well-validated and actionable targets and the onset of chemoresistant metastasis. The 5-year overall survival rate for TNBC is only 60%-70% and IBC is only 62% -68% with multimodality treatment, largely due to a lack of U.S. Food and Drug Administration (FDA)-approved targeted therapies. Poor TNBC and IBC outcomes are related to the propensity of these two tumor types to metastasize.

Our previous studies show that a that a protein called MELK (maternal embryonic leucine zipper kinase) is present in higher amounts in TNBC and IBC than in other breast cancer types. Further, the amount of time patients with breast cancer survived was correlated to the amount of MELK in their tumor cells: the lower the amount of MELK, the longer the survival time and vice versa. These findings suggest that drugs that reduce the level of MELK in tumors will allow patients with TNBC and IBC to live longer. Therefore, we identified MELK inhibitors that reduce the amount of MELK in TNBC and IBC cells and reduce metastasis. The proposed project builds up on the findings of our preliminary data and will focus on (i) whether combining a MELK inhibitor with the standard of care (paclitaxel) in TNBC and IBC preclinical animal models will prevent the spread of TNBC and IBC to distant sites, (ii) the clinical significance of MELK as a biomarker and in patient outcomes, and (iii) understanding how MELK promotes metastasis by modulating the tumor cell microenvironment.

The following three specific aims will be pursued:

Aim 1: Evaluate MELK as a novel therapeutic target in models of metastatic TNBC and TN-IBC.

Aim 2: Determine the prognostic and predictive significance of MELK in TNBC and IBC.

Aim 3: Determine if MELK modulation of fibronectin affects tumor cell microenvironment in aggressive breast cancers.

Because our inhibitor specifically targets MELK, we expect that MELK-targeted therapy would have low toxicity with fewer side effects than are seen with conventional therapies. We will use cell culture-based approaches and mouse models to identify how MELK modifies the microenvironment and promotes metastasis. We will also test the MELK inhibitor in patient-derived xenograft mouse models that harbor tumors from patients with breast cancer and humanized mice to ensure the clinical relevance of the proposed study.

Impact: These contributions will have a significant impact because they will address the need for targeted therapies to reduce mortality due to metastasis in patients with TNBC and IBC. Our study is highly translational as we expect that this research will lead to a novel approach and will help significantly reduce TNBC and IBC-related deaths by adding a new treatment option and establishing biomarkers by validating

protein substrates and efficacy data of inhibitors. Our work is expected to lead to an effective clinical trial with a MELK inhibitor in combination with standard of care in the next 3-5 years.

This unique grant is in line with Fiscal Year 2022 Overarching Challenges: (1) Identify why some breast cancers become metastatic. (2) Eliminate the mortality associated with metastatic breast cancer with state-of-the-art drug development to address aggressive cancers. We are grateful for this opportunity to work toward conquering this highly fatal, aggressive disease through our research.

Proposal Title:	Changing the Paradigm of Breast Cancer Treatment: MBQ-167 as a First-in-Class Metastatic Breast Cancer Therapeutic
Log Number:	BC220292
Current PI Name:	Jose Rodriguez
Award Number:	HT9425-23-1-0381
Current Contracting Organization:	MBQ Pharma, Inc.
Current Performing Organization:	MBQ Pharma, Inc.
Web Approval Date:	05-16-2023

The critical challenges of breast cancer treatment are: (i) management of advanced primary disease with or without regional and/or distant metastases and (ii) the prevention of metastasis, which typically manifests itself in a more aggressive manner where none of the currently available standard treatments can cure the disease.

Moreover, while the 5-year survival of early-stage disease has reached nearly 99%, this achievement, unfortunately, comes at the price of frequent overdiagnosis and overtreatment, leading to significant morbidity and a markedly reduced quality of life, if not premature death. Lastly, although given to most breast cancer patients, current adjuvant therapies have been shown to deliver only a marginal impact on survival. The recurrent disease often demonstrates acquired resistance to previously effective breast cancer chemotherapy agents. Notably, at this time, there is no treatment to eradicate or prevent the formation of metastasis, the latter being linked strongly to the recurrence of breast cancer.

MBQ Pharma has set out to address these unmet medical needs in breast cancer treatment by targeting related signaling molecules that play key roles in regulating tumor growth, metastasis formation, and conferring resistance to standard tumor therapies. Rac1 and Cdc42, when overactive, appear to be causal in these pathogenetic processes, not currently addressed by existing therapies. As a first success, the MBQ Pharma lead compound, MBQ-167, is an unparalleled high-affinity dual-action inhibitor of both Rac1 and Cdc42 and has been shown to markedly reduce tumor growth and eradicate and prevent metastasis formation in breast cancer animal models. In addition, MBQ-167 markedly inhibited the growth of a panel of solid tumor cell lines. MBQ-167 also reduced stem cell-like mammosphere growth of triple-negative breast cancer cells, a micrometastases model that can lay dormant for many years with dangerous potential for recurrence. Therefore, this study will address the overarching challenges to revolutionize treatment regimens by replacing them with more effective, less toxic alternatives, improve quality of life, and reduce mortality.

Subsequently, MBQ Pharma developed a pharmaceutical MBQ-167 orally administered formulation. Notably, the preclinical program revealed an excellent safety profile, good bioavailability, and pharmacokinetics. Based on the encouraging preclinical data, which points towards a paradigm-changing potential of MBQ-167 and its excellent safety profile, the U.S. Food and Drug Administration (FDA) approved Investigational New Drug (IND) for a phase 1 open-label, first-in-human trial of oral MBQ-167 as single agent in subjects with advanced breast cancer. The primary objective of the phase 1 study is to determine the maximally tolerated dose of MBQ-167 as a single agent administered orally, twice daily, continuously for 21 days in subjects with advanced breast cancer. Approximately 72 participants will be screened to achieve enrollment in the phase 1 for an estimated total of 24-48 evaluable participants. As secondary parameters, pharmacokinetics and pharmacodynamics profiles will be assessed. Following completion of the phase 1 trial, the further development program will aim to assess the efficacy of MBQ-167 in treating breast cancer metastasis both in early and advanced stages of the disease. Ultimately, the role of

MBQ-167 in preventing disease recurrence will be assessed. Following successful completion of the phase 1 trial, where we will determine the maximum tolerated dose, we plan to conduct phase 2 trials for MBQ-167 and expect to achieve a patient-related outcome in the next 5 years.

If successful, MBQ-167 and other potential pipeline candidates have excellent prospects to effectively treat advanced metastatic breast cancer, solve the issue of recurrent disease arising from dormant cancer cells, and overcome tumor-acquired resistance to current standard treatment. Based on the targeted pathogenetic process, MBQ Pharma is firmly convinced that MBQ-167 and the pipeline compounds have an excellent opportunity to have an unparalleled positive impact on the overall survival and quality of life of breast cancer patients and impact the Breast Cancer Research Program’s mission of ending breast cancer.

Proposal Title:	FADD: A Mechanistic Basis and Therapeutic Opportunity for Overcoming CDK4/6 Inhibitor Resistance
Log Number:	BC220344
Current PI Name:	Alnawaz Rehemtulla
Award Number:	HT9425-23-1-0210
Current Contracting Organization:	Michigan, University of
Current Performing Organization:	Michigan, University of
Web Approval Date:	03-22-2023

Endocrine therapy has been used for the treatment of estrogen receptor-positive (ER+) breast cancer for many years. However, the development of resistance to this therapy led to the inclusion of inhibitors of the CDK4/6 kinases, including palbociclib, ribociclib, and abemaciclib, in combination with endocrine therapy for women with ER+ breast cancer. This combination therapy has demonstrated significantly improved survival compared to endocrine therapy alone in ER+ advanced breast cancer, resulting in the approval of CDK4/6i with endocrine therapy in the first-line setting. However, some patients have tumors that do not respond to CDK4/6i, and a significant number of patients with tumors that initially respond to these drugs will succumb to disease due to the development of resistance, underscoring the need to new approaches to block the growth of tumors cells. Our team has identified a novel signaling pathway that bypasses CDK4/6 inhibitors. Our preliminary findings show that FADD protein, when phosphorylated, can overcome CDK4/6 inhibitor effectiveness and promote the growth of cancer cells. We believe that Casein Kinase 1a (CK1a) plays a critical role in this resistance and the CDK 4/6 inhibitor resistance can be overcome with CK1a inhibition. Importantly, drugs that inhibit the protein that phosphorylates FADD, CK1a, have been approved for the treatment of patients with myelodysplastic syndrome (MDS), and additional improved drugs targeting CK1a are being tested in patients. The studies proposed in this grant application will investigate the safety, efficacy, and mechanism by which CK1a inhibitors can prevent the development of resistance to endocrine therapy and CDK4/6 inhibitors. If successful, these studies will provide improved survival for breast cancer patients in the Armed Forces and the general public.

Proposal Title:	FADD: A Mechanistic Basis and Therapeutic Opportunity for Overcoming CDK4/6 Inhibitor Resistance
Log Number:	BC220344P1
Current PI Name:	Corey Speers
Award Number:	HT9425-23-1-0211
Current Contracting Organization:	Case Western Reserve University
Current Performing Organization:	Case Western Reserve University
Web Approval Date:	03-22-2023

Endocrine therapy has been used for the treatment of estrogen receptor-positive (ER+) breast cancer for many years. However, the development of resistance to this therapy led to the inclusion of inhibitors of the CDK4/6 kinases, including palbociclib, ribociclib, and abemaciclib, in combination with endocrine therapy for women with ER+ breast cancer. This combination therapy has demonstrated significantly improved survival compared to endocrine therapy alone in ER+ advanced breast cancer, resulting in the approval of CDK4/6i with endocrine therapy in the first-line setting. However, some patients have tumors that do not respond to CDK4/6i, and a significant number of patients with tumors that initially respond to these drugs will succumb to disease due to the development of resistance, underscoring the need to new approaches to block the growth of tumors cells. Our team has identified a novel signaling pathway that bypasses CDK4/6 inhibitors. Our preliminary findings show that FADD protein, when phosphorylated, can overcome CDK4/6 inhibitor effectiveness and promote the growth of cancer cells. We believe that Casein Kinase 1a (CK1a) plays a critical role in this resistance and the CDK 4/6 inhibitor resistance can be overcome with CK1a inhibition. Importantly, drugs that inhibit the protein that phosphorylates FADD, CK1a, have been approved for the treatment of patients with myelodysplastic syndrome (MDS), and additional improved drugs targeting CK1a are being tested in patients. The studies proposed in this grant application will investigate the safety, efficacy, and mechanism by which CK1a inhibitors can prevent the development of resistance to endocrine therapy and CDK4/6 inhibitors. If successful, these studies will provide improved survival for breast cancer patients in the Armed Forces and the general public.

Proposal Title:	Exploiting Epigenetic Vulnerabilities in Breast Cancers with Chromosomal Instability
Log Number:	BC220470
Current PI Name:	Samuel Bakhoum
Award Number:	HT9425-23-1-0199
Current Contracting Organization:	Sloan Kettering Institute for Cancer Research
Current Performing Organization:	Sloan Kettering Institute for Cancer Research
Web Approval Date:	03-23-2023

The heterogeneity that exists among cells in any given tumor constitutes one of the most conspicuous features of aggressive breast cancers. This diversity allows cancer cells to constantly change and adapt to the environment. This capability is central to two of the deadliest properties of breast cancer: drug resistance and distant metastasis.

This heterogeneity derives from genomic instability, where cells can rapidly change their genome or DNA content each time they divide. As such, they constantly acquire new properties that enable them to resist therapies and spread outside of the breast to distant organs. One major form of genomic instability is called chromosomal instability, where cells make mistakes each time they divide their chromosomes, and this leads to unequal distribution of chromosomes. Chromosomal instability exists in over 80% of human tumors, and it is a distinctive hallmark of triple-negative breast cancer as well as a considerable subset of estrogen receptor-positive and Her2-positive breast cancers. Breast tumors with chromosomal instability have a much higher chance to spread to distant organs and relapse after therapy.

It has been assumed that the way chromosomal instability generates cell-to-cell variability is through shuffling of the genetic material. When cells in the same tumor have a diverse number of copies of the same genetic material, they can make different amounts of RNA and protein, thus leading to heterogeneity. We made the interesting observation that in addition to changing the genetic content, chromosomal instability can also lead to alteration in the epigenetic context in breast cancer cells. The same genetic material can be read or interpreted differently by each cell. Changes in this epigenetic state promote yet an additional layer of heterogeneity that facilitates breast cancer cell adaptation.

In this proposal, we hope to better understand how epigenetic abnormalities arising from chromosomal instability impact the level of cancer promoting genes in breast cancer. We also aim to test whether these changes can also lead to additional structural changes in the chromosome. Finally, we will test whether preventing some of these epigenetic changes can be used to suppress breast cancer metastasis.

Our goal is to deepen our understanding of the key processes by which chromosomal instability shapes breast cancer cell behavior and use this knowledge to intervene and prevent breast cancer progression.

Proposal Title:	Targeting Creatine Kinase Brain Isoform (CKB) to Inhibit Brain and Bone Metastasis
Log Number:	BC220493
Current PI Name:	Tiffany Seagroves
Award Number:	HT9425-23-1-0091
Current Contracting Organization:	Tennessee, University of, Health Science Center
Current Performing Organization:	Tennessee, University of, Health Science Center
Web Approval Date:	02-06-2023

Overarching Challenges: This Breakthrough Funding Level 2 proposal will: (1) identify why some breast cancers become metastatic, (2) eliminate mortality associated with metastatic breast cancer (MBC), and (3) revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival.

Rationale: The overall survival (OS) rate of patients diagnosed with MBC remains dismal. Breast cancer deaths are due to spread ("metastasis"), then growth, of tumor cells that left the breast to seed and grow in distant organs. Therefore, to improve survival rates, cancer researchers must refocus our efforts on finding safe, effective and specific "targeted" drug therapies to block the pathways in the tumor that drive metastasis and to stop metastatic tumor cells from surviving and/or growing. Researchers must also strive to test promising new therapies in the most appropriate animal ("preclinical") models available, using only those breast tumor models in the laboratory setting that consistently spread to, then grow in, the same organs in mice as in patients. Rigorously proving anticancer efficacy in animals that develop relevant sites of metastasis is needed before initiating new clinical trials and enrolling breast cancer patients, in order to improve likelihood of drug success and ultimately, U.S. Food and Drug Administration (FDA) approval.

Types of Patients, or At-Risk Individuals, Research Will Help:

• Patients with triple-negative breast cancer (TNBC), who have limited treatment options except general cell killing ("cytotoxic") chemotherapies, such as Taxol or Adriamycin (doxorubicin). Patients developing resistance to these drugs is common; different strategies are needed to stop cancer growth again.

• Patients with metastasis to brain and to bone since anti-creatine kinase pathway targeting drugs, like cyclocreatine (older generation agent), and like RGX-202-01 (a new agent, made by Inspirna), cross the blood-brain barrier and cyclocreatine can prevent bone loss in rodents in the non-cancer setting. The bone data suggest that creatine kinase inhibitors could attack bone-destroying breast cancer cells, helping patients with "osteolytic breast cancer."

• All patients with breast tumors that express the biomarker (creatine kinase [CK], brain isoform, or CK brain isoform [CKB]).

• Cancer patients diagnosed with other cancer types that also express CKB (glioma, colon cancer, pancreatic cancer, ovarian cancer, prostate cancer).

Potential Clinical Applications, Benefits and Risks, and Potential Breakthroughs Relevant to Mission of Ending Breast Cancer:

• Efficacy of anti-creatine kinase agents (like cCr and RGX-202-01) appears to be most potent for blocking metastasis, which is the stage of cancer that kills breast cancer patients, although these drugs also prevent primary tumor cell growth.

• RGX-202-01 is made for pill form, and is already in early clinical trials, to be taken by mouth twice per day.

• RGX-202-01 is reported to be well-tolerated in early phase clinical trials, and since RGX-202-01 is made into pill form, this dosing route avoids the need for intravenous (IV) infusion in a clinic. These features lower the risks of treatment-induced adverse reactions for patients.

• Combination of an anti-CK agent, like RGX-202-01, with conventional chemotherapies, like Taxol or doxorubicin, seems to be more effective in breast cancer cells than either drug alone, potentially allowing the dose of Taxol or doxorubicin to be lowered. These two drugs have known adverse side effects and also require IV infusion (Taxol: dose-dependent risk of peripheral neuropathy, or nerve numbness/burning/pain; and doxorubicin: dose-limiting cardiotoxicity, or the risk of future heart failure).

• Understanding how CKB and anti-creatine kinase pathway drugs work to inhibit metastasis (i.e., which genes/proteins predict if a CKB+ tumor will respond well to therapy) may be useful to identify those patients most likely to respond to treatment, in a precision medicine approach, increasing the benefits to patients.

• Targeting the creatine kinase pathway to block metastasis is expected to significantly improve progression-free and overall survival, particularly for TNBC and HER2+ patients with brain lesions.

Projected Time to Achieve Patient-Related Outcomes: Generating strong preliminary data showing effectiveness of RGX-202-01 in mouse models of stage IV breast cancer, and understanding how inhibiting CKB works at the molecular level, is essential to justify beginning new clinical trials enrolling breast cancer patients. RGX-202-01 is now enrolling subjects in phase 2 studies to test efficacy in colon and pancreatic cancers; therefore, trials enrolling patients with metastatic breast disease could begin within 5 years of initiating this proposal (by 2027). We will share our results as they are generated with our local oncologist consultant (Dr. Chokshi, University of Tennessee Health Science Center) and with Inspirna to ensure this could happen as soon as possible.

Proposal Title:	Driving Systemic Antitumor Immune Responses Against Breast Cancer Through Precision Medicine
Log Number:	BC220499
Current PI Name:	Matthew Spitzer
Award Number:	HT9425-23-1-0161
Current Contracting Organization:	California, University of, San Francisco
Current Performing Organization:	California, University of, San Francisco
Web Approval Date:	02-28-2023

Background and Vision: Of the treatment options for patients with breast cancer, immunotherapies have the unique ability to provide long-term protection from tumor recurrence by acting as a living drug. When immune cells called T cells are properly activated, they can selectively recognize cancer cells and kill them. In addition, a subset of immune cells called memory cells can persist for a patient's lifetime and prevent cancer cells from growing back again. Therefore, it would be ideal for all patients to benefit from these unique benefits of immunotherapies. However, only a small fraction of patients with breast cancer respond to currently available immunotherapies, which are called immune checkpoint inhibitors (ICIs). ICIs block inhibitory signals that shut off immune cells. They are currently approved by the U.S. Food and Drug Administration for a subset of patients with breast cancer. Patients who have already mounted a substantial immune response to their tumor are more likely to respond to ICIs. In contrast, patients with "cold" tumors, which do not have many T cells in the tumor prior to treatment, are unlikely to benefit.

In contrast to the status quo, I envision a future where each and every patient with breast cancer will receive an immunotherapy that is effective for them. An optimal immunotherapy should drive an anti-tumor immune response capable of clearing a patient's tumor with minimal toxicity and should also create immune memory that protects from cancer recurrence. Transforming this vision into reality requires two fundamental advances: (1) We must be able to predict which patients will benefit from ICIs and who will require a different type of immunotherapy. We have developed methods to measure the types of immune cells circulating in a patient's blood and to measure how those cells are activated. We will apply these methods to predict response to ICIs in patients with breast cancer. (2) We must develop immunotherapy strategies that are more effective at driving new anti-tumor immune responses, particularly for women with cold tumors that do not respond to ICIs.

Research Goals and Approach: My proposed studies address the Breast Cancer Research Program challenge to "revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival."

In our first aim, we will measure the types and activation of immune cells in the peripheral blood of patients with breast cancer before and after treatment with immunotherapy. We hypothesize that a detailed analysis of immune cells in peripheral blood can guide immunotherapy treatment selection for patients with breast cancer to identify patients who will benefit from ICIs and those who will not. We will test this hypothesis using research methods we have developed for immune monitoring technology in collaboration with the I-SPY 2 clinical trial. Activated immune cells circulating in the blood often come from lymph nodes, where they receive the signals that promote their activation. Sentinel lymph nodes are often removed from patients with breast cancer to determine if cancer cells have spread into these lymph nodes, but the immune response in these lymph nodes is not typically measured to guide treatment decisions. We will use innovative imaging technologies to measure immune responses in these lymph nodes to determine which patients are currently benefiting from immunotherapy.

Aim 2: Develop new immunotherapies that drive strong systemic anti-tumor immune responses. The main cells responsible for activating T cells are called dendritic cells. Our prior studies revealed that dendritic cells are not activated properly in mice with breast cancer, resulting in poor activation of T cell responses. Here, we will understand the underlying mechanism(s) and target them to develop a new generation of cancer immunotherapy that can drive new immune responses against breast cancers.

Impact: We will tackle two fundamental challenges that currently limit the clinical benefit that patients with breast cancer experience from immunotherapy: (1) advancing precision medicine for immunotherapy by monitoring immune responses in the blood and lymph nodes, and (2) improving immunotherapy strategies to drive strong, new immune responses against cold tumors.

Proposal Title:	Germline Variants and the Population-Wide Risk of Breast Cancer
Log Number:	BC220523
Current PI Name:	Olivier Lichtarge
Award Number:	HT9425-23-1-0078
Current Contracting Organization:	Baylor College of Medicine
Current Performing Organization:	Baylor College of Medicine
Web Approval Date:	02-06-2023

Background: Breast cancer often appears multiple times in families, meaning that a first- or second-degree relative has the disease. This tendency reflects a familial predisposition that is linked to genetic causes. That is, there are defects in the DNA that makes up our genetic code – genes – and this causes some women to have a much greater risk than others to develop breast cancer. The exact nature of these defects is known for some genes such as BRCA1, BRCA2, TP53, and PTEN, but they account for only 25% of the familiar risk. So, by and large the majority of breast cancer-related DNA gene defects remain unknown.

Overarching Challenge: To improve prevention and early detection of breast cancer, genetic tests have been developed to detect the DNA defects that are already known. But because the known gene defects are relatively few, meaning that they cover only a small fraction of all those that are relevant, the tests have not been very effective so far. Therefore, the overarching challenges our proposal address are (1) identify determinants of breast cancer initiation, risk, or susceptibility and (2) prevent breast cancer. Specially, we aim to identify many more novel DNA defects that are at the root of breast cancer and get passed on from one generation to the next. The discovery of these risk factors would improve our ability to predict which women are at increased risk for breast cancer before they develop it and hence enable us to screen much more effectively.

Objectives and Hypothesis: The crux of the matter is how to detect more effectively than is currently possible the DNA defects that predispose to breast cancer. This is very difficult because all individuals carry a large number of DNA defects, and only a small fraction of these affect breast cancer risk. So, finding those by conventional methods is tantamount to looking for a needle in a haystack. What is innovative and impactful about our approach is that we will attempt to discern the causative DNA defects by developing and applying a new way to measure the strength of a DNA defect in causing cancer. The idea is that most DNA defects in healthy women will be negligible in raising cancer risk and can safely be discarded as irrelevant. But in women who later developed breast cancer, there will be a clearly recognizable set of DNA defects that are impactful, and that tend to occur again and again across patients. Those impactful DNA defects should, we believe, be directly relevant to disease risk.

Specific Aims: This study is in two main parts. In the first part, our computational team led by the Initiating Principal Investigator (PI) Dr. Olivier Lichtarge will measure properly the strength of a DNA defect. For this, we have unique tools that go back and look at evolutionary history to infer which DNA changes were “mild” and which were “impactful.” Once this is measured, we will develop and merge a series of complementary computer programs (algorithms), including machine learning methods, to identify segments of DNA that carry impactful gene defects in patients with breast cancer but not in healthy women, and these defects will then be used as a fingerprint to recognize other women who are at risk of the disease. In a second part of the study, our laboratory team led by the Partnering PI Dr. Yi Li and the co-investigator Dr. Larry Donehower will first use human breast cells to rigorously test the leading DNA gene defects we identified that are novel. After this round of laboratory testing in cultured cells, we will further examine the five leading candidate mutants in our mouse models of human breast cancer that we have specifically designed and developed for this task of validation.

Impact: (1) Our results will identify new DNA defects that predispose to the genetic risk of developing breast cancer. (2) In turn, this will lead to new methods to predict risk and thereby develop genetic tests to screen the general population in order to identify a subset of individuals at increased risk of breast cancer who should undergo more intensive follow-up for prevention and early detection. (3) The DNA defects we identify will suggest new mechanisms for further molecular research and which hold the potential to develop new drugs. (4) This study is in breast cancer, but the tools it will develop are entirely general. So, the same tools could be applied broadly to other cancers and, in fact, to improve risk prediction for many other diseases that affect adults across their lifespan.

Proposal Title:	Germline Variants and the Populationwide Risk of Breast Cancer
Log Number:	BC220523P1
Current PI Name:	Yi Li
Award Number:	HT9425-23-1-0079
Current Contracting Organization:	Baylor College of Medicine
Current Performing Organization:	Baylor College of Medicine
Web Approval Date:	02-06-2023

Background: Breast cancer often appears multiple times in families, meaning that a first- or second-degree relative has the disease. This tendency reflects a familial predisposition that is linked to genetic causes. That is, there are defects in the DNA that makes up our genetic code – genes – and this causes some women to have a much greater risk than others to develop breast cancer. The exact nature of these defects is known for some genes such as BRCA1, BRCA2, TP53, and PTEN, but they account for only 25% of the familiar risk. So, by and large the majority of breast cancer-related DNA gene defects remain unknown.

Overarching Challenge: To improve prevention and early detection of breast cancer, genetic tests have been developed to detect the DNA defects that are already known. But because the known gene defects are relatively few, meaning that they cover only a small fraction of all those that are relevant, the tests have not been very effective so far. Therefore, the overarching challenges our proposal address are (1) identify determinants of breast cancer initiation, risk, or susceptibility and (2) prevent breast cancer. Specially, we aim to identify many more novel DNA defects that are at the root of breast cancer and get passed on from one generation to the next. The discovery of these risk factors would improve our ability to predict which women are at increased risk for breast cancer before they develop it and hence enable us to screen much more effectively.

Objectives and Hypothesis: The crux of the matter is how to detect more effectively than is currently possible the DNA defects that predispose to breast cancer. This is very difficult because all individuals carry a large number of DNA defects, and only a small fraction of these affect breast cancer risk. So, finding those by conventional methods is tantamount to looking for a needle in a haystack. What is innovative and impactful about our approach is that we will attempt to discern the causative DNA defects by developing and applying a new way to measure the strength of a DNA defect in causing cancer. The idea is that most DNA defects in healthy women will be negligible in raising cancer risk and can safely be discarded as irrelevant. But in women who later developed breast cancer, there will be a clearly recognizable set of DNA defects that are impactful, and that tend to occur again and again across patients. Those impactful DNA defects should, we believe, be directly relevant to disease risk.

Specific Aims: This study is in two main parts. In the first part, our computational team led by the Initiating Principal Investigator (PI) Dr. Olivier Lichtarge will measure properly the strength of a DNA defect. For this, we have unique tools that go back and look at evolutionary history to infer which DNA changes were “mild” and which were “impactful.” Once this is measured, we will develop and merge a series of complementary computer programs (algorithms), including machine learning methods, to identify segments of DNA that carry impactful gene defects in patients with breast cancer but not in healthy women, and these defects will then be used as a fingerprint to recognize other women who are at risk of the disease. In a second part of the study, our laboratory team led by the Partnering PI Dr. Yi Li and the co-investigator Dr. Larry Donehower will first use human breast cells to rigorously test the leading DNA gene defects we identified that are novel. After this round of laboratory testing in cultured cells, we will further examine the five leading candidate mutants in our mouse models of human breast cancer that we have specifically designed and developed for this task of validation.

Impact: (1) Our results will identify new DNA defects that predispose to the genetic risk of developing breast cancer. (2) In turn, this will lead to new methods to predict risk and thereby develop genetic tests to screen the general population in order to identify a subset of individuals at increased risk of breast cancer who should undergo more intensive follow-up for prevention and early detection. (3) The DNA defects we identify will suggest new mechanisms for further molecular research and which hold the potential to develop new drugs. (4) This study is in breast cancer, but the tools it will develop are entirely general. So, the same tools could be applied broadly to other cancers and, in fact, to improve risk prediction for many other diseases that affect adults across their lifespan.

Proposal Title:	Translational Development of MBQ-167 as Targeted Therapy for Metastatic Breast Cancer
Log Number:	BC220526
Current PI Name:	Suranganie Dharmawardhane
Award Number:	HT9425-23-1-0166
Current Contracting Organization:	Puerto Rico, University of, Medical Sciences Campus
Current Performing Organization:	Puerto Rico, University of, Medical Sciences Campus
Web Approval Date:	03-23-2023

This proposal will further the development of a targeted therapeutic for metastatic breast cancer and address the following Fiscal Year 2022 (FY22) Breast Cancer Research Program (BCRP) overarching challenges: (i) revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival, and (ii) eliminate mortality associated with metastatic breast cancer.

Rationale: Breast cancer mortality is typically from metastatic disease. We are limited by targeted therapeutic options for metastasis, especially in triple-negative breast cancer (TNBC). Due to the inherent heterogeneity and lack of targetable receptors, a uniform therapeutic approach for TNBC has been challenging. Our solution is to target the related metastasis drivers Rac and Cdc42 proteins that drive metastatic TNBC and HER2 breast cancer, which until the development of our drug, have not been successfully targeted.

The Principal Investigator (PI), a breast cancer biologist, in collaboration with medicinal chemists, has developed and characterized the drug MBQ-167 to target Rac and Cdc42. The PI and Partnering PI, breast cancer expert Dr. Robert J. Schneider, have clearly demonstrated the efficacy of MBQ-167 in preclinical mouse models: (i) MBQ-167 inhibits breast tumor growth and metastasis by ~90% to all organs from mice with breast tumors established from both human and mouse HER2-type and TNBC cells. (ii) MBQ-167 blocks direct establishment of metastases when TNBC cells are introduced into the blood vessels and allowed to colonize lungs. (iii) MBQ-167 chemosensitizes established TNBC metastases to Paclitaxel as well as those seeded during primary tumor growth. We were recently funded by the BCRP to investigate the mechanism of chemo-sensitization of breast cancer therapeutics including Paclitaxel, and that grant will end in 2022. Contracted by the company MBQ Pharma, Inc. (founded by the PI and collaborators with Dr. Schneider as chair of Scientific Advisory Board), MBQ-167 was shown to be completely safe in rat and dog models up to 1000 mg/kg BW, which is much higher than its effective concentration. Therefore, we recently submitted an Investigational New Drug (IND) application (IND148376) to the U.S. Food and Drug Administration (FDA) to conduct phase 1 clinical trials in metastatic breast cancer patients, independent of this application.

The objective of the present application is to further the translational development of MBQ-167.

Aim 1 will identify and characterize, for the first time, total expression and levels of biochemically active Rac, Cdc42, and their direct downstream effector p21-activated kinase (PAK) as well as cell proliferation regulator Cyclin D in breast cancer patient tissues from Puerto Rico and New York. We will correlate active Rac/Cdc42/PAK/Cyclin D status with breast cancer grade (I-IV), receptor status (ER, PR, HER2, TNBC), and ethnicity (Hispanic, non-Hispanic Caucasian, African American). The outcome of this aim will characterize the patient population that may best benefit from MBQ-167 therapy and aid in patient stratification for phase 2 clinical studies for MBQ-167 efficacy. Since available histopathological data do not have much information from Puerto Rican patients, we expect to obtain new data pertaining to the large underrepresented Hispanic and African American populations. Therefore, this study may elucidate a

molecular basis for the health disparity of African American women in TNBC and identify a new drug that will forward personalized medicine for the medically underserved.

Aim 2 will test the applicability of using active phosphorylated PAK (p-PAK) and Cyclin D expression as markers for MBQ-167 efficacy using mice with tumors from TNBC cells. MBQ Pharma, Inc. will then utilize a certified diagnostic development Research Organization to develop and validate a Clinical Laboratory Improvement Amendments (CLIA) certified diagnostic test for MBQ-167 patient selection and efficacy in our planned clinical trials in breast cancer patients.

Aim 3 will test the effect of MBQ-167 directly in human breast cancer patient tissue. We will collect fresh breast cancer tissue from patients undergoing surgery, and culture whole sections in the lab and test MBQ-167 for effects on tumor cell death and immune infiltration that mediates either anti-tumor response or progression. We will provide data indicating that MBQ-167 is effective in the human tumor microenvironment.

Clinical Applications: The patients who will benefit from this drug are expected to be metastatic advanced stage breast cancer patients.

Projected Time: Phase 1 trials for MBQ-167 will be initiated in 2022, independent of this application. This proposal will identify the patient population who will best benefit from MBQ-167 therapy, develop a biomarker/diagnostic for MBQ-167 efficacy, and validate the potential of MBQ-167 as an anti-breast cancer therapeutic. Therefore, we will generate information for phase 2 clinical trials of MBQ-167 in high-grade breast cancer patients (in 2023) and accelerate the BCRPs mission of ending breast cancer within the next 5 years.

Proposal Title:	Translational Development of MBQ-167 as Targeted Therapy for Metastatic Breast Cancer
Log Number:	BC220526P1
Current PI Name:	Robert Schneider
Award Number:	HT9425-23-1-0167
Current Contracting Organization:	New York University School of Medicine
Current Performing Organization:	New York University School of Medicine
Web Approval Date:	03-23-2023

This proposal will further the development of a targeted therapeutic for metastatic breast cancer and address the following Fiscal Year 2022 (FY22) Breast Cancer Research Program (BCRP) overarching challenges: (i) revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival, and (ii) eliminate mortality associated with metastatic breast cancer.

Rationale: Breast cancer mortality is typically from metastatic disease. We are limited by targeted therapeutic options for metastasis, especially in triple-negative breast cancer (TNBC). Due to the inherent heterogeneity and lack of targetable receptors, a uniform therapeutic approach for TNBC has been challenging. Our solution is to target the related metastasis drivers Rac and Cdc42 proteins that drive metastatic TNBC and HER2 breast cancer, which until the development of our drug, have not been successfully targeted.

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The objective of the present application is to further the translational development of MBQ-167.

Aim 1 will identify and characterize, for the first time, total expression and levels of biochemically active Rac, Cdc42, and their direct downstream effector p21-activated kinase (PAK) as well as cell proliferation regulator Cyclin D in breast cancer patient tissues from Puerto Rico and New York. We will correlate active Rac/Cdc42/PAK/Cyclin D status with breast cancer grade (I-IV), receptor status (ER, PR, HER2, TNBC), and ethnicity (Hispanic, non-Hispanic Caucasian, African American). The outcome of this aim will characterize the patient population that may best benefit from MBQ-167 therapy and aid in patient stratification for phase 2 clinical studies for MBQ-167 efficacy. Since available histopathological data do not have much information from Puerto Rican patients, we expect to obtain new data pertaining to the large underrepresented Hispanic and African American populations. Therefore, this study may elucidate a

molecular basis for the health disparity of African American women in TNBC and identify a new drug that will forward personalized medicine for the medically underserved.

Aim 2 will test the applicability of using active phosphorylated PAK (p-PAK) and Cyclin D expression as markers for MBQ-167 efficacy using mice with tumors from TNBC cells. MBQ Pharma, Inc. will then utilize a certified diagnostic development Research Organization to develop and validate a Clinical Laboratory Improvement Amendments (CLIA) certified diagnostic test for MBQ-167 patient selection and efficacy in our planned clinical trials in breast cancer patients.

Aim 3 will test the effect of MBQ-167 directly in human breast cancer patient tissue. We will collect fresh breast cancer tissue from patients undergoing surgery, and culture whole sections in the lab and test MBQ-167 for effects on tumor cell death and immune infiltration that mediates either anti-tumor response or progression. We will provide data indicating that MBQ-167 is effective in the human tumor microenvironment.

Clinical Applications: The patients who will benefit from this drug are expected to be metastatic advanced stage breast cancer patients.

Projected Time: Phase 1 trials for MBQ-167 will be initiated in 2022, independent of this application. This proposal will identify the patient population who will best benefit from MBQ-167 therapy, develop a biomarker/diagnostic for MBQ-167 efficacy, and validate the potential of MBQ-167 as an anti-breast cancer therapeutic. Therefore, we will generate information for phase 2 clinical trials of MBQ-167 in high-grade breast cancer patients (in 2023) and accelerate the BCRPs mission of ending breast cancer within the next 5 years.

Proposal Title:	Turning Breast Cancer Cells Against Themselves as the Next-Generation Immunotherapy
Log Number:	BC220541
Current PI Name:	Ming-Ru Wu
Award Number:	HT9425-23-1-0031
Current Contracting Organization:	Dana-Farber Cancer Institute
Current Performing Organization:	Dana-Farber Cancer Institute
Web Approval Date:	01-12-2023

Scientific Objective: Our goal is to develop a transformative new strategy – Synthetic Tumor Recruited Immuno-Cellular Therapy (STRICT) – for treating triple-negative breast cancer (TNBC). STRICT forces tumors to recruit immune cells that kill primary tumors and metastasis. To achieve this goal, we will design artificial gene circuits activated explicitly in TNBC cells. These gene circuits will command TNBC cells to secrete immune modulators that attract immune cells to target the tumors for destruction. This effect should also induce long-term immune memory against cancer relapses. Here, we will develop, optimize, and validate the effectiveness of STRICT within in vitro and in vivo mouse models of TNBC.

Rationale: The immune system has been harnessed to treat a variety of blood cancers, including acute leukemia and multiple myeloma, via cell-based therapies. These strategies require isolation, engineering, and expansion of immune cells from each patient, which is expensive and labor-intensive. Furthermore, these approaches have not yet been applied successfully against TNBC, which poses additional therapeutic challenges due to the heterogeneity found in the tumors. Thus, there is an urgent need for novel, safe, and effective therapies.

We aim to develop novel therapies that act from within tumors to recruit and activate immune cells into tumors – a Trojan horse approach. Specifically, we will design genetic circuits that can be delivered locally or systemically, sense when they are inside cancer cells, and respond by producing combinations of complementary immune modulators from within tumors. These immune modulators shall recruit immune cells into the tumors, thus harnessing the immune system to target TNBC and establishing long-lasting protection against metastasis and recurrent cancer. STRICT can be modulated and shut off if needed, thus providing controllable safety switches. Furthermore, STRICT does not require custom cellular engineering for every patient, thus enabling greater patient access and reduced burden on health care infrastructure. STRICT can also be used with other cancer therapies to achieve enhanced efficacy.

Aims: In Aim 1, we will engineer synthetic gene circuits to specifically express immunomodulators within tumors to recruit immune cells to kill tumors. We shall validate the effectiveness of these gene circuits in vitro in TNBC models. We will also optimize STRICT to target heterogeneous tumors. In Aim 2, we will identify the optimal therapeutic output combination that confers the strongest efficacy. We will also determine the minimal percentage of cancer cells that need to be targeted by the synthetic gene circuits to achieve therapeutic efficacy in mouse models of TNBC. In Aim 3, we will elucidate the immune response triggered by STRICT. We will also test the ability of STRICT to eliminate primary and metastatic TNBCs in fully immunocompetent mice. Furthermore, we will measure whether STRICT can trigger immune memory to prevent tumor relapses. This work will establish key parameters needed for successful immunotherapy against TNBC and enable the optimization of designs for future preclinical and clinical trials.

Overarching Challenges: We aim to address two overarching challenges: (1) revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival; and (2) eliminate the mortality associated with metastatic breast cancer.

What types of patients will be helped and how? This work will benefit TNBC patients, especially ones with metastatic disease or cancer relapse, with a new and potentially powerful therapy.

What are potential clinical applications, benefits, and risks? Our strategy has the potential to become a new clinical therapy for TNBC. The potential benefits of this technology include providing highly effective treatment for metastatic TNBC and protection against future relapse. The potential risks of this technology include the challenge of targeting heterogeneous solid tumors, although we have outlined a comprehensive plan to minimize this risk with a set of alternative strategies.

What Is the Projected Time for Patient-Related Outcomes/Interim Outcomes? We anticipate that by the end of the 3-year period of this grant, we will have constructed and optimized our therapeutic circuits and validated their activity within mouse models of cancer. If successful, we anticipate that preclinical and clinical development can commence immediately thereafter, thus accelerating the time scale to impacting patient health.

What Is the Impact on Ending Breast Cancer? If successful, this research will provide a novel therapeutic strategy for ending TNBC, with the potential to replace existing treatments that have toxicities and target metastatic and recurrent TNBC, which are major causes of mortality.

Proposal Title:	Novel Combination Immune Therapy Regimens for Metastatic Breast Cancer
Log Number:	BC220542
Current PI Name:	Andrei Bakin
Award Number:	HT9425-23-1-0059
Current Contracting Organization:	Health Research Inc., Roswell Park Division
Current Performing Organization:	Health Research Inc., Roswell Park Division
Web Approval Date:	01-26-2023

Overarching Challenge: The proposal addresses two challenges: (a) eliminate the mortality associated with metastatic breast cancer and (b) revolutionize existing treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival.

Immunotherapy with agents called immune checkpoint inhibitors (ICIs) has provided new promise for cancer patients. However, ICI therapy showed low response rates in breast cancer (BC), including metastatic BC (MBC). This problem was linked to the highly immune suppressive environment that detracts the positive benefits of ICI therapy. New approaches to improve ICI therapy are needed such as targeting myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs), major immune suppressive cells in the immune environment.

Most MBCs carry mutations inactivating the tumor suppressor p53. Mutant p53 drives cancer progression and metastasis in part by promoting inflammation and the build-up of immune suppressive MDSC/TAM cells. At present, there are no effective treatment options for p53-mutant BC. We developed a novel therapeutic strategy for selective damaging p53-mutant tumors that takes advantage of DNA repair dysregulation in those tumors. Our data demonstrate that this novel anti-mutant-p53 regimen effectively blocks tumor growth and metastasis in p53-mutant MBC models without adverse effects. This new anti-mutant-p53 strategy is now being tested in the first-in-human phase 1 clinical trial for advanced colorectal cancer (CRC) (NCT04511039). The results from the first six patients showed good tolerance to this treatment.

New data suggest that our anti-mutant-p53 treatment may affect immune suppressive cells and improve response to ICI therapy. The current study will test the hypothesis that this anti-mutant-p53 regimen can stimulate antitumor immune responses and improve the ICI efficacy by acting via both tumor-intrinsic and tumor-extrinsic mechanisms affecting the immune environment in p53-mutant MBC.

The proposal includes three specific aims. Aim 1 will define the effects of our anti-mutant-p53 regimen on the various immune cell populations in p53-mutant MBC models. Aim 2 will assess the contribution of anti-tumor immune cells and immune suppressive cells to the antitumor action of our anti-mutant-p53 therapy. Aim 3 will test this anti-mutant-p53 treatment in combination with ICI agents in p53-mutant MBC.

This work matters for thousands of patients with advanced BC, such as MBC, which is presently incurable. The results of this work may lead to a new treatment option for breast cancers with mutant p53 accounting for majority of MBC. Excitingly, our new therapeutic strategy can be quickly translated into the clinical trials as all components are already approved for cancer treatment but never been combined, to the best of our knowledge.

Proposal Title:	m6A Epitranscriptome Drivers of Endocrine-Resistant Breast Cancer
Log Number:	BC220564
Current PI Name:	Carolyn Klinge
Award Number:	HT9425-23-1-0017
Current Contracting Organization:	University of Louisville Research Foundation, Inc.
Current Performing Organization:	University of Louisville Research Foundation, Inc.
Web Approval Date:	12-08-2022

Rationale for Proposed Research: This project specifically addresses why some breast cancer patients develop acquired endocrine-resistant, recurrent, metastatic cancer. The current standard adjuvant treatment for patients with estrogen receptor a positive (ER+) breast cancer depends on menopausal status with aromatase inhibitors for post-menopausal and tamoxifen for pre-menopausal women. While these endocrine therapies have greatly increased the survival rate of patients with ER+ breast cancer, unfortunately, a major limitation is the development of acquired resistance in ~40% of initially responsive patients. The goal of our study is to understand how changes in “chemical decorations” or modifications added onto transcribed RNA, specifically adenosine methylation (m6A), and their downstream consequences affect the evolution of breast tumors from endocrine-sensitive to endocrine resistance and metastatic disease. Ultimately, these RNA chemical modifications change the expression of multiple proteins that regulate biological activities in a process termed “epitranscriptomics.” We have recently demonstrated that a cellular “reader” of these marks, HNRNPA2B1, is required to drive endocrine-resistant ER+ BC and that it alters a specific metabolic pathway that we have also shown influences tamoxifen efficacy. We now intend to globally assess these RNA marks dynamically impacts pathways of endocrine resistance, metastasis, cellular metabolism between endocrine-sensitive and -resistant ER+ BC. Our objectives will be achieved by completion of two distinct aims that will (1) use state-of-the-art genetic sequencing, proteomics, and bioinformatic technologies to definitively characterize RNA modifications and subsequent protein changes that influence endocrine sensitivity, and (2) determine relevance of epitranscriptomic control of serine metabolism in acquired resistance using advanced metabolomics tracing approaches. These studies will be performed in complementary relevant models of ER+ BC that have defined endocrine sensitivity or resistance, including established breast cancer cell lines and patient-derived ER+ BC tissues (PDX). We will also employ a pharmaceutical inhibitor of the enzyme that generates the m6A marks in RNA to determine if diminished m6A deposition is clinically relevant against BC cell and PDX tumor growth and whether it is a potential avenue for altering endocrine resistance. The resulting data will be the first direct identification of the requirement for changes in the epitranscriptome that regulates endocrine resistance and ER+ BC progression and will also address the overarching challenge to identify why some breast cancers become metastatic.

Ultimate Applicability for Proposed Research: Prior studies have attempted to elucidate mechanisms that drive endocrine therapy resistance, yet the majority of these have focused on selective protein changes. Here we take a novel, global approach in describing the significance of the m6A epitranscriptome in ER+ BC progression. Further, this work will provide proof of principle that epitranscriptomic control of key pathways established to contribute to endocrine resistance and the serine synthetic metabolic pathway that is also necessary to promote endocrine resistance. This study will validate the relevance of RNA modifications in regulating biochemical pathways that promote recurrent disease, which occurs all too often after initially successful hormonal therapy. While these studies will interrogate the basic biological understanding of these mechanisms in ER+ BC, these findings have the potential for near-future clinical translation. Notably, inhibitors of RNA epitranscriptome modifiers are currently under development. Defining specific changes in RNA modifications that drive cellular activities that are necessary for ER+ BC progression will provide novel selective targets as a therapeutic strategy against endocrine-resistant metastatic disease, which ultimately is aimed at a second overarching challenge in eliminating the mortality associated with metastatic breast cancer.

Proposal Title:	m6A Epitranscriptomic Drivers of Endocrine-Resistant Breast Cancer
Log Number:	BC220564P1
Current PI Name:	Brian Clem
Award Number:	HT9425-23-1-0018
Current Contracting Organization:	University of Louisville Research Foundation, Inc.
Current Performing Organization:	University of Louisville Research Foundation, Inc.
Web Approval Date:	12-08-2022

Rationale for Proposed Research: This project specifically addresses why some breast cancer patients develop acquired endocrine-resistant, recurrent, metastatic cancer. The current standard adjuvant treatment for patients with estrogen receptor a positive (ER+) breast cancer depends on menopausal status with aromatase inhibitors for post-menopausal and tamoxifen for pre-menopausal women. While these endocrine therapies have greatly increased the survival rate of patients with ER+ breast cancer, unfortunately, a major limitation is the development of acquired resistance in ~40% of initially responsive patients. The goal of our study is to understand how changes in “chemical decorations” or modifications added onto transcribed RNA, specifically adenosine methylation (m6A), and their downstream consequences affect the evolution of breast tumors from endocrine-sensitive to endocrine resistance and metastatic disease. Ultimately, these RNA chemical modifications change the expression of multiple proteins that regulate biological activities in a process termed “epitranscriptomics.” We have recently demonstrated that a cellular “reader” of these marks, HNRNPA2B1, is required to drive endocrine-resistant ER+ BC and that it alters a specific metabolic pathway that we have also shown influences tamoxifen efficacy. We now intend to globally assess these RNA marks dynamically impacts pathways of endocrine resistance, metastasis, cellular metabolism between endocrine-sensitive and -resistant ER+ BC. Our objectives will be achieved by completion of two distinct aims that will (1) use state-of-the-art genetic sequencing, proteomics, and bioinformatic technologies to definitively characterize RNA modifications and subsequent protein changes that influence endocrine sensitivity, and (2) determine relevance of epitranscriptomic control of serine metabolism in acquired resistance using advanced metabolomics tracing approaches. These studies will be performed in complementary relevant models of ER+ BC that have defined endocrine sensitivity or resistance, including established breast cancer cell lines and patient-derived ER+ BC tissues (PDX). We will also employ a pharmaceutical inhibitor of the enzyme that generates the m6A marks in RNA to determine if diminished m6A deposition is clinically relevant against BC cell and PDX tumor growth and whether it is a potential avenue for altering endocrine resistance. The resulting data will be the first direct identification of the requirement for changes in the epitranscriptome that regulates endocrine resistance and ER+ BC progression and will also address the overarching challenge to identify why some breast cancers become metastatic.

Ultimate Applicability for Proposed Research: Prior studies have attempted to elucidate mechanisms that drive endocrine therapy resistance, yet the majority of these have focused on selective protein changes. Here we take a novel, global approach in describing the significance of the m6A epitranscriptome in ER+ BC progression. Further, this work will provide proof of principle that epitranscriptomic control of key pathways established to contribute to endocrine resistance and the serine synthetic metabolic pathway that is also necessary to promote endocrine resistance. This study will validate the relevance of RNA modifications in regulating biochemical pathways that promote recurrent disease, which occurs all too often after initially successful hormonal therapy. While these studies will interrogate the basic biological understanding of these mechanisms in ER+ BC, these findings have the potential for near-future clinical translation. Notably, inhibitors of RNA epitranscriptome modifiers are currently under development. Defining specific changes in RNA modifications that drive cellular activities that are necessary for ER+ BC progression will provide novel selective targets as a therapeutic strategy against endocrine-resistant metastatic disease, which ultimately is aimed at a second overarching challenge in eliminating the mortality associated with metastatic breast cancer.

Proposal Title:	A Direct RAS Pan-Inhibitor as a Novel Strategy for Luminal B Breast Cancer
Log Number:	BC220575
Current PI Name:	Geoffrey Clark
Award Number:	HT9425-23-1-0060
Current Contracting Organization:	University of Louisville Research Foundation, Inc.
Current Performing Organization:	University of Louisville Research Foundation, Inc.
Web Approval Date:	01-12-2023

Rationale: Luminal B breast cancer is almost as dangerous as triple-negative breast cancer yet is much less studied and has no good therapeutic options. Recently, it has been shown that many luminal B cancers are driven by loss of function of negative regulators of the RAS oncoprotein. This results in abnormally high RAS oncoprotein activity, which drives the tumor. Therefore, a targeted inhibitor of RAS could be a highly effective approach to luminal B tumors. There are currently no clinically approved, direct wild-type RAS inhibitors and only a few published in the literature. None have been tried on breast cancer. We have developed a RAS inhibitor that works particularly well on luminal B experimental systems.

Objective: To determine if our RAS inhibitors are effective against luminal B breast cancer systems, alone and in combination with standard-of-care agents.

Aims: We propose two aims, the first focuses on testing the RAS inhibitor drug against a series of human luminal B cell line experimental tumor/metastasis systems, in combination with other targeted therapies used for luminal B breast cancer in the clinic. The second aim proposes to test the inhibitor in combinations in more physiological tumor model systems. Primary tumor grafts and transgenic mice. The latter experiments involve Immune checkpoint inhibitors.

Ultimate Applicability: Ultimately, we intend to develop the agents into the stage where we can perform a clinical trial on luminal B breast cancer. We currently have industry financial support to develop the compounds into a clinical agent for use in lung and pancreatic cancer. If positive, we could extend the trial to any breast tumor that exhibits over-activation of RAS.

Overarching Challenge: “Revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival” and ”Eliminate the mortality associated with metastatic breast cancer.”

Patient Benefit: The proposal directly addresses patients with luminal B breast cancer, but it also is applicable to any breast cancer that is driven by deregulated wild-type RAS; this could include Her2-positive tumors and triple-negative tumors driven by loss of the NF1 RASGAP (about 15%). If successful, the proposal could lead to a novel and highly effective, low toxicity targeted therapy for luminal B breast cancer.

Clinical Applications: We envisage a low toxicity, oral available therapeutic emerging from the technology, that can used in combination with immune checkpoint inhibitors. As RAS activity has been closely associated with the development of metastasis, we would hope to see activity against metastatic lesions. These would include brain metastases as we have confirmed our compound can cross the blood-brain barrier. Potential risks include unanticipated toxicity in humans manifesting, despite the lack of toxicity we have observed so far in mice.

Projected Time It May Take to Achieve a Patient-Related Outcome: We currently have a sponsored research agreement with a biotech company whose funding proposes that we reach the stage ready for an Investigational New Drug (IND) filing within 18 months. This is an aggressive schedule, but not entirely unrealistic. Therefore, we may be in a position to evaluate patient outcome within the medium term.

What is the likely impact of this study on the Breast Cancer Research Program's mission of ending breast cancer? If successful, the project could lead to a new, low toxicity, high efficacy therapy for luminal B breast cancer.

Proposal Title:	Targeting APE1 Interaction with DNA G-Quadruplex to Prevent Metastasis in Triple-Negative Breast Cancer
Log Number:	BC220592
Current PI Name:	Kishor Bhakat
Award Number:	HT9425-23-1-0051
Current Contracting Organization:	Nebraska, University of, Medical Center
Current Performing Organization:	Nebraska, University of, Medical Center
Web Approval Date:	01-12-2023

Cancer remains one of the leading cause of deaths among Americans. Breast cancer is the second leading cause of cancer-related deaths in women. According to American Cancer Society, about 287,850 women will be diagnosed of invasive breast cancer in year 2022. An estimated 43,250 breast cancer deaths in women are expected in year 2022.

Although over past decade we have made significant advancement in early diagnosis and targeted therapy for majority of breast cancer patients, about 10% to 20% of breast cancers that are called triple-negative breast cancer (TNBC) do not respond to conventional hormonal or antibody therapies due to lack of estrogen receptors, progesterone receptors, and ErbB2 overexpression. This subtype of breast cancer has a tendency to grow fast and spread (metastasize) more quickly to lungs and brain than other subtypes of breast cancer. TNBC is frequently observed in younger women and threaten patients' median overall survival to 9-13 months due to its high rate of metastasis and recurrence. Standard chemotherapy and radiation do not work effectively, and metastasis is responsible for greater than 80% of death of women with TNBC. Therefore, we need more research to identify and characterize the factors that drive metastasis to allow the development of new therapeutic agents to inhibit TNBC metastasis.

Overarching Challenges: Our research will address the following overarching challenges: "Identify why some breast cancers become metastatic" and "Eliminate the mortality associated with metastatic breast cancer" by elucidating the mechanisms by which TNBC cells drive distant metastasis and by identifying novel therapeutic targets to prevent metastasis.

We have identified AP-endonuclease (APE1), a protein that repairs DNA damages and controls gene expression, is elevated in TNBC, and patient with a high level of APE1 in tumors cells had a shorter survival due to fast spreading of tumors from primary site (breast) to lungs, bone, and brain. To begin to understand how overexpression of this protein promotes TNBC metastasis, we discovered a novel pathway, the subject of this proposal. APE1 protein binds to a four-stranded DNA structures known as quadruplexes (G4) present in gene promoters and facilitates expression of a set of genes that drive migrations of tumor cells from primary site (breast) to distant organs. DNA is usually depicted as double-stranded, but recent research shows that parts of the genome that adopt four-stranded structures known as G4 control expression of a set of proteins needed for migration of tumor cells and their colonization/growth in other organs. APE1 binds to this special G4 DNA structure to alter the expression of genes. If tumor cells do not have APE1 protein, then they fail to migrate. In this proposal, we are interested in learning more about how these elevated G4-DNA structures and APE1 drive tumor cells migration and metastatic progression, and if we can find a way to disrupt APE1's function in binding with G4 DNA, we might be able to modulate gene expression in tumor cells and stop their migration and invasion. Significantly, a small molecule, called TMPyP4, can bind to G4 DNA and block APE1 binding to G4, which has a significant effect on gene expression and affects cell migration. We predict that this small molecule can inhibit tumor cells migration and colonization to lungs

and brain and prevent metastasis. To test this idea, we will generate TNBC in mice and examine whether treatment with TMPyP4 can inhibit tumor spreading to lungs. If successful, this will represent a novel therapeutic strategy to prevent metastasis in TNBC patients.

Successful completion of this project will establish that elevated levels of APE1 and G4 DNA promote metastasis-related genes expression in TNBC, and small molecule TMPyP4 (G4 ligand) can be used to prevent TNBC metastasis and improve patients' outcomes.

Proposal Title:	Targeting APE1 Interaction with DNA G-Quadruplex to Prevent Metastasis in Triple-Negative Breast Cancer
Log Number:	BC220592P1
Current PI Name:	Vimla Band
Award Number:	HT9425-23-1-0052
Current Contracting Organization:	Nebraska, University of, Medical Center
Current Performing Organization:	Nebraska, University of, Medical Center
Web Approval Date:	01-12-2023

Cancer remains one of the leading cause of deaths among Americans. Breast cancer is the second leading cause of cancer-related deaths in women. According to American Cancer Society, about 287,850 women will be diagnosed of invasive breast cancer in year 2022. An estimated 43,250 breast cancer deaths in women are expected in year 2022.

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We have identified AP-endonuclease (APE1), a protein that repairs DNA damages and controls gene expression, is elevated in TNBC, and patient with a high level of APE1 in tumors cells had a shorter survival due to fast spreading of tumors from primary site (breast) to lungs, bone, and brain. To begin to understand how overexpression of this protein promotes TNBC metastasis, we discovered a novel pathway, the subject of this proposal. APE1 protein binds to a four-stranded DNA structures known as quadruplexes (G4) present in gene promoters and facilitates expression of a set of genes that drive migrations of tumor cells from primary site (breast) to distant organs. DNA is usually depicted as double-stranded, but recent research shows that parts of the genome that adopt four-stranded structures known as G4 control expression of a set of proteins needed for migration of tumor cells and their colonization/growth in other organs. APE1 binds to this special G4 DNA structure to alter the expression of genes. If tumor cells do not have APE1 protein, then they fail to migrate. In this proposal, we are interested in learning more about how these elevated G4-DNA structures and APE1 drive tumor cells migration and metastatic progression, and if we can find a way to disrupt APE1's function in binding with G4 DNA, we might be able to modulate gene expression in tumor cells and stop their migration and invasion. Significantly, a small molecule, called TMPyP4, can bind to G4 DNA and block APE1 binding to G4, which has a significant effect on gene expression and affects cell migration. We predict that this small molecule can inhibit tumor cells migration and colonization to lungs

and brain and prevent metastasis. To test this idea, we will generate TNBC in mice and examine whether treatment with TMPyP4 can inhibit tumor spreading to lungs. If successful, this will represent a novel therapeutic strategy to prevent metastasis in TNBC patients.

Successful completion of this project will establish that elevated levels of APE1 and G4 DNA promote metastasis-related genes expression in TNBC, and small molecule TMPyP4 (G4 ligand) can be used to prevent TNBC metastasis and improve patients' outcomes.

Proposal Title:	Moving PARP Inhibitors Beyond BRCAness as Immune Modulating Agents for TNBC Prevention
Log Number:	BC220706
Current PI Name:	Powel Brown
Award Number:	HT9425-23-1-0055
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	01-10-2023

Despite the advances in the treatment of breast cancer, too many women with breast cancer must undergo chemotherapy, and still some women still die of this disease. To eliminate this disease, we seek to develop effective, but minimally toxic, ways to prevent this disease and eliminate the need for toxic chemotherapy. Basic science research has uncovered new information about breast cancer biology and the immune system that now supports the development of targeted therapies for breast cancer treatment and prevention. Clinical trials have shown that estrogen receptor (ER)-positive breast cancer prevention is possible, although this preventive therapy is still too toxic for most women. However, there are no preventive therapies for triple-negative breast cancer (TNBC), the most aggressive form of breast cancer that occurs often in young women, African American women, and women who carry BRCA1 mutations. In this study, we will test novel targeted therapies for the ability to alter immune pathways and to prevent TNBC development in mice.

Poly (ADP-ribose) polymerase (PARP) inhibitors (PARPis) are approved for the treatment of advanced breast tumors with BRCA1 or BRCA2 (BRCA1/2) mutations. In March 2022, the U.S. Food and Drug Association (FDA) approved PARPi (Olaparib) for the adjuvant treatment of high-risk early breast cancer in women with BRCA mutations. Consistent with this notion, recent studies have shown that PARPis can prevent the development of BRCA1-deficient TNBC tumors in mice. However, women with germline BRCA1/2 mutations are a very small subset of women that develop TNBC. Laboratory studies from our group uncovered a previously unknown activity of PARPis. We have shown that PARPis activate the immune system through an important pathway called the c-GAS-STING- type I IFN innate immune response pathway. Our preliminary data suggest that the efficacy of PARPis is through regulating the expression of a key immune gene, STING, which suggests that PARPis will prevent many TNBCs, and not just those arising in women carrying BRCA1/2 mutations.

Based on results from our clinical and laboratory studies, we wish to now study the following:

(1) Determine how PARPis induce the expression of the key immune gene STING and how they affect the innate immune response,

(2) Determine whether PARPis induce changes in the c-GAS-STING-type I IFN innate immune response pathway, which can serve as biomarkers for PARPis' ability to induce anti-tumor immunity,

(3) Evaluate whether PARPis prevent the development of TNBC in two mouse models (one that develops BRCA1-deficient tumors and one that develops BRCA1 wild-type tumors).

Upon completion of this project, we will obtain essential preclinical data to support the development of clinical trials to test the ability of PARPis to prevent the development of all TNBCs, not just those that arise due to BRCA1/2 mutations.

In this application, we study PARPis in TNBC models as proof-of-principle experiments. Innate immune response is a fundamental biological pathway in cancer immunology of all breast cancer subtypes and in breast cancers at all development and progression stages. By identifying the new mechanisms underlying PARPis' ability to activate the innate immune system, our study will benefit breast cancer patients, women at risk of TNBC, as well as women who develop this devastating disease.

Proposal Title:	Moving PARP Inhibitors Beyond BRCAness as Immune Modulating Agents for TNBC Prevention
Log Number:	BC220706P1
Current PI Name:	Guang Peng
Award Number:	HT9425-23-1-0056
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	01-10-2023

Despite the advances in the treatment of breast cancer, too many women with breast cancer must undergo chemotherapy, and still some women still die of this disease. To eliminate this disease, we seek to develop effective, but minimally toxic, ways to prevent this disease and eliminate the need for toxic chemotherapy. Basic science research has uncovered new information about breast cancer biology and the immune system that now supports the development of targeted therapies for breast cancer treatment and prevention. Clinical trials have shown that estrogen receptor (ER)-positive breast cancer prevention is possible, although this preventive therapy is still too toxic for most women. However, there are no preventive therapies for triple-negative breast cancer (TNBC), the most aggressive form of breast cancer that occurs often in young women, African American women, and women who carry BRCA1 mutations. In this study, we will test novel targeted therapies for the ability to alter immune pathways and to prevent TNBC development in mice.

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(1) Determine how PARPis induce the expression of the key immune gene STING and how they affect the innate immune response,

(2) Determine whether PARPis induce changes in the c-GAS-STING-type I IFN innate immune response pathway, which can serve as biomarkers for PARPis' ability to induce anti-tumor immunity,

(3) Evaluate whether PARPis prevent the development of TNBC in two mouse models (one that develops BRCA1-deficient tumors and one that develops BRCA1 wild-type tumors).

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In this application, we study PARPis in TNBC models as proof-of-principle experiments. Innate immune response is a fundamental biological pathway in cancer immunology of all breast cancer subtypes and in breast cancers at all development and progression stages. By identifying the new mechanisms underlying PARPis' ability to activate the innate immune system, our study will benefit breast cancer patients, women at risk of TNBC, as well as women who develop this devastating disease.

Proposal Title:	Intracellular Bacterial Delivery of Vaccine Antigens to Establish T-Cell Antitumor Immunity in Triple-Negative Breast Cancer
Log Number:	BC220717
Current PI Name:	Neil Forbes
Award Number:	HT9425-23-1-0067
Current Contracting Organization:	Massachusetts, University of, Amherst
Current Performing Organization:	Massachusetts, University of, Amherst
Web Approval Date:	01-31-2023

Background: In the proposed research, we will create a bacterial therapy to treat immune-resistant breast cancer. Most breast tumors, and especially triple-negative breast cancers (TNBCs), do not respond to immune checkpoint inhibitors (ICI). These immune therapies are effective in only about 5% of TNBC patients. TNBC is an aggressive form of breast cancer that responds poorly to conventional therapies and has a poor prognosis. Most triple-negative tumors grow quickly and rapidly spread to the bone, liver, lung, and brain. Metastatic spread is the primary cause of death from breast cancer. The average 5-year survival for TNBC is 65%, which drops to 11% once tumors have spread. Developing new therapies for TNBC and metastatic disease are critical medical needs.

Overarching Challenge: The primary goal of the research is to create a bacterial immunotherapy to treat TNBC and metastatic disease. We propose to develop a therapy that refocuses vaccine memory CD8 T cells to generate antitumor immunity. We will create antigen-delivering Salmonella (ADS) to deliver the measles nucleoprotein directly into the cytoplasm of cancer cells. This antigen is a component of the measles, mumps, and rubella (MMR) vaccine, a common childhood vaccine that is administered to 91% of the U.S. population. ADS therapy utilizes new technology that we recently developed to deliver antigens specifically into cells in tumors.

We have strong evidence that this strategy will work. In preliminary research, we used ADS to deliver a model antigen to mice with immunologically cold pancreatic tumors. The bacterially delivered antigen activated vaccine T cells, cleared primary tumors, and established immunity against the cancer. The core goal of the proposed research is to develop this strategy into an effective treatment for immune-resistant breast cancer.

Type of Patients: ADS therapy would be effective for a broad range of breast cancer patients, especially women of African ancestry and women under 40. Women in these groups have increased mortality and a higher risk of developing TNBC. Military women, who are predominantly (>90%) under 40, have a 20% -40% increased risk of breast cancer. African American women have similarly poor survival, with mortality rates 41% higher than European Americans. Triple-negative tumors from African American women have high densities of CD8 T cells and immunosuppressive macrophages, indicating T cell exhaustion and resistance to immune clearance. By targeting memory T cells (from prior vaccination) that are less likely to become exhausted, ADS therapy would avoid these limitations.

Potential Clinical Applications: In its primary application, ADS therapy would be used for patients with late-stage breast cancer. For the majority (~75%) of these patients, resected tumors have recurred, spread to secondary sites, and chemotherapy is no longer effective. Clinically, we anticipate that ADS will be administered with intravenous doses once a week, similar to preclinical animal trials. If the therapy performs as observed in animal models, it will colonize all tumor masses in a patient, deliver the measles antigen into cancer cells, and educate the immune system to the tumor’s neoantigen signature. This immune induction would eliminate all existing lesions and prevent new ones from forming. A secondary preventative strategy

could impact a greater number of patients. In this strategy, ADS would be administered prior to excision of the primary tumor to promote colonization and identification of tumor neoantigens. Generating antitumor immunity before surgery would have a prophylactic effect, reducing the incidence of both recurrence and metastatic disease.

Projected Time to Patient-Related Outcomes: During the 3-year research period, the proposed experiments will demonstrate the safety and efficacy of using ADS to treat breast cancer. Results from these experiments will be the basis for Investigational New Drug (IND)-enabling studies that we will perform in collaboration with our industrial partner, Ernest Pharmaceuticals. We are currently preparing ADS for clinical trials by developing Good Manufacturing Practice (GMP) manufacturing, regulatory filings, and clinical protocols. We anticipate that breast cancer clinical trials could begin within a year of completing the proposed research.

Impact on Breast Cancer Research Program's mission: ADS therapy could have a dramatic impact on breast cancer by providing a treatment for metastatic disease. Almost all (90%) breast cancer deaths are due to metastatic disease, and yet treatments for metastases do not exist. Despite over 2,000 breast cancer clinical trials, there has been little impact on cancer mortality. This deficiency is compounded by the limited funding to develop treatments for metastatic disease. If successful, ADS would provide a therapy for patients who have few treatment options. As an "off-the- shelf" therapy, ADS would not need turning for each patient's tumor and would be effective for most breast cancer subtypes.

Proposal Title:	Intracellular Bacterial Delivery of Vaccine Antigens to Establish T-Cell Antitumor Immunity in Triple-Negative Breast Cancer
Log Number:	BC220717P1
Current PI Name:	Lisa Minter
Award Number:	HT9425-23-1-0068
Current Contracting Organization:	Massachusetts, University of, Amherst
Current Performing Organization:	Massachusetts, University of, Amherst
Web Approval Date:	01-31-2023

Background: In the proposed research, we will create a bacterial therapy to treat immune-resistant breast cancer. Most breast tumors, and especially triple-negative breast cancers (TNBCs), do not respond to immune checkpoint inhibitors (ICI). These immune therapies are effective in only about 5% of TNBC patients. TNBC is an aggressive form of breast cancer that responds poorly to conventional therapies and has a poor prognosis. Most triple-negative tumors grow quickly and rapidly spread to the bone, liver, lung, and brain. Metastatic spread is the primary cause of death from breast cancer. The average 5-year survival for TNBC is 65%, which drops to 11% once tumors have spread. Developing new therapies for TNBC and metastatic disease are critical medical needs.

Overarching Challenge: The primary goal of the research is to create a bacterial immunotherapy to treat TNBC and metastatic disease. We propose to develop a therapy that refocuses vaccine memory CD8 T cells to generate antitumor immunity. We will create antigen-delivering Salmonella (ADS) to deliver the measles nucleoprotein directly into the cytoplasm of cancer cells. This antigen is a component of the measles, mumps, and rubella (MMR) vaccine, a common childhood vaccine that is administered to 91% of the U.S. population. ADS therapy utilizes new technology that we recently developed to deliver antigens specifically into cells in tumors.

We have strong evidence that this strategy will work. In preliminary research, we used ADS to deliver a model antigen to mice with immunologically cold pancreatic tumors. The bacterially delivered antigen activated vaccine T cells, cleared primary tumors, and established immunity against the cancer. The core goal of the proposed research is to develop this strategy into an effective treatment for immune-resistant breast cancer.

Type of Patients: ADS therapy would be effective for a broad range of breast cancer patients, especially women of African ancestry and women under 40. Women in these groups have increased mortality and a higher risk of developing TNBC. Military women, who are predominantly (>90%) under 40, have a 20% -40% increased risk of breast cancer. African American women have similarly poor survival, with mortality rates 41% higher than European Americans. Triple-negative tumors from African American women have high densities of CD8 T cells and immunosuppressive macrophages, indicating T cell exhaustion and resistance to immune clearance. By targeting memory T cells (from prior vaccination) that are less likely to become exhausted, ADS therapy would avoid these limitations.

Potential Clinical Applications: In its primary application, ADS therapy would be used for patients with late-stage breast cancer. For the majority (~75%) of these patients, resected tumors have recurred, spread to secondary sites, and chemotherapy is no longer effective. Clinically, we anticipate that ADS will be administered with intravenous doses once a week, similar to preclinical animal trials. If the therapy performs as observed in animal models, it will colonize all tumor masses in a patient, deliver the measles antigen into cancer cells, and educate the immune system to the tumor’s neoantigen signature. This immune induction would eliminate all existing lesions and prevent new ones from forming. A secondary preventative strategy

could impact a greater number of patients. In this strategy, ADS would be administered prior to excision of the primary tumor to promote colonization and identification of tumor neoantigens. Generating antitumor immunity before surgery would have a prophylactic effect, reducing the incidence of both recurrence and metastatic disease.

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Impact on Breast Cancer Research Program's mission: ADS therapy could have a dramatic impact on breast cancer by providing a treatment for metastatic disease. Almost all (90%) breast cancer deaths are due to metastatic disease, and yet treatments for metastases do not exist. Despite over 2,000 breast cancer clinical trials, there has been little impact on cancer mortality. This deficiency is compounded by the limited funding to develop treatments for metastatic disease. If successful, ADS would provide a therapy for patients who have few treatment options. As an "off-the- shelf" therapy, ADS would not need turning for each patient's tumor and would be effective for most breast cancer subtypes.

Proposal Title:	Elucidating and Exploiting SAMHD1 for Breast Cancer Therapy
Log Number:	BC220744
Current PI Name:	David Yu
Award Number:	HT9425-23-1-0192
Current Contracting Organization:	Emory University
Current Performing Organization:	Emory University
Web Approval Date:	04-04-2023

Breast cancer is the most commonly diagnosed non-skin cancer in women and the second leading cause of cancer death in women in the United States. Despite recent advances in improving treatment outcomes, the overall impact of breast cancer remains devastating due to how common it is. Major challenges for the eradication of breast cancer include the need to conquer the problems of overtreatment and the need to revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival. This is especially important for patients with triple-negative breast cancer (TNBC) who do not respond to more tumor-specific treatments such as tamoxifen or herceptin and thus are treated with less tumor-specific treatments such as chemotherapy and radiation therapy and have poor clinical outcomes. In recent years, immune therapy has emerged as a promising treatment option for patients with breast cancer; however, only about 10% of breast cancer patients will respond. Thus, there is an urgent need to understand why many breast cancer patients do not respond so that novel approaches can be developed to improve the efficacy of immune therapy. Our lab and others have found that SAMHD1, a protein that functions as a HIV-1 restriction factor and is mutated in Aicardi Goutières syndrome (AGS), a rare autoimmune disorder, has a new role in resecting the ends of DNA double-strand breaks (DSB) or stalled DNA replication forks to prevent the accumulation of DNA in the cytoplasm of cells that activates the cGAS-STING pathway to induce an innate immune response that facilitates the recruitment and stimulation of CD8+ T cells that attack cancer cells. Significantly, SAMHD1 is overexpressed in up to 27% of breast cancers and its high expression is associated with poor outcomes in breast cancer patients, suggesting that SAMHD1 may be a promising therapeutic target for cancer therapy. We have also identified a novel immunologic niche in breast cancer consisting of stem-cell like CD8+ T cells that are essential for a robust immune response and that is associated with improved clinical outcomes. Finally, we developed a novel therapeutic strategy for targeting SAMHD1 in tumors whereby a natural viral accessory protein called Vpx, which targets SAMHD1 for degradation, is packaged in virus-like particles (VLP) and show that it can degrade SAMHD1 in tumors. We hypothesize that SAMHD1 governs the resistance of breast cancer to immune therapy at least in part through its role in suppressing the accumulation of cytosolic DNA and activation of the innate immune response that potentiates immune therapy by stimulating stem cell-like CD8+ T cells. Targeting SAMHD1 for degradation with VLPs containing Vpx sensitizes resistant breast cancer to immune therapy. We propose to determine the mechanism by which SAMHD1 directs immunologic dynamics underlying response to immune therapy in breast cancer and establish proof of concept that targeting SAMHD1 with VLPs containing Vpx sensitizes resistant breast cancer tumors to immune therapy. We will use multiple approaches, including with human breast cancer patient samples and breast cancer mouse models. Completion of this research will provide new insights into how SAMHD1 directs immunologic dynamics underlying anti-tumor immunity in breast cancer via stimulation of stem cell-like CD8+ T cells. This work will also elucidate the significance of SAMHD1 as a potential rationale-driven biomarker for selecting breast patients who may benefit from immune therapy. Finally, this work will establish proof of concept for the use of VLPs containing Vpx in targeting SAMHD1 as a novel therapeutic approach for overcoming breast cancer resistance to immune therapy that may significantly improve clinical outcomes for patients with breast cancer. Our research team, which includes two board-certified physician-scientists as Partnering Principal Investigators, is well positioned to rapidly translate knowledge gained from this highly innovative and impactful research to clinical practice.

Proposal Title:	Elucidating and Exploiting SAMHD1 for Breast Cancer Therapy
Log Number:	BC220744P1
Current PI Name:	Zachary Buchwald
Award Number:	HT9425-23-1-0193
Current Contracting Organization:	Emory University
Current Performing Organization:	Emory University
Web Approval Date:	04-04-2023

Breast cancer is the most commonly diagnosed non-skin cancer in women and the second leading cause of cancer death in women in the United States. Despite recent advances in improving treatment outcomes, the overall impact of breast cancer remains devastating due to how common it is. Major challenges for the eradication of breast cancer include the need to conquer the problems of overtreatment and the need to revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival. This is especially important for patients with triple-negative breast cancer (TNBC) who do not respond to more tumor-specific treatments such as tamoxifen or herceptin and thus are treated with less tumor-specific treatments such as chemotherapy and radiation therapy and have poor clinical outcomes. In recent years, immune therapy has emerged as a promising treatment option for patients with breast cancer; however, only about 10% of breast cancer patients will respond. Thus, there is an urgent need to understand why many breast cancer patients do not respond so that novel approaches can be developed to improve the efficacy of immune therapy. Our lab and others have found that SAMHD1, a protein that functions as a HIV-1 restriction factor and is mutated in Aicardi Goutières syndrome (AGS), a rare autoimmune disorder, has a new role in resecting the ends of DNA double-strand breaks (DSB) or stalled DNA replication forks to prevent the accumulation of DNA in the cytoplasm of cells that activates the cGAS-STING pathway to induce an innate immune response that facilitates the recruitment and stimulation of CD8+ T cells that attack cancer cells. Significantly, SAMHD1 is overexpressed in up to 27% of breast cancers and its high expression is associated with poor outcomes in breast cancer patients, suggesting that SAMHD1 may be a promising therapeutic target for cancer therapy. We have also identified a novel immunologic niche in breast cancer consisting of stem-cell like CD8+ T cells that are essential for a robust immune response and that is associated with improved clinical outcomes. Finally, we developed a novel therapeutic strategy for targeting SAMHD1 in tumors whereby a natural viral accessory protein called Vpx, which targets SAMHD1 for degradation, is packaged in virus-like particles (VLP) and show that it can degrade SAMHD1 in tumors. We hypothesize that SAMHD1 governs the resistance of breast cancer to immune therapy at least in part through its role in suppressing the accumulation of cytosolic DNA and activation of the innate immune response that potentiates immune therapy by stimulating stem cell-like CD8+ T cells. Targeting SAMHD1 for degradation with VLPs containing Vpx sensitizes resistant breast cancer to immune therapy. We propose to determine the mechanism by which SAMHD1 directs immunologic dynamics underlying response to immune therapy in breast cancer and establish proof of concept that targeting SAMHD1 with VLPs containing Vpx sensitizes resistant breast cancer tumors to immune therapy. We will use multiple approaches, including with human breast cancer patient samples and breast cancer mouse models. Completion of this research will provide new insights into how SAMHD1 directs immunologic dynamics underlying anti-tumor immunity in breast cancer via stimulation of stem cell-like CD8+ T cells. This work will also elucidate the significance of SAMHD1 as a potential rationale-driven biomarker for selecting breast patients who may benefit from immune therapy. Finally, this work will establish proof of concept for the use of VLPs containing Vpx in targeting SAMHD1 as a novel therapeutic approach for overcoming breast cancer resistance to immune therapy that may significantly improve clinical outcomes for patients with breast cancer. Our research team, which includes two board-certified physician-scientists as Partnering Principal Investigators, is well positioned to rapidly translate knowledge gained from this highly innovative and impactful research to clinical practice.

Proposal Title:	Clinical Relevance of Needle Biopsy-Induced Prometastatic Phenotypic Changes in Breast Cancer
Log Number:	BC220773
Current PI Name:	Takemi Tanaka
Award Number:	HT9425-23-1-0710
Current Contracting Organization:	Oklahoma, University of, Health Sciences Center
Current Performing Organization:	Oklahoma, University of, Health Sciences Center
Web Approval Date:	10-02-2023

Delay of breast cancer (BC) surgery has become increasingly common over the past decades in the U.S. A group of investigators, including us, have raised concerns about the increased risk of disease progression and mortality for early-stage BC patients who wait for surgery over 60 days after diagnosis. Accordingly, the American College of Surgeons Commission on Cancer introduced a new recommendation for therapeutic surgery within 60 days of diagnostic biopsy, effective in 2023.

A question remains, however, as to what causes such rapid disease progression that can negatively impact survival among generally good prognostic early-stage breast cancer.

Currently, no biological mechanisms explain the risk of rapid increase in mortality after diagnostic biopsy. Our funded Breakthrough Award developed a radically novel, paradigm-shifting concept that needle biopsy provokes progressive prometastatic changes that lead to systemic dissemination of breast cancer. Successful completion of the project provided coherent evidence to support this novel concept: (1) biopsy of tumor leaves an unhealed wound dominated by prometastatic M2 macrophages that concomitantly promote the phenotypic conversion of BC cells, angiogenesis, and metastasis; (2) sustained elevation of cyclooxygenase-2 (COX-2) cascade in the wound stroma, accompanied by low oxygen hypoxia tension, favor M2 macrophage dominance; and (3) oral administration of COX-2 inhibitors alleviate biopsy-induced metastasis. In addition, our epidemiologic analysis showed the emergence of a conspicuous increase of mortality risk at 53 days after needle biopsy that followed by an exponential rise, growing from 6% to 30% higher risk each subsequent week, which translates to approximately 5-, 24-, and 54-times higher mortality rates over a 14-year period for patients with biopsy-to-surgery intervals of 60, 90, and 120 days, respectively, compared to those who had surgery within 30 days of diagnosis. Our funded project concept is contrary to the current clinical dogma and altered the prevailing view of (1) the absolute safety of needle biopsy, and (2) the contraindication of nonsteroidal anti-inflammatory drugs (NSAIDs) after biopsy.

How has the mortality risk associated with needle biopsy been overlooked this long? Early studies conducted in the 1960s to 1980s granted the safety of needle biopsy, although the average biopsy-to-surgery interval used in those studies was 7 to 30 days, well within the risk-free first 53 days in our study. Therefore, such risk was previously undetected. However, soaring treatment delays beyond the "safety window" in the modern U.S. clinical setting has uncovered increased mortality risk associated with surgery delay after biopsy. Thus, the new recommendation for timely surgery undoubtedly alleviates the mortality risk posed by surgery delay; however, significant challenges remain since many delayed cases are involuntary. Our cohort analysis showed disproportionate surgery delay in racial minorities, socioeconomically disadvantaged women, and those needing additional medical procedures. For example, first-time enrollment in Medicaid for qualifying, newly diagnosed women in Oklahoma typically takes up 7 to 8 weeks before surgery can be approved (depending on the state of residence), and the average wait time for surgery in one well-regarded cancer center is 11 weeks since the vast majority of their patients have sought second opinions there. With

the diverse causes of surgery delay, the recommendation of a timeline does not simply solve the issue; and there remains a current pressing need to implement a safe, affordable prevention strategy for "post-biopsy care" to mitigate negative effects in patients for whom delay may be unavoidable.

Leveraging the preclinical data obtained in the Breakthrough Award, the Expansion Award seeks to advance the clinical relevance of this problem. Using 100 surgically resected clinical BC cases and their matched biopsy samples, we aim to validate whether the abundance of M2 macrophages is correlated with the emergence of an invasive phenotype. We have identified 50 cases each for early or delayed surgery to capture the spatial and temporal difference in prometastatic changes after biopsy (Aim 1). We will also perform a cohort study using the Surveillance, Epidemiology, and End Results (SEER)-Medicare database to evaluate whether the mortality increase posed by surgery delay is attenuated among patients who use NSAIDs (Aim 2). Additional evidence from the proposed clinical case analyses and cohort study will corroborate our preclinical findings to support the rapid prometastatic progression of breast tumors after biopsy. The success of the Expansion Award holds potential for direct clinical impact by providing evidence of the prometastatic nature of needle biopsy and mitigation of disease progression after biopsy through NSAIDs.

Needle biopsy is indispensable for accurate diagnosis; our data support its safety as long as the surgery is performed promptly. Timely surgery, in parallel with "post-biopsy care," holds the direct potential to improve survival for women with early-stage BC. Therefore, this project will address the overarching challenge in the fiscal year 2022 Breast Cancer Research Program to eliminate mortality associated with metastatic BC and identify what drives breast cancer growth and determine how to stop it.

Proposal Title:	Chemotherapy Dosing in Patients with Obesity: Are Oncologists Optimally Balancing Risks and Benefits to Avoid Overtreatment and Undertreatment?
Log Number:	BC220896
Current PI Name:	Mary Schroeder
Award Number:	HT9425-23-1-0653
Current Contracting Organization:	Iowa, University of
Current Performing Organization:	Iowa, University of
Web Approval Date:	10-02-2023

Almost 300,000 new cases of invasive breast cancer will be diagnosed this year, and military active-duty females have up to 40% higher risk of breast cancer than the general population. The vast majority of these are considered curable with a combination of surgery, radiation, chemotherapy, and/or targeted therapy. However, balancing the benefit and harms of chemotherapy has always been challenging. There is currently no perfect way to predict which patients will benefit from chemotherapy, and the toxicities associated with the treatment can be severe, permanent, and even life-threatening.

Unfortunately, maximally tolerated chemotherapy doses are required for optimal survival. In real-world practice, 13% to 20% of patients are hospitalized, 23% to 55% require dose reductions, and up to 67% report experiencing moderate-to-severe toxicity, all as a result of their chemotherapy treatment. These negative outcomes are even more pronounced for patients with obesity, who are more likely to experience treatment-induced toxicity as well as breast cancer recurrence and death. The optimal chemotherapy dose that balances better survival with tolerable toxicity is not clear for patients with obesity, as guidelines are based on randomized clinical trials that enrolled younger healthier patients and lack head-to-head comparisons of the chemotherapy regimen recommended and used in clinical practice today. This uncertainty in the efficacy and safety of chemotherapy for patients with obesity may result in overtreatment and/or unnecessary toxicity. Over 42% of adults are now obese, defined by body mass index (BMI) >30, and almost 10% have BMI >40.

There is an urgent need to evaluate the use of chemotherapy and its outcomes in current practice for patients with and without obesity. We propose a population-based, observational study of chemotherapy use in a large sample of real-world breast cancer patients (minimum estimated study cohort of 18,000), using a centralized multimodal database of interoperable electronic health records (EHRs) from 13 large health care systems with a catchment population of over 34 million. Our research team is uniquely positioned and qualified to address the overarching challenges of overtreatment and toxicity with chemotherapy.

In our first Aim, we will identify the study cohort and characterize the various combinations and doses of chemotherapy ("regimen"), resulting in the most up-to-date estimates of the use of chemotherapy in current, real-world practice for a large, population-based sample of breast cancer patients. We will also estimate the extent of non-guideline chemotherapy use for patients with and without obesity. In Aim 2, we will assess the clinical appropriateness of the chemotherapy treatment choice and identify whether deviations from guidelines are explained by obesity itself, treatment-induced toxicity, comorbidity, or lack of supportive care medications. Finally in Aim 3, we will assess the results of these chemotherapy choices and the impact of obesity, toxicity, and dose reductions on survival. We will also develop a framework for overtreatment and undertreatment in chemotherapy to assess the full balance of treatment benefits and harms experienced by patients with and without obesity in current practice.

Upon successful completion of our aims, we will have identified which combinations of chemotherapy (and what doses) result in excess toxicity or worse outcomes in patients with and without obesity. We will also identify any unmet needs with regards to the use of supportive care medications that help manage symptoms of nausea and vomiting, as well as protect against damage to blood cells. These and other findings will be immediately applicable and help inform treatment decision-making in real-world clinical practice and achieve outcomes important to patients.

This proposed project could also lead to a major breakthrough in the use of chemotherapy to cure breast cancer. Although the first randomized clinical trials were initiated over 60 years ago, the specific chemotherapy regimens (combinations and doses) listed in clinical guidelines have remained virtually unchanged in the last 15 years. Evidence from this project could inform future randomized clinical trials aimed at optimizing the effect and safety of chemotherapy for patients with and without obesity.

Proposal Title:	Chemotherapy Dosing in Patients with Obesity: Are Oncologists Optimally Balancing Risks and Benefits to Avoid Overtreatment and Undertreatment?
Log Number:	BC220896P1
Current PI Name:	Joan Neuner
Award Number:	HT9425-23-1-0654
Current Contracting Organization:	Wisconsin, Medical College of
Current Performing Organization:	Wisconsin, Medical College of
Web Approval Date:	10-02-2023

Almost 300,000 new cases of invasive breast cancer will be diagnosed this year, and military active-duty females have up to 40% higher risk of breast cancer than the general population. The vast majority of these are considered curable with a combination of surgery, radiation, chemotherapy, and/or targeted therapy. However, balancing the benefit and harms of chemotherapy has always been challenging. There is currently no perfect way to predict which patients will benefit from chemotherapy, and the toxicities associated with the treatment can be severe, permanent, and even life-threatening.

Unfortunately, maximally tolerated chemotherapy doses are required for optimal survival. In real-world practice, 13% to 20% of patients are hospitalized, 23% to 55% require dose reductions, and up to 67% report experiencing moderate-to-severe toxicity, all as a result of their chemotherapy treatment. These negative outcomes are even more pronounced for patients with obesity, who are more likely to experience treatment-induced toxicity as well as breast cancer recurrence and death. The optimal chemotherapy dose that balances better survival with tolerable toxicity is not clear for patients with obesity, as guidelines are based on randomized clinical trials that enrolled younger healthier patients and lack head-to-head comparisons of the chemotherapy regimen recommended and used in clinical practice today. This uncertainty in the efficacy and safety of chemotherapy for patients with obesity may result in overtreatment and/or unnecessary toxicity. Over 42% of adults are now obese, defined by body mass index (BMI) >30, and almost 10% have BMI >40.

There is an urgent need to evaluate the use of chemotherapy and its outcomes in current practice for patients with and without obesity. We propose a population-based, observational study of chemotherapy use in a large sample of real-world breast cancer patients (minimum estimated study cohort of 18,000), using a centralized multimodal database of interoperable electronic health records (EHRs) from 13 large health care systems with a catchment population of over 34 million. Our research team is uniquely positioned and qualified to address the overarching challenges of overtreatment and toxicity with chemotherapy.

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Proposal Title:	Precision Glycocalyx Editing of Metastatic Bone Microenvironment
Log Number:	BC220912
Current PI Name:	Xiang Zhang
Award Number:	HT9425-23-1-0493
Current Contracting Organization:	Baylor College of Medicine
Current Performing Organization:	Baylor College of Medicine
Web Approval Date:	10-01-2023

This proposal will address the following overarching challenges: (1) Revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival; (2) Eliminate the mortality associated with metastatic breast cancer; (3) Identify what drives breast cancer growth; determine how to stop it; and (4) Identify why some breast cancers become metastatic.

Twenty to forty percent of breast cancer (BCa) survivors will eventually suffer metastases to distant organs, sometimes years after initial treatment. We will focus on metastatic breast cancers (mBCa) in bone, which are involved in about 70% of all metastatic diseases. In more than two-thirds of cases, metastases will not remain only in bones, but rather spread to other organs and ultimately result in the mortality of patients. Recent analyses elucidated that bone is not the final destination of dissemination but may serve as a launch pad of further metastasis. Thus, developing treatments that target bone minimal residue disease is key not only to the cure of metastasis therein, but also to the prevention of multi-organ secondary metastases.

Recently, targeted antibody therapy and immunotherapy have emerged as new avenues for mBCa therapy, but their efficacies on bone metastases are disappointing. For example, trastuzumab (Herceptin) and pertuzumab (Perjeta), antibodies targeting human epidermal growth factor receptor 2 (HER2), have been used to treat patients under adjuvant and metastatic conditions. Although some mBCa patients benefit from these treatments, a high number of mBCa patients with bone metastasis progress within 1 year of starting treatment and few experience prolonged remission. Besides antibody-based therapy, chimeric antigen receptor (CAR)-engineered immune cell therapy that endows patient's immune cells with reactivity for tumor cell surface molecules is emerging as an effective and innovative treatment for BCa. However, homing of therapeutic T cells to the disease site also remains a key limiting factor, especially for tumor cells in their bone niche. Thus, therapies with improved outcomes for BCa patients with bone metastases are highly desired. Previous collaboration between the Zhang lab (Baylor College of Medicine) and the Xiao lab (Rice University) has effectively overcome the inaccessibility issue of bone metastasis. We invented a bone-targeting approach to enable enrichment and retention of antibodies and peptides in the bone microenvironment. In this project, we will apply this approach to a novel target as elaborated below.

We will study how a "glyco-immune checkpoint" in the bone metastatic niche may drive bone metastasis progression, and therefore, provide a therapeutic target. This "glyco-immune checkpoint" is called Siglec-15. As PD1 and CTLA-4, Siglec-15 is expressed in cancer cells and some immune cells. When binding certain "sugar molecules" on cell surface, Siglec-15 suppresses antitumor immunity, hence the term "glycol (Greek prefix of "sugar")-immune checkpoint" (GIC). Besides its immune functions, Siglec-15 is enriched in bone and also involves in resorption of bone, which is a hallmark of breast cancer bone metastasis. Thus, targeting Siglec-15 may inhibit bone metastasis with multiple mechanisms.

Three aims are proposed in this collaborative project. In Aim 1, we will further evaluate the therapeutic efficacy of GIC inhibitors for the treatment of BCa bone metastasis. Aim 2 will focus on the molecular mechanisms underlying the regulatory roles of Siglec-15 in the metastatic bone microenvironment. In Aim 3, the combinations of the bone-targeting GIC inhibitors and other immunotherapies will be further evaluated

for the treatment of BCa bone metastasis. The resulting regiments from this study will have an enhanced efficacy for preventing and curing bone mBCa and further multi-organ metastases seeded from bone lesions. Thus, the fulfillment of the proposed research will reduce the mortality of BCa.

Taken together, this project will, for the first time, reveal how GIC blockade in bone can affect bone metastasis and subsequent multi-organ metastases. Our goal is to provide a rationale and establish feasibility for phase 1 clinical trials. Positive outcome of this project will immediately benefit at least 9% of BCa patients carrying bone-only metastases. This benefit may extend to mBCa patients with other site metastases since secondary metastases from bone lesions are prevalent. For more than two-thirds of metastatic patients, bone metastases will further metastasize to other organs and eventually progress to death of patients. In summary, this study is well aligned with the mission of the Breast Cancer Research Program to apply innovative, creative, collaborative, and high-impact approaches to end breast cancer.

Proposal Title:	Precision Glycocalyx Editing of Metastatic Bone Microenvironment
Log Number:	BC220912P1
Current PI Name:	Han Xiao
Award Number:	HT9425-23-1-0494
Current Contracting Organization:	Rice University
Current Performing Organization:	Rice University
Web Approval Date:	10-01-2023

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Proposal Title:	Early Assessment of Treatment Response in Patients Undergoing Neoadjuvant Therapy Using an Automated Liquid Biopsy-Breast Cancer Methylation (LBx-BCM) Assay
Log Number:	BC220929
Current PI Name:	Saraswati Sukumar
Award Number:	HT9425-23-1-0425
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	09-06-2023

Rationale: Women with localized or large breast tumors (cancer limited to the breast and/or lymph nodes) are often treated with a relatively new regimen called neoadjuvant therapy (NAT). NAT has a major advantage in that therapy before surgical resection (a) allows large tumors to shrink so that extent of surgery is limited, and (b) allows pathologic examination of the smaller tumor/tumor bed, enabling an objective assessment of how effective the neoadjuvant was on the tumor. Those who achieve a pathologic complete response (pCR) do better in the long run -- they have longer recurrence-free survival and longer overall survival. Currently, depending on the subtype, many women have an incomplete pathologic response and must go through additional therapy. So why do oncologists not continue treatment with the same or some other agent until the patient's tumor shrinks completely? This is because they have no way of telling if the tumor is shrinking or growing. Many groups are trying hard to develop "intermediate response markers" to address this unmet need. Testing blood for mutated DNA has been researched extensively, but gives sensitivity of detection of

Methylated genes in breast cancer, on the other hand, are common, and we have reported that the pattern of methylation is retained from the primary tumor to distant metastasis, like a barcode for that tumor. Detecting tumor-specific methylated DNA in blood has required the development of techniques in our lab that can detect less than 50 copies in 300 µl of cancer plasma. In collaboration with the diagnostic company, Cepheid, we have converted this lab assay into an automated

Objectives: Our objective is to provide a sensitive and specific molecular blood test to the oncologist to assess response of the patient to the therapy at intervals during the neoadjuvant regimen. Our assay worked well in detecting and monitoring tumor load in Stage 4 metastatic breast cancer. But we know that the amount of DNA shed decreases with decreasing stage. Usually, the smaller the tumor, the less circulating DNA is shed by the tumor. So, our first objective is to increase the sensitivity of the cartridge test. We will test new ways to optimize DNA retrieval from plasma and test a newly developed 10-fluorophore detection cartridge, which will reduce cost and maximize use of the DNA (year 1). Our second objective is to test the performance of the optimized test. We will determine if methylation levels in blood measured using the LBx-BCM assay at two weekly intervals during NAT correlate with pathologic response in the surgically resected lesion. We will select one of these time points which best correlates with pathologic response for future work (years 2-3).

Ultimate Applicability: The target population for the application of the test developed in this proposal is women and men with Stage 3 cancer who will undergo NAT. Currently, there are no blood biomarkers to assess whether a patient is or is not responding to therapy, or whether an alternate therapy may be better. The endpoint is what the tumor looks like at the end of the treatment, upon surgical resection. It is critical to have

a complete pathologic response, since long-term outcome is dependent on it. Subsequent treatment is given to patients who have incomplete or partial pathologic response, but there is no way of predicting the outcome. A reliable blood test can act as a surrogate for tumor load. Methylated DNA has a short half-life, and is stable, so repeated measures at short intervals can be made with confidence. The LBx-BCM cartridge assay can be performed by people with little expertise, and has few hands-on steps, so it can be implemented in any pathology laboratory. Optimizing the sensitivity of the assay, which is already very specific, will help not only patients with Stage 3, but eventually women with Stage 2 and Stage 1 breast cancer.

Overarching Challenge: Quantitative measurement of circulating methylated DNA that would reflect tumor burden in real time is essential, and the use of LBx-BCM assay for this purpose is promising. It is time to address the overarching challenge of "Developing reliable, quantitative, specific, and sensitive liquid biopsy biomarkers to continue effective therapies or to discontinue ineffective ones in a timely manner."

Proposal Title:	Developing Combined Therapies with Hemichannel-Targeting Antibodies and FDA-Approved Therapies in Treating Breast Cancer Bone Metastasis
Log Number:	BC220939
Current PI Name:	Jean Jiang
Award Number:	HT9425-23-1-0843
Current Contracting Organization:	Texas, University of, Health Science Center at San Antonio
Current Performing Organization:	Texas, University of, Health Science Center at San Antonio
Web Approval Date:	10-02-2023

Breast cancer is the second leading cause of cancer death for women in the U.S. Cancer that spreads to bones, bone metastases, are the most frequent complications, occurring in up to 80% of patients with advanced breast cancers. Bone metastases, like other metastatic diseases, typically make the disease incurable. Almost all patients with bone metastases suffer from various complications varying from severe bone pain and pathological fractures to general physical disability, which rapidly deteriorate the quality of life. Treatment options are very limited. These include chemotherapy combined with the administration of bisphosphonates (zoledronate [Zometa®] and pamidronate [Aredia®]) and an anti-RANKL antibody (Denosumab®). These therapies reduce bone metastasis-associated symptoms. However, none of the current therapies cures skeletal breast cancer metastasis. Patients' responses to the treatments vary. Some patients have adverse effects or develop drug resistance. New PD-1 targeted immunotherapies (such as Keytruda®, pembrolizumab), although showing efficacies in triple-negative breast cancer types, have limited success in treating breast cancer bone metastasis. Therefore, there is an urgent medical need for a new specific treatment option with improved therapeutic effectiveness to help patients with this deadly disease.

Studies in our research team show that opening of a type of membrane channels, called connexin hemichannels, in bone cells can effectively defend bone tissues against breast cancer cell invasion. Development of drugs that can maximize this potential by activating hemichannels, represents a new therapy for treatment of breast cancer bone metastases. With the support from the parental Breast Cancer Research Program (BCRP) grant, we have developed an antibody drug that targets hemichannels and suppresses bone metastasis of various subtypes of breast cancer. Through the collaboration with our commercial partner, we obtained a regulatory approval for Investigational New Drug (IND) and a clinical trial was initiated and is currently ongoing, and the results generated thus far from the clinical trial show that this antibody drug candidate is safe for human testing. In the current proposed research, we plan to expand the drug efficacy with combined therapy approaches by using this antibody drug along with the other two U.S. Food and Drug Administration (FDA)-approved therapies for the treatment of bone metastasis of breast cancer.

The major objective of this drug development proposal is to optimize the treatment regimens in treating breast cancer bone metastasis by assessing efficacies of the combined therapies. Two overarching challenges will be addressed: (1) Revolutionizing treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival; and (2) Elimination of the mortality associated with metastatic breast cancer. In this study, we propose two specific aims. First, we will determine the efficacy of combined treatment of this antibody drug with zoledronate in various breast cancer subtypes at various combined treatment regimens. Second, we will assess the efficacy of combined immunotherapy treatment regimens with this antibody and PD-1 antibody, and the drug responses of cancer immunity. Results from these two aims will provide guidance for clinical trials and treatment alternatives.

The outcome from this drug-development proposal will generate an entirely new class of antibody therapies that target novel mechanisms with high efficacy and low toxicity. Approximately 170,000 women in the U. S. currently live with breast cancer metastasis and about 50,000 die from this disease each year, including active-duty U.S. Military personnel, retirees, Veterans, and their family members. The drug developed in this proposal will offer enormous benefits to these patients with the reduction of breast cancer bone metastasis-associated symptoms and improvement of survival rate.

The estimated time for the proposed project is 3 years. Within the 3 years, we will provide research data on the efficacies of combined therapies of this antibody drug with zoledronate and PD-1 antibody in treating bone metastasis of various subtypes of breast cancer. In the late part of year 3, we will prepare an IND package for combined therapies and subsequent clinical trials.

Proposal Title:	Epigenome Editing for Targeted Reactivation of Tumoral FOXP3 to Treat Breast Cancers
Log Number:	BC221000
Current PI Name:	Lizhong Wang
Award Number:	HT9425-23-1-0696
Current Contracting Organization:	Alabama, University of, at Birmingham
Current Performing Organization:	Alabama, University of, at Birmingham
Web Approval Date:	10-02-2023

Metastatic breast cancer (mBCa), also known as stage IV or advanced breast cancer, is evident when BCa has spread to other organs in the body, including the lungs, bones, liver, and/or the brain. The 5-year survival rate for women with mBCa is 27%, causing the majority of deaths from BCa. Currently, there is no cure for patients with mBCa. Recently, we found that the FOXP3 gene has dual roles in tumor cells and immune cells; and is involved in tumor metastasis and in BCa patient survival. In BCa cells, FOXP3 stops the spread of cancer, whereas in immune cells, FOXP3 is a promoter of metastasis. In our original research project, fiscal year 2016 Breakthrough Award Level 1, we demonstrated that activation of FOXP3 in BCa cells inhibits their growth and metastasis. In the proposed study, we will continue to elucidate the effects of FOXP3-mediated metastatic mechanisms as well as new targeted FOXP3 gene therapy for eliminating the mortality associated with mBCa.

Our preliminary analysis of more than 500 human BCa samples revealed that the combination of FOXP3-negative tumor cells and high numbers of FOXP3-positive immune cells is associated with the worst survival and in tumor metastasis. Conversely, patients with FOXP3-positive tumor cells and low numbers of FOXP3-positive immune cells show no evidence of distant metastases and no cancer-related deaths. However, the underlying mechanism remains poorly understood. Here, we hypothesize that FOXP3 is a key regulator in tumor-immune interactions for tumor metastasis and that targeting reactivation of FOXP3 in BCa cells can inhibit tumor metastasis.

First, we will investigate, with cell models and animal models, the mechanisms of FOXP3-mediated tumor metastasis. Next, we will use newly developed tumor-specific technologies to target the FOXP3 gene for its reactivation in tumor cells and eliminate tumor metastasis.

In future clinical applications, our identified mechanisms will not only help to understand how lethal mBCa originates and develops, but also provide the potential to yield new gene therapy for eliminating the mortality associated with mBCa.

We will address the following challenges: identify why some breast cancers become metastatic; revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival; and eliminate the mortality associated with metastatic breast cancer.

What types of patients will it help and how will it help them? Since the lack of FOXP3 is associated with a higher propensity for triple-negative BCa (TNBC), we will focus on TNBC. Our identified mechanism will help TNBC patients with early diagnosis, prediction of tumor metastasis, and recurrence after surgery. If metastasis and recurrence are predicted after surgery, appropriate adjuvant treatment (e.g., chemotherapy and immunotherapy) will reduce patient deaths from metastasis and recurrence. On the other hand, patients with negative biomarkers can forego any unnecessary adjuvant treatment, and overtreatment can be prevented. Also, our developed FOXP3 gene therapy has potential to prevent or eliminate tumor metastasis for BCa patients with a high risk of developing metastases.

What are the potential clinical applications, benefits, and risks? Our identified mechanism will assist clinicians in early detection of aggressive BCa (with FOXP3 defect), in early prediction of tumor recurrence, and will benefit patients for appropriate selection of adjuvant treatments after surgery. If a low risk of metastasis and recurrence is predicted, patients will not need any adjuvant therapy and will avoid overtreatment. Also, our FOXP3 gene therapy will benefit BCa patients by reducing metastasis. We will develop a tumor-specific targeted gene therapy, leading to limited side effects.

What is the projected time it may take to achieve a patient-related outcome? It will likely take 2 to 3 years for clinical application of our developed gene therapy.

What is the likely impact of this study on the Breast Cancer Research Program's (BCRP's) mission of ending breast cancer? This research will provide more chances for early and appropriate adjuvant treatments after surgery to reduce death from metastasis and recurrence, which relates to the BCRP's mission of ending breast cancer.

Proposal Title:	Dual CD24/CD276 Targeting Therapies for Triple-Negative Breast Cancer
Log Number:	BC221012
Current PI Name:	Lizhong Wang
Award Number:	HT9425-23-1-0647
Current Contracting Organization:	Alabama, University of, at Birmingham
Current Performing Organization:	Alabama, University of, at Birmingham
Web Approval Date:	10-02-2023

Breast cancer affects about one in eight women worldwide, and targeted therapies to three common receptors (ER, PR, and HER2) on breast cancer cells have decreased the death rate by about 40% over the last 25 years. Unfortunately, these receptors are not expressed in "triple-negative" breast cancers (TNBC, ER-/PR-/HER2-), which affects 10% to 20% of diagnosed patients. The standard chemotherapies (e.g., doxorubicin, paclitaxel, and gemcitabine) available for patients with TNBC have poor clinical benefit, severe adverse effects, and the challenge of recurrence due to drug resistance. Recently, therapies that combine specific anti-cancer antibodies with toxic drugs have been developed. These therapies, called antibody-drug conjugates (ADCs), can deliver toxic drugs specifically to the tumor cells and not to healthy cells, maximizing antitumor effects while minimizing adverse effects. However, ADCs that eliminate TNBC cells have not yet been developed.

In our preliminary studies, we first identified targetable cell surface receptors in TNBC cells (CD24 and CD276). We also developed tumor-specific antibodies and identified toxic drugs that kill TNBC cells. We then developed ADCs that bear cytotoxic drugs and target TNBCs, and we validated them for in vivo safety and in vitro anti-TNBC efficacy. Our objective-central hypothesis is that the specific targeting and killing of primary TNBC cells, achieved by administering our potent ADC-based therapy after surgery, can effectively eliminate TNBC and prevent its metastasis and recurrence.

We will rigorously evaluate our ADCs to treat TNBC. For our ADCs that target CD24, we will identify the most efficient strategy by use of cell lines and mouse models. Next, we will evaluate the capacity of combined CD24/276 ADCs to eliminate TNBC and prevent tumor recurrence, and tumor spreading in mouse models. Finally, we will use preclinical animal models, including patient-derived xenograft models, to test the clinical applications of these ADCs by administering them in combination to treat metastatic cells after surgical removal of the primary TNBC tumor.

A challenge in TNBC therapy is to prevent tumor recurrence while minimizing adverse effects. Our ADC combination therapy can deliver highly toxic drugs into TNBC cells, limit adverse effects, reduce drug resistance, enhance antitumor immunity, prevent recurrence and spreading, and improve the quality of life and the survival of patients who suffer from TNBC. Thus, our proposed work will provide a breakthrough for new TNBC therapeutic approaches by addressing two fiscal year 2022 (FY22) Depart of Defense (DOD) Breast Cancer Research Program (BCRP) overarching challenges: "Revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival," and "Eliminate the mortality associated with metastatic breast cancer."

What types of patients will it help and how will it help them? This research will help to eliminate tumor recurrence and metastasis for patients with CD24+ and/or CD276+ TNBC.

What are the potential clinical applications, benefits, and risks? This study will develop targeted clinical therapy (after surgery) that benefits TNBC patients with fewer toxic effects.

What is the projected time it may take to achieve a patient-related outcome? When effective therapies are developed in the proposed study, we will immediately assess and validate these therapies through a clinical trial, which will take 3 to 5 years for the clinical application of our findings.

What is the likely impact of this study on the BCRP's mission of ending breast cancer? Our developed therapy, which is more specific, effective, and less toxic, will prevent TNBC recurrence and metastasis, and thereby contribute to the BCRP's mission of ending breast cancer.

Proposal Title:	Dual CD24/CD276 Targeting Therapies for Triple-Negative Breast Cancer
Log Number:	BC221012P1
Current PI Name:	Runhua Liu
Award Number:	HT9425-23-1-0648
Current Contracting Organization:	Alabama, University of, at Birmingham
Current Performing Organization:	Alabama, University of, at Birmingham
Web Approval Date:	10-02-2023

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What types of patients will it help and how will it help them? This research will help to eliminate tumor recurrence and metastasis for patients with CD24+ and/or CD276+ TNBC.

What are the potential clinical applications, benefits, and risks? This study will develop targeted clinical therapy (after surgery) that benefits TNBC patients with fewer toxic effects.

What is the projected time it may take to achieve a patient-related outcome? When effective therapies are developed in the proposed study, we will immediately assess and validate these therapies through a clinical trial, which will take 3 to 5 years for the clinical application of our findings.

What is the likely impact of this study on the BCRP's mission of ending breast cancer? Our developed therapy, which is more specific, effective, and less toxic, will prevent TNBC recurrence and metastasis, and thereby contribute to the BCRP's mission of ending breast cancer.

Proposal Title:	Pretargeting Bacterial Therapy of HER2+ Breast Cancer
Log Number:	BC221019
Current PI Name:	Dmitri Artemov
Award Number:	HT9425-23-1-0467
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	05-18-2023

The major goal of the project is development of specifically targeted therapy for HER2-positive breast cancer using functionalized bacteria, as therapeutic agent. Bacterial therapy is a long-standing concept in cancer management that has been originally proposed in the late 19th century, and currently there is a renewed interest with novel advancements. Bacteria-mediated tumor therapy is orthogonal to standard anticancer therapies and therefore can overcome some of their limitations, such as limited efficacy, development of drug resistance, as well as severe side effects. The major premise of bacterial cancer therapy is that the systemically injected bacteria can home to the tumor, penetrate, invade, colonize, and eventually destroy the tumor. In this project, we intend to develop a novel strategy of bacterial therapy for HER2-positive breast cancer that is based on (i) specific targeting of bacteria to the tumor by pretargeting of the HER2 receptors expressed on cancer cells; (ii) non-pathogenic form of E. coli bacteria will be engineered to produce a specific enzyme bacterial cytosine deaminase that can convert a non-toxic compound 5-fluorocytosine to a standard cytotoxic chemotherapeutic drug 5-fluorouracil (5-FU); and (iii) bacterial colonization of the target tumor will be monitored in vivo by noninvasive PET imaging. Real-time imaging will allow precise evaluation of the tumor bacterial load, as well as immediate termination if an uncontrolled proliferation of bacteria in non-tumor body locations is detected. This proof-of-concept study will be performed in preclinical mouse models of breast cancer.

By exploring a new strategy for pretargeting bacterial therapy, the project has high relevance to developing novel forms of breast cancer therapy and is specifically responsive to Breast Cancer Research Program (BCRP) overarching challenges: “revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival” and “eliminate mortality associated with metastatic breast cancer.”

Breast cancer is the most commonly detected non-dermatologic cancer in women in the United States with an estimated 290,000 new cases diagnosed per year and approximately 43,780 deaths. HER2-positive breast cancer has an aggressive clinical phenotype with generally unfavorable prognosis. While several first- and second-line targeted therapies including novel forms of immunotherapy exist for this cancer type exist, the cancer ultimately becomes resistant. Eventually, the stage IV metastatic breast cancer is incurable and fatal. Therefore, new, efficient, and less toxic treatment strategies are urgently needed to improve outcomes in patients with HER2-positive breast cancer. We envision that future clinical applications of bacterial cancer therapy will be initially tested and will show efficacy in patients who have already been treated with multiple therapies and likely have partly suppressed immune system and drug refractory HER2-positive breast cancer. We anticipate that these clinically translatable technologies, membrane surface modified pretargeted bacteria, and complementary bacterial PET imaging could be used to assess colonization and safety of microbial-based therapy in clinical trials as well as a precision medicine approach for patient care.

The current proposal is focused on the development and proof of principle of the pretargeting bacterial therapeutic strategy in preclinical models. If successful, future clinical translation would require additional validation and toxicity evaluation in small and large animals, for which we would seek additional funding. We estimate the projected time to achieve patient-related outcomes is within 5 to 10 years.

BCRP’s mission to end breast cancer requires supported development of novel therapies that can be applied to the most dangerous metastatic cancers that escape local control and for which there is no current effective treatment options. Unfortunately for aggressive disease, even if treated to remission, the risk of distant recurrence is high (~10% over 15 years) even in women with small, low-grade cancers and no nodal involvement at presentation. Therefore, early detection technology and improved surgical procedures need to be accompanied by advanced therapeutic strategies. Our project is aimed at the development of a novel HER2-pretargeting bacterial therapy that can be an important step in the development of a breakthrough treatment to accelerate progress toward the BCRP’s mission of ending breast cancer.

Proposal Title:	Pretargeting Bacterial Therapy of HER2+ Breast Cancer
Log Number:	BC221019P1
Current PI Name:	Sanjay Jain
Award Number:	HT9425-23-1-0468
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	05-18-2023

The major goal of the project is development of specifically targeted therapy for HER2-positive breast cancer using functionalized bacteria, as therapeutic agent. Bacterial therapy is a long-standing concept in cancer management that has been originally proposed in the late 19th century, and currently there is a renewed interest with novel advancements. Bacteria-mediated tumor therapy is orthogonal to standard anticancer therapies and therefore can overcome some of their limitations, such as limited efficacy, development of drug resistance, as well as severe side effects. The major premise of bacterial cancer therapy is that the systemically injected bacteria can home to the tumor, penetrate, invade, colonize, and eventually destroy the tumor. In this project, we intend to develop a novel strategy of bacterial therapy for HER2-positive breast cancer that is based on (i) specific targeting of bacteria to the tumor by pretargeting of the HER2 receptors expressed on cancer cells; (ii) non-pathogenic form of E. coli bacteria will be engineered to produce a specific enzyme bacterial cytosine deaminase that can convert a non-toxic compound 5-fluorocytosine to a standard cytotoxic chemotherapeutic drug 5-fluorouracil (5-FU); and (iii) bacterial colonization of the target tumor will be monitored in vivo by noninvasive PET imaging. Real-time imaging will allow precise evaluation of the tumor bacterial load, as well as immediate termination if an uncontrolled proliferation of bacteria in non-tumor body locations is detected. This proof-of-concept study will be performed in preclinical mouse models of breast cancer.

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Proposal Title:	Targeting the PIM Kinase Pathway to Eliminate Breast Cancer Metastasis
Log Number:	BC221024
Current PI Name:	Andrei Goga
Award Number:	HT9425-23-1-0539
Current Contracting Organization:	California, University of, San Francisco
Current Performing Organization:	California, University of, San Francisco
Web Approval Date:	09-06-2023

Breast cancers that lack expression of the HER2, estrogen, and progesterone receptors, so called "triple-negative" breast cancers (TNBC), represent the tumor type with the poorest outcome and for which targeted agents are limited. Likewise, luminal B breast cancers represent a particularly aggressive subtype of ER+ tumors which have limited response to anti-hormone therapy. Both TNBC and luminal B are also associated with worse-response to existing therapies and increased risk of breast cancer metastasis. Our laboratory discovered that MYC is disproportionally expressed in the vast majority of triple-negative breast cancers and also in many luminal B tumors (Horiuchi, et al., Journal of Experimental Medicine, 2012). Likewise, increased MYC activity is associated with poor patient outcome across all subtypes of breast cancer (Horiuchi, et al., Journal of Experimental Medicine, 2012). Thus, MYC is a high-interest target for therapy of TNBCs, luminal B, and other aggressive breast cancers. Unfortunately, specific small-molecule inhibitors that directly target MYC are not available for clinical use. Furthermore, targeting MYC directly might be expected to cause substantial toxicity to rapidly-dividing normal cells, such as the intestinal cells, within a patient's body. Therefore, therapeutic strategies that target specific proteins that MYC-elevated tumors cannot live without might be an effective method to selectively kill the tumor cells.

As part of the Breakthrough Award, our laboratory identified that the kinase PIM1 can selectively kill breast cancer cells that have high expression of MYC (Horiuchi, et al., Nature Medicine, 2016). This is important because loss of the PIM family of kinases does not appear to be toxic when these genes are lost in mice, suggesting that normal cells would not be significantly affected by PIM kinase inhibition. In our Department of Defense-supported Breakthrough studies, we have discovered that PIM kinase inhibitors developed for clinical studies blocked cancer metastasis and improved immunotherapies in advanced preclinical models of breast cancer.

The Expansion Award proposal will build on these discoveries and address two overarching challenges: First, we seek to eliminate the mortality associated with metastatic breast cancer. Second, we intent to revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival.

We seek a better understanding of the biology for how PIM inhibition eliminates metastatic colonization of the lung and other tissues. We have identified candidate molecules that are diminished by PIM inhibition and that may be critical for eliminating disseminated tumor cells (DTCs) and metastatic colonization. Our discoveries from the parent Breakthrough Award have also revealed that PIM inhibitors can be effectively combined with immune therapies. Since PIM inhibitors are an oral drug, this suggests that improved immune therapies may be achieved via oral and perhaps safer medications.

As part of the proposal, a team of clinicians and physician-scientists, as well as experienced patient-advocates, will work together to test PIM inhibitors and move them forward to an early phase clinical trial. We have also received enthusiastic support of our ideas from pharma, who agree to provide novel drugs and look forward to collaborating on an investigator-initiated clinical trial of PIM inhibitors.

This work will have direct and immediate clinical impact by determining if PIM inhibitors, already in clinical development, can block metastasis of TNBCs and luminal B breast cancers that overexpress MYC. Moreover, the proposed studies will determine if immunotherapies can be made more effective and less toxic for patients. Finally, the proposed studies will inform, guide, and accelerate clinical translation of PIM inhibitors to the clinic.

The initiating PI, Dr. Andrei Goga, is a medical oncologist at the University of California, San Francisco (UCSF) Breast Oncology Program. In collaboration with Dr. Mehrdad Matloubian and other clinician-scientists at UCSF, we anticipate results from this proposal will rapidly inform clinical translation of PIM inhibitors to clinical trials for breast cancer patients. Indeed, a Phase I study of PIM inhibitors is already planned at UCSF with support from NewBay Pharma, and the proposed studies will help us characterize the mechanisms of action of PIM inhibitors and understand their roles in metastasis and immunotherapies.

Proposal Title:	Hedgehog Inhibition Disrupts the Immune Privileges of Breast Cancer
Log Number:	BC221039
Current PI Name:	Lalita Shevde
Award Number:	HT9425-23-1-0779
Current Contracting Organization:	Alabama, University of, at Birmingham
Current Performing Organization:	Alabama, University of, at Birmingham
Web Approval Date:	10-03-2023

Through studies proposed in this application, we will address the overarching challenge of eliminating the mortality associated with metastatic breast cancer.

Cancer cells interact with other cells in their surroundings and adapt to their environment. This niche contains abundant immune cells and is commonly referred to as the 'tumor immune microenvironment.' We expect that our immune system will eliminate the tumor. However, cancer cells take over the immune system and create a tumor-promoting environment.

This is even more obvious in triple-negative breast cancer (TNBC), which has a very potent tumor-promoting immune cell composition. This further exacerbates the complications of TNBC that does not have any markers for targeted treatment. While a combination of immunotherapy and chemotherapy seems to benefit a subgroup of early-stage TNBC patients, there are still a majority of patients for whom we need to do better by broadening the arsenal of chemotherapeutics. Informed by these challenges and the need for more effective treatment options for TNBC, my lab has a long-standing research program focused on dysregulated Hedgehog (Hh) signaling in TNBC.

What is the ultimate applicability of the research, what patient population would it benefit, and how will it impact the BCRP’s mission of ending breast cancer? What is the feasibility of translational potential? We discovered that the U.S. Food and Drug Administration (FDA)-credentialed, orally available pharmacological Hedgehog (Hh) inhibitor, Vismodegib, reduces tumor-promoting immune cells in the triple-negative tumor and increases the population of tumor-eradicating immune cells. In addition to discovering the molecular changes that orchestrate these immune alterations, we will test the possibility that this altered immune environment of the Vismodegib-treated mammary tumor will be more responsive to immune checkpoint therapy. We will test a neoadjuvant strategy with Vismodegib in combination with anti-PD1 treatment with the goal of impeding metastasis and improving outcomes to decrease the mortality associated with TNBC. Our investigations are carefully designed to simulate a newly diagnosed TNBC patient and as such, our studies are positioned to benefit the TNBC patient community. Our goals are attainable because anti-PD1 therapy and Vismodegib are FDA-approved, thereby allowing us an immediately translatable path to the breast cancer clinic.

What are the benefits and risks? What is the projected time it may take to achieve a patient-related outcome? Vismodegib and anti-PD-1 are FDA-credentialed with manageable side effects. As such, there seem to be no foreseeable risks or delays in achieving a patient-centric outcome. Thus, the outcomes will be transformative, fundamentally evolved, and have the potential to make a tangible impact.

Proposal Title:	Novel Tools to Improve Responses to DNA-Damaging Therapeutics in Metastatic Breast Cancer
Log Number:	BC221043
Current PI Name:	Daniel Higginson
Award Number:	HT9425-23-1-0533
Current Contracting Organization:	Sloan Kettering Institute for Cancer Research
Current Performing Organization:	Sloan Kettering Institute for Cancer Research
Web Approval Date:	09-06-2023

DNA-damaging chemotherapies and radiation are commonly used in cancer management, including breast cancer. These include drugs and radiation that cause DNA double strand breaks (DSBs) in both cancer cells and normal cells, and unfortunately these agents can cause substantial side effects due to their effects in normal cells. The doses of DNA-damaging agents are already maximized in terms of what patients can tolerate.

Another emerging class of treatment is called an antibody drug conjugate (ADC). These are antibodies that recognize antigens or proteins on the surface of cancer cells and in most cells these antigens are not present on the surface of non-cancer cells. After the ADC binds the cancer cell surface, it is internalized, and an attached (conjugated) drug is released into the cancer cell. These ADCs have demonstrated clinical efficacy in breast adenocarcinomas and non-small cell lung cancers. A total of 11 ADCs are now U.S. Food and Drug Administration-approved, including several in breast cancer. However, many conventionally used DNA-damaging agents in oncology are not suitable for use as ADC payloads because they lack the drug potency required.

In this proposal, we seek to further develop novel ADC molecules with small molecule inhibitors of the DNA-damage response (DDRi). We propose that in this format, taking advantage of the tumor-targeting nature of an ADC, a broad range of systemically administered DNA-damaging therapies that induce DSBs can be rendered more effective if the DDR is selectively inhibited in antigen-expressing cancer cells. In preliminary data, we have synthetized ADCs containing small molecule inhibitors of the ATM kinase and DNA-PK, protein enzymes highly involved in cellular responses to DNA damage. The ATM kinase is a sensor of DSBs, activating downstream processes that help the cell survive DNA damage. The DNA-PK enzyme participates in non-homologous end joining, the most important pathway for DNA repair of two-ended DSBs and likewise enable the cell to survive DNA-damaging agents. Using complementary assays, we demonstrated high potency of these DDRi ADCs in a range consistent with clinically successful ADCs.

In this proposal, we seek to create an additional category of ADC molecules with DDR inhibitor payloads and an antibody that recognizes the cell surface protein TROP2. The new therapeutics could help patients with both early and advanced breast cancer by improving the responses to these DNA-damaging agents. We believe this is a novel approach with potential to meet the overarching DOD challenge to "revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival."

In specific aim 1 (SA1), we will characterize the activity of the new ADCs in breast cancer cells with the TROP2 protein. In SA2, we will test combinations of the ADCs with DNA-damaging agents called doxorubicin, ionizing radiation, topoisomerase inhibitors, crosslinking agents, and PARP inhibitors. In SA3, we will test optimal combinations of the ADCs and DNA-damaging agents in mouse models of metastatic breast cancer. The overall goal of this proposal is to develop a novel means of improving the efficacy of DNA-damaging agents with the tumor-targeting nature of an ADC.

Proposal Title:	Therapeutic Targeting of Genomic Instability in Triple-Negative Breast Cancer
Log Number:	BC221065
Current PI Name:	Samuel Aparicio
Award Number:	HT9425-23-1-0820
Current Contracting Organization:	Provincial Health Services Authority
Current Performing Organization:	Provincial Health Services Authority
Web Approval Date:	10-03-2023

This proposal addresses two fiscal year 2022 (FY22) Breast Cancer Research Program (BCRP) Overarching Challenges: (1) Revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival; and (2) Eliminate the mortality associated with metastatic triple-negative breast cancer (TNBC).

TNBC is an aggressive form of the disease, which is associated with a high risk of relapse and short survival in the metastatic setting. Currently, it has fewer treatment options than other forms of breast cancer. Our objective is to lower mortality and improve treatment for patients affected by TNBC by developing biological and computational methods that will identify treatment strategies to overcome the cancer evolution that is responsible for the development of drug resistance and the failure of existing treatments.

Cancers are adaptable systems constantly changing in response to evolutionary pressure. These processes occur differently across the individual cells of a single tumor. Genome sequencing methods to evaluate patient tumors typically assess the tumor as a single blended genome, obscuring information about important processes occurring in single cells. This proposal builds on work from our groups and our collaborators over several years, which has laid a strong foundation of sophisticated single-cell genome sequencing and computational analysis, along with laboratory models of breast cancer which mimic the behavior of clinical disease one to 15. Altogether, these tools enable the study of cancer as a mosaic of distinct genomes: sequencing of these single-cell genomes can reveal information about a cancer's response to drug treatments. In recent work, we have shown that these single-cell processes can lead to resistance to platinum salts, a standard chemotherapy treatment used today for people with TNBC.

This proposal seeks to use novel single-cell genome sequencing methods in TNBC tumors transplanted from patients and grown in the lab within specialized strains of mice or as cells in a laboratory culture under customized growth conditions. We will not only study the behavior of these patient-derived cancers as they evolve over time, but we will also study these TNBC genomes as they respond to drug treatment, drug withdrawal, and under direct genetic editing of cancer cells. We aim to inform treatment decisions and improve treatment options by identifying combinations of existing therapeutics and those positioned to be tested in patients in the near future.

We expect several outcomes that will lead to near-term clinical benefits for patients with early and metastatic TNBC. This proposal will: (1) identify new strategies that can be tested in clinical trials, together with predictive markers, to select drugs for patients based on associations between their tumor's genetic markers and the relative effectiveness of each drug in that context; and (2) identify combinations of existing and emerging drugs that, when informed by genetic testing of patients, can overcome single-drug resistance. Our work will also create a better understanding of cancer evolution and a general framework for the investigation of new drugs for breast cancer, which will advance and accelerate ongoing progress towards durable breast cancer treatment and cures.

Proposal Title:	Therapeutic Targeting of Genomic Instability in Triple-Negative Breast Cancer
Log Number:	BC221065P1
Current PI Name:	David Cescon
Award Number:	HT9425-23-1-0821
Current Contracting Organization:	University Health Network
Current Performing Organization:	University Health Network
Web Approval Date:	10-03-2023

This proposal addresses two fiscal year 2022 (FY22) Breast Cancer Research Program (BCRP) Overarching Challenges: (1) Revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival; and (2) Eliminate the mortality associated with metastatic triple-negative breast cancer (TNBC).

TNBC is an aggressive form of the disease, which is associated with a high risk of relapse and short survival in the metastatic setting. Currently, it has fewer treatment options than other forms of breast cancer. Our objective is to lower mortality and improve treatment for patients affected by TNBC by developing biological and computational methods that will identify treatment strategies to overcome the cancer evolution that is responsible for the development of drug resistance and the failure of existing treatments.

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Proposal Title:	Targeting Chromatin Regulator WDR5 to Treat Breast Cancer Metastasis
Log Number:	BC221109
Current PI Name:	Qin Yan
Award Number:	HT9425-23-1-0602
Current Contracting Organization:	Yale University
Current Performing Organization:	Yale University
Web Approval Date:	10-02-2023

In the United States, breast cancer is the most common type of cancer and the second most common cause of cancer death in women. Advanced breast cancer is associated with significant mortality because it metastasizes or spreads to other vital organs. The available treatment options only have limited clinical benefits for a small subset of patients. Thus, it is critical to identify and validate new drug targets for the development of efficacious therapies. Similar to genetic aberrations, a different type of change that occurs without a change of DNA sequence, termed epigenetic alterations, are likely the major drivers of breast cancer metastasis. However, these changes are not well understood in the context of breast cancer metastasis. Our long-term goal is to elucidate how these alterations contribute to cancer development. Because epigenetic changes are reversible, enzymes that regulate these changes are very attractive targets for cancer therapy.

To this end, we recently identified an epigenetic protein WDR5 as a new therapeutic target for breast cancer metastasis. This protein can be removed, using newly developed small molecules called degraders, from tumor cells to treat triple-negative breast cancer (TNBC). We showed that the WDR5 regulated pathway correlates with poor survival of breast cancer patients. We showed that WDR5 is required for tumor metastasis in multiple animal models of cancer. However, further dissection of the roles of WDR5 in breast cancer progression and metastasis, and evaluation of WDR5 targeting strategies, are needed to translate these findings to the clinic. Therefore, the objectives of this project are to determine whether and how WDR5 depletion can be used to treat metastatic breast cancer, and to develop WDR5 targeting strategies. In Aims 1 and 2, we will further study how WDR5 promotes tumor growth and metastasis in TNBC models. In Aim 3, we will evaluate the efficacy of WDR5 degrader monotherapy and combination therapy with everolimus (an approved drug to treat hormone receptor positive, HER2 negative breast cancer) on metastatic TNBC.

Regarding the ultimate applicability of the proposed research, our proposed studies will address several overarching challenges related to metastatic breast cancer, including: eliminating the mortality; identifying drivers of cancer growth and metastasis; preventing lethal recurrence; and developing novel treatment regimens.

Our study to understand and target breast cancer metastasis will benefit breast cancer patients with metastatic disease. Since our preclinical mouse models are based on TNBC, patients with TNBC, the most aggressive subtype of disease, will likely benefit the most from our research. In addition, we have shown that WDR5 is critical for the growth of ER+ and HER2+ breast cancer, and our findings could also benefit patients with these subtypes of breast cancer.

Our proposed translational studies could lead to new treatment regimens to treat metastatic breast cancer and reveal new prognostic and/or predictive biomarkers of metastatic breast cancer. We will achieve a patient-related impact by validating WDR5 degrader as a new therapeutic strategy and revealing biomarkers of this drug for metastatic breast cancer in 2 to 3 years. Our collaborator Dr. Jian Jin is working closely with Cullgen, a biotech company, to further develop WDR5 degraders for clinical use. As the Principal

Investigators (PIs) collaborate closely with clinicians, including Dr. Lajos Pusztai and Dr. Patricia LoRusso who run phase I breast cancer clinical trials at the Yale Cancer Center, this strategy could be translated rapidly to the clinic. Relevant to this, PI Dr. Qi Yan and Dr. Pusztai communicate frequently as scientific co-directors of the Center for Breast Cancer at Yale Cancer Center and Smilow Cancer Hospital.

In summary, our studies are expected to reveal a paradigm-changing treatment strategy, which could be rapidly tested in the clinic for treating breast cancer patients within 3 years. Collectively, the results of our studies will have major impact on reducing breast cancer mortality and significantly advance the field toward the goal of ending breast cancer.

Proposal Title:	Targeting Chromatin Regulator WDR5 to Treat Breast Cancer Metastasis
Log Number:	BC221109P1
Current PI Name:	Don Nguyen
Award Number:	HT9425-23-1-0603
Current Contracting Organization:	Yale University
Current Performing Organization:	Yale University
Web Approval Date:	10-02-2023

In the United States, breast cancer is the most common type of cancer and the second most common cause of cancer death in women. Advanced breast cancer is associated with significant mortality because it metastasizes or spreads to other vital organs. The available treatment options only have limited clinical benefits for a small subset of patients. Thus, it is critical to identify and validate new drug targets for the development of efficacious therapies. Similar to genetic aberrations, a different type of change that occurs without a change of DNA sequence, termed epigenetic alterations, are likely the major drivers of breast cancer metastasis. However, these changes are not well understood in the context of breast cancer metastasis. Our long-term goal is to elucidate how these alterations contribute to cancer development. Because epigenetic changes are reversible, enzymes that regulate these changes are very attractive targets for cancer therapy.

To this end, we recently identified an epigenetic protein WDR5 as a new therapeutic target for breast cancer metastasis. This protein can be removed, using newly developed small molecules called degraders, from tumor cells to treat triple-negative breast cancer (TNBC). We showed that the WDR5 regulated pathway correlates with poor survival of breast cancer patients. We showed that WDR5 is required for tumor metastasis in multiple animal models of cancer. However, further dissection of the roles of WDR5 in breast cancer progression and metastasis, and evaluation of WDR5 targeting strategies, are needed to translate these findings to the clinic. Therefore, the objectives of this project are to determine whether and how WDR5 depletion can be used to treat metastatic breast cancer, and to develop WDR5 targeting strategies. In Aims 1 and 2, we will further study how WDR5 promotes tumor growth and metastasis in TNBC models. In Aim 3, we will evaluate the efficacy of WDR5 degrader monotherapy and combination therapy with everolimus (an approved drug to treat hormone receptor positive, HER2 negative breast cancer) on metastatic TNBC.

Regarding the ultimate applicability of the proposed research, our proposed studies will address several overarching challenges related to metastatic breast cancer, including: eliminating the mortality; identifying drivers of cancer growth and metastasis; preventing lethal recurrence; and developing novel treatment regimens.

Our study to understand and target breast cancer metastasis will benefit breast cancer patients with metastatic disease. Since our preclinical mouse models are based on TNBC, patients with TNBC, the most aggressive subtype of disease, will likely benefit the most from our research. In addition, we have shown that WDR5 is critical for the growth of ER+ and HER2+ breast cancer, and our findings could also benefit patients with these subtypes of breast cancer.

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In summary, our studies are expected to reveal a paradigm-changing treatment strategy, which could be rapidly tested in the clinic for treating breast cancer patients within 3 years. Collectively, the results of our studies will have major impact on reducing breast cancer mortality and significantly advance the field toward the goal of ending breast cancer.

Proposal Title:	Targeting a Lipid Kinase Regulator of Nuclear Akt in Breast Cancer
Log Number:	BC221114
Current PI Name:	Vincent Cryns
Award Number:	HT9425-23-1-0553
Current Contracting Organization:	Wisconsin, University of, Madison
Current Performing Organization:	Wisconsin, University of, Madison
Web Approval Date:	10-01-2023

The p53 gene (TP53) is mutated in more than half of all human tumors. Similarly, a molecule known as Akt that regulates cell growth is abnormally active in the majority of cancers. Mutant p53 and Akt drive tumor progression and metastasis, the spread of tumor cells to distant organs. TP53 mutations occur in 80% of triple-negative breast cancers (TNBCs), an aggressive type of breast cancer that is not hormonally driven and is defined by the lack of three receptors (estrogen receptor, progesterone receptor, and HER2) which are commonly expressed in other types of breast cancer. Abnormal activation of Akt is also a common feature of TNBC. TNBC disproportionately strikes young African American and Hispanic women, and often spreads rapidly to the lungs and brain despite treatment. Although there are a few alternatives to chemotherapy recently approved for TNBC, there is an urgent need to develop new therapies for this deadly disease.

Recently, my colleague, Dr. Richard Anderson, and I discovered a new pathway that directly links mutant p53 and Akt, two key cancer genes that were not known to interact. We discovered that a molecule called inositol polyphosphate multikinase (IPMK) modifies mutant p53 and enables it to bind and activate Akt. Importantly, we also showed that inhibiting IPMK blocks nuclear Akt and triggers breast cancer cells to die, thereby pointing to IPMK as a promising drug target in breast tumors with mutant p53 and/or abnormally active Akt. This work was published in the prestigious journal Nature Cell Biology and received extensive media attention. Notably, IPMK has not been studied in breast cancer. We also recently discovered that a U. S. Food and Drug Administration (FDA)-approved antidepressant vilazodone, an IPMK inhibitor, blocks the nuclear Akt pathway, suggesting we may be able to "repurpose" this drug to treat breast cancer.

In the proposed experiments, Dr. Anderson and I will partner and bring our different backgrounds to study IPMK as a new drug target and vilazodone as a potential "breakthrough" drug in breast cancer. Dr. Anderson is an expert in basic cell biology who has made landmark discoveries about the Akt pathway, and I am an expert in translational breast cancer research who has taken my lab discoveries into clinical trials in breast cancer. Our partnership has been very productive, resulting in two Department of Defense BCRP Breakthrough Awards and several publications, including two in the prestigious journal Nature Cell Biology. We will use our complementary expertise to investigate our hypothesis that inhibiting IPMK with drugs like vilazodone or by genetic strategies will block nuclear Akt activation and cause breast cancer cells to die, thereby stopping breast tumor growth and the spread of tumor cells ("metastasis") to other organs. As such, our proposal addresses two BCRP Overarching Challenges: Revolutionizing treatment with less toxic and more effective options; and eliminating the mortality of metastatic breast cancer.

We will test our hypothesis with three aims. In Aim 1, we will examine the effects of drugs such as vilazodone, which inhibit IPMK, and genetic strategies to turn off the IPMK gene required for the nuclear Akt pathway in TNBC cells. In Aim 2, we will use these same approaches (drugs and genetic strategies) to inhibit IPMK and examine the effects on the growth, survival, and movement of TNBC cells. In Aim 3, we will evaluate the effects of inhibiting IPMK (vilazodone and genetic strategies) to kill TNBC cells and stop the growth and metastasis of breast tumors in mice, using clinically relevant models such as patient-derived tumors and metastatic models of TNBC. Drug toxicity will be carefully investigated. These studies have been carefully designed to obtain critical proof-of-principle data to support a future clinical trial of

vilazodone or other IPMK inhibitors in breast cancer. Indeed, we will review data with our clinical collaborator, Dr. Kari Wisinski, an expert in breast cancer clinical trials, to accelerate the clinical translation of our work.

To summarize, we have discovered a new pathway and drug target (IPMK) that directly links two of the most common cancer-causing pathways (p53 and Akt). We have also discovered that the FDA-approved antidepressant vilazodone blocks this pathway, and we will explore its activity in clinically relevant breast cancer models. As such, the proposed studies may revolutionize treatment approaches for breast cancer by pointing to IPMK as a novel therapeutic target and repurposing an FDA-approved drug for breast cancer.

Proposal Title:	Targeting a Lipid Kinase Regulator of Nuclear Akt in Breast Cancer
Log Number:	BC221114P1
Current PI Name:	Richard Anderson
Award Number:	HT9425-23-1-0554
Current Contracting Organization:	Wisconsin, University of, Madison
Current Performing Organization:	Wisconsin, University of, Madison
Web Approval Date:	10-01-2023

The p53 gene (TP53) is mutated in more than half of all human tumors. Similarly, a molecule known as Akt that regulates cell growth is abnormally active in the majority of cancers. Mutant p53 and Akt drive tumor progression and metastasis, the spread of tumor cells to distant organs. TP53 mutations occur in 80% of triple-negative breast cancers (TNBCs), an aggressive type of breast cancer that is not hormonally driven and is defined by the lack of three receptors (estrogen receptor, progesterone receptor, and HER2) which are commonly expressed in other types of breast cancer. Abnormal activation of Akt is also a common feature of TNBC. TNBC disproportionately strikes young African American and Hispanic women, and often spreads rapidly to the lungs and brain despite treatment. Although there are a few alternatives to chemotherapy recently approved for TNBC, there is an urgent need to develop new therapies for this deadly disease.

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Proposal Title:	Perturbomics Identification of Critical Genetic Targets in NK Cells for Breast Cancer Therapy
Log Number:	BC221122
Current PI Name:	Sidi Chen
Award Number:	HT9425-23-1-0472
Current Contracting Organization:	Yale University
Current Performing Organization:	Yale University
Web Approval Date:	10-02-2023

Breast cancer is the most common cancer and the second leading cause of cancer death in women. Although standard of care have good prognosis, in general, late-stage and metastatic breast cancers are still lethal. Therefore, new and better treatment options are unmet urgent needs for breast cancer patients. Cell therapy is a powerful means of cancer treatment; however, currently existing cell therapies face major challenges in solid tumors including breast cancer. Natural killer (NK) cell is an innate immune cell type that serves at the first level of defense against pathogens and cancer. The development of genetically engineered chimeric antigen receptor (CAR) cells has shown great therapeutic potential in NK cells. However, despite all these efforts, there is no approved CAR-NK therapy to date. Current forms of NK cell-based immunotherapy candidates face a number of obstacles. NK cells encode the same collection of about 20,000 protein coding genes in their genome, many of which might play critical roles in regulating or limiting the antitumor function of NK cells. We hypothesize that systems approaches can identify critical regulators of NK cells to enhance NK-based cell therapy against breast cancer. Our goal is to use unbiased in vivo screens and single-cell sequencing to produce maps of genetic regulators of tumor-infiltrating NK cells, then perform gene editing of these regulators to enhance the function and antitumor efficacy of CAR-NKs against breast cancer.

This project addresses the fiscal year 2022 (FY22) Breast Cancer Research Program (BCRP) overarching challenge "Revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival." This project’s research output will help late-stage breast cancer patients, especially those with cancer antigen positive population, such as HER2 positive or MUC1 positive. This is a 3-year project. The direct outcome of this project will provide fundamental new knowledge on natural killer cells in breast cancer, plus new cell therapy candidates for breast cancer treatment. The new cell therapy candidates can progress to further development and translational studies shortly after the completion of this project to approach clinical stage. These novel cell therapy candidates initially developed here, if successfully developed into clinical products in future years, will provide new treatments for late-stage breast cancer patients who are often out of options. This project is directly relevant to the health and well-being of Service Members, Veterans, their family members, and all people impacted by breast cancer.

Proposal Title:	Novel Immunologic Discovery Platforms to Prevent Metastasis and Recurrence in Breast Cancer
Log Number:	BC221209
Current PI Name:	Judith Agudo
Award Number:	HT9425-23-1-0815
Current Contracting Organization:	Dana-Farber Cancer Institute
Current Performing Organization:	Dana-Farber Cancer Institute
Web Approval Date:	10-02-2023

Metastasis is when cancer spreads from its original location to a different part of the body. Breast cancer deaths are not caused by the primary tumor in the breast but by metastasis, as it often occurs in vital organs such as the brain, liver, and lungs. For many patients, these metastatic tumors grow after a treatment that initially looked successful; and it can occur even years later. This is the result of breast cancer cells that escape the primary tumor mass in the breast and travel to other organs where they become the seeds of more breast cancer. Disseminated tumor cells that re-grow the breast cancer after initial treatment are inherently resistant to conventional systemic therapies, such as chemotherapy. Our vision is to identify strategies to eradicate these seeds before they bring the disease back and spread it throughout the body. Thus, the challenges we aim to address are to eliminate the mortality associated with metastatic breast cancer and determine how to prevent lethal recurrence. If we succeed in our goal, this will save the lives of many breast cancer patients, avoid unnecessary suffering from treatments once the disease re-appears, and alleviate the associated economic costs. Moreover, if physicians could guarantee that the disease has no means to come back, this would save patients from years of emotional stress wondering whether and when the disease will return.

Approaches to eradicate therapy-resistant disseminated breast cancer cells is a critical unmet clinical need. Since these "seeds" of metastases and recurrence survive during conventional therapies, completely different therapies are necessary. Here we propose to leverage the patients' own immune system to prevent metastatic disease rather than to treat it. During early stages of breast cancer, immune cells recognize and eliminate a significant fraction of breast cancer cells that escape the tumor and travel throughout the body. However, some of these disseminated breast cancer cells evolve means to hide from patrolling immune cells. If we discover how they trick immune cells and survive, this will allow us to develop approaches to ensure that the patients' inherent immune surveillance can detect and target all breast cancer cells. Of note, immune cells can be thought of like live drugs with the property of specificity and memory, and hence they can patrol the patients' bodies for many years.

In summary, I believe that metastasis must be prevented, and so, when patients are treated for a tumor in their breast, approaches should be available to ensure their disease is never coming back. Immune cells harbor the power to recognize and attack cancer cells. My goal is to discover means to exploit immune cells to find breast cancer cells that spread during early stages of the disease before they can grow new tumors.

Proposal Title:	Sustained-Release STING Agonist Implants Activate Antitumor Immunity to Treat Advanced Breast Cancer in Combination with PARPi
Log Number:	BC221248
Current PI Name:	Needa Brown
Award Number:	HT9425-23-1-0813
Current Contracting Organization:	Northeastern University
Current Performing Organization:	Northeastern University
Web Approval Date:	10-02-2023

Triple-negative breast cancer (TNBC) is the most aggressive form of breast cancer, with a higher risk of recurrence and worse prognosis after relapse than hormone receptor-positive cancers (median survival 12 to 18 months versus 50 to 60 months). Until recently, conventional treatment options for TNBC patients involve either a mastectomy or a lumpectomy followed by radiation and/or chemotherapy. When given the option for breast conservation therapy (BCT), 72% of patients preferred some form of BCT over surgical options, especially younger women. Poly (ADP-ribose) polymerase inhibitors (PARPi) have emerged as a promising therapy for these patients, however, resistance emerges and responses are not durable, necessitating research into quick translational strategies.

Immune checkpoint blockade (ICB) has revolutionized the treatment of some cancer types but has demonstrated only modest benefit in TNBC as a monotherapy. ICB has been recently U.S. Food and Drug Administration (FDA)-approved for TNBC in combination with chemotherapy, however, the approval is only for PD-L1-positive metastatic TNBC, and while there are responses, the majority of the patients eventually progress. The limited response rates (5% to 20%) suggest that tumor-related immunosuppression cannot be fully overcome by ICB blockade alone.

T cell infiltration and activation has been the focus of ICB, however, the largest population of infiltrating immune cells are tumor-associated macrophages (TAMs) that link innate and adaptive immunity. TAM-targeting strategies have been slow to be approved for clinical care, likely due to lack of clinical rationale and testing of appropriate combinations. Dr. Guerriero’s team recently demonstrated that PARPi drives development of a subset of suppressive TAMs that restrict antitumor T cell function. In the absence of tumorigenic TAMs, PARPi induce robust recruitment of cytotoxic T cells and durable antitumor responses. The breakthrough discovery by Dr. Guerriero’s team has set the stage for next-generation innate immune modulatory therapies to reprogram TAMs, specifically targeting of the cGAS/STING pathway with exogenous stimulator of interferon genes (STING) agonist. Dr. Guerriero’s team and others have recently shown that intratumoral STING agonists reprogram TAMs from protumor M2-like to anticancer M1-like phenotypes and synergize with PARPi, leading to 100% complete regression.

Given that all cells have some degree of cGAS/STING sensing pathway, a key limitation to translation of STING agonist is the need for recurrent intratumoral injections to achieve high disease site drug dose and limit systemic toxicity. Chronic systemic activation of the STING pathway is linked to an autoinflammatory condition, STING-associated vasculopathy with onset in infancy (SAVI). Given the promising preliminary results of intratumoral STING agonist and PARPi, rapid clinical translation is necessary, however, hurdles of STING agonist delivery must be overcome. Sustained-release polymer-drug formulations are promising alternatives to recurrent intratumoral injections. Dr. Brown’s team has formulated sustained-release SMAART (Sustained Modulation and Activation of Anticancer Responses and Targets) implants from FDA-approved poly (lactic-co-glycolic) acid (PLGA) polymer that have linear release kinetics over 28 days and prolong duration of drug action.

This partnering PI proposal will combine our expertise in drug delivery and nanoformulation (Brown), and immunology and breast tumor biology (Guerriero), to test the efficacy of sustained STING agonist implants to activate innate and adaptive immunity compared to periodic injections. This work will fill in gaps in knowledge about: (1) tumor immune microenvironment shifts following sustained versus periodic STING pathway activation (Aim 1); (2) how primary site cGAS/STING activation impacts the immune microenvironment at secondary, metastatic sites (Aim 2); and (3) the synergistic impact of sustained STING pathway activation with PARPi to eradicate advanced breast cancer (Aim 2). Successful completion of this work will provide the platform necessary to translate STING agonist into the clinic by overcoming barriers for continuous delivery.

Our preliminary data support the central hypothesis that sustained activation of the STING pathway using biodegradable STING agonist implants will activate anticancer innate and adaptive immunity within the tumor microenvironment, compared to periodic STING agonist injections. To ensure that our research produces a direct impact on breast cancer patient treatment, we will test the translational hypothesis that sustained STING pathway modulation amplifies the antitumor efficacy of PARPi therapy at primary and secondary metastatic sites, for rapid translation to advanced breast cancer patients. In this translational proposal, we will revolutionize sustained STING agonist delivery to activate innate and adaptive anticancer immunity at primary and secondary metastatic sites that will eradicate advanced breast cancer in combination with PARPi therapy.

Proposal Title:	Sustained-Release STING Agonist Implants Activate Antitumor Immunity to Treat Advanced Breast Cancer in Combination with PARPi
Log Number:	BC221248P1
Current PI Name:	Jennifer Guerriero
Award Number:	HT9425-23-1-0814
Current Contracting Organization:	Brigham and Women's Hospital, Inc.
Current Performing Organization:	Brigham and Women's Hospital, Inc.
Web Approval Date:	10-02-2023

Triple-negative breast cancer (TNBC) is the most aggressive form of breast cancer, with a higher risk of recurrence and worse prognosis after relapse than hormone receptor-positive cancers (median survival 12 to 18 months versus 50 to 60 months). Until recently, conventional treatment options for TNBC patients involve either a mastectomy or a lumpectomy followed by radiation and/or chemotherapy. When given the option for breast conservation therapy (BCT), 72% of patients preferred some form of BCT over surgical options, especially younger women. Poly (ADP-ribose) polymerase inhibitors (PARPi) have emerged as a promising therapy for these patients, however, resistance emerges and responses are not durable, necessitating research into quick translational strategies.

Immune checkpoint blockade (ICB) has revolutionized the treatment of some cancer types but has demonstrated only modest benefit in TNBC as a monotherapy. ICB has been recently U.S. Food and Drug Administration (FDA)-approved for TNBC in combination with chemotherapy, however, the approval is only for PD-L1-positive metastatic TNBC, and while there are responses, the majority of the patients eventually progress. The limited response rates (5% to 20%) suggest that tumor-related immunosuppression cannot be fully overcome by ICB blockade alone.

T cell infiltration and activation has been the focus of ICB, however, the largest population of infiltrating immune cells are tumor-associated macrophages (TAMs) that link innate and adaptive immunity. TAM-targeting strategies have been slow to be approved for clinical care, likely due to lack of clinical rationale and testing of appropriate combinations. Dr. Guerriero’s team recently demonstrated that PARPi drives development of a subset of suppressive TAMs that restrict antitumor T cell function. In the absence of tumorigenic TAMs, PARPi induce robust recruitment of cytotoxic T cells and durable antitumor responses. The breakthrough discovery by Dr. Guerriero’s team has set the stage for next-generation innate immune modulatory therapies to reprogram TAMs, specifically targeting of the cGAS/STING pathway with exogenous stimulator of interferon genes (STING) agonist. Dr. Guerriero’s team and others have recently shown that intratumoral STING agonists reprogram TAMs from protumor M2-like to anticancer M1-like phenotypes and synergize with PARPi, leading to 100% complete regression.

Given that all cells have some degree of cGAS/STING sensing pathway, a key limitation to translation of STING agonist is the need for recurrent intratumoral injections to achieve high disease site drug dose and limit systemic toxicity. Chronic systemic activation of the STING pathway is linked to an autoinflammatory condition, STING-associated vasculopathy with onset in infancy (SAVI). Given the promising preliminary results of intratumoral STING agonist and PARPi, rapid clinical translation is necessary, however, hurdles of STING agonist delivery must be overcome. Sustained-release polymer-drug formulations are promising alternatives to recurrent intratumoral injections. Dr. Brown’s team has formulated sustained-release SMAART (Sustained Modulation and Activation of Anticancer Responses and Targets) implants from FDA-approved poly (lactic-co-glycolic) acid (PLGA) polymer that have linear release kinetics over 28 days and prolong duration of drug action.

This partnering PI proposal will combine our expertise in drug delivery and nanoformulation (Brown), and immunology and breast tumor biology (Guerriero), to test the efficacy of sustained STING agonist implants to activate innate and adaptive immunity compared to periodic injections. This work will fill in gaps in knowledge about: (1) tumor immune microenvironment shifts following sustained versus periodic STING pathway activation (Aim 1); (2) how primary site cGAS/STING activation impacts the immune microenvironment at secondary, metastatic sites (Aim 2); and (3) the synergistic impact of sustained STING pathway activation with PARPi to eradicate advanced breast cancer (Aim 2). Successful completion of this work will provide the platform necessary to translate STING agonist into the clinic by overcoming barriers for continuous delivery.

Our preliminary data support the central hypothesis that sustained activation of the STING pathway using biodegradable STING agonist implants will activate anticancer innate and adaptive immunity within the tumor microenvironment, compared to periodic STING agonist injections. To ensure that our research produces a direct impact on breast cancer patient treatment, we will test the translational hypothesis that sustained STING pathway modulation amplifies the antitumor efficacy of PARPi therapy at primary and secondary metastatic sites, for rapid translation to advanced breast cancer patients. In this translational proposal, we will revolutionize sustained STING agonist delivery to activate innate and adaptive anticancer immunity at primary and secondary metastatic sites that will eradicate advanced breast cancer in combination with PARPi therapy.

Proposal Title:	G0S2 as a Master Regulator of the Local Estrogenic Environment During Breast Cancer Progression and Antiestrogen Therapy
Log Number:	BC221269
Current PI Name:	Michael Spinella
Award Number:	HT9425-23-1-0530
Current Contracting Organization:	Illinois, University of, Champaign/Urbana
Current Performing Organization:	Illinois, University of, Champaign/Urbana
Web Approval Date:	09-06-2023

The overarching challenges are: (1) Identify what drives breast cancer growth; determine how to stop; and (2) Eliminate the mortality associated with metastatic breast cancer.

Antiestrogen therapy in the form of drugs, including tamoxifen and aromatase inhibitors like anastrozole, exemestane, and letrozole, are a mainstay for the treatment of estrogen receptor positive (ER+) breast cancer. However, many women, especially those with advances disease, become resistant to these therapies and die from their disease. We found through unbiased approaches that a protein called G0S2 can alter the levels of estrogen in breast cancer cells by directly altering the machinery the makes and degrades estradiol, the most potent estrogen. We also showed that those patients that have high levels of G0S2 have a lower rate of recurrence after antiestrogen therapy. Our objective is to provide further evidence that G0S2 modulates the estrogen biosynthetic pathway to suppress the growth of ER+ breast cancer, and that the loss of G0S2 that is typically seen in breast cancer compared to normal breast tissue promotes ER+ breast cancer by increasing the tumor levels of estradiol. We also want to understand how G0S2 alters the estrogen biosynthesis pathway. To uncover new alternative targeted therapies for breast cancers that fail antiestrogen therapy, it is vital to further understand the mechanisms of recurrence. Our discovery that G0S2 expression is frequently repressed in human breast cancer, and that this repression is associated with recurrence and alteration in estradiol bioavailability, may have major impact in predicting recurrence and mechanisms of sensitivity and resistance to antiestrogen therapy. If our hypothesis is proven correct, we can obtain additional funding, including breakthrough 3 and 4 awards, to bring G0S2 into the clinic as a biomarker of antiestrogen therapy response and to develop drugs that will induce G0S2 levels in breast cancer patients.

Proposal Title:	Integrative Single-Cell Analyses of Circulating Tumor Cells in Metastatic Breast Cancer
Log Number:	BC221274
Current PI Name:	Chonghui Cheng
Award Number:	HT9425-23-1-0753
Current Contracting Organization:	Baylor College of Medicine
Current Performing Organization:	Baylor College of Medicine
Web Approval Date:	10-02-2023

Breast cancer affects one in eight women in the United States during her lifetime and claims approximately 41,000 lives per year. Many of these deaths are due to metastatic disease. Triple-negative breast cancer (TNBC) is more likely to affect younger people and minority populations and can be more aggressive, making it more difficult to treat and more likely to recur or metastasize. While many types of breast tumors can be treated by hormone therapy or drugs that target common receptors (ER, PR, HER2), TNBC still lacks effective targeted therapeutic options.

Metastasis occurs when cells from the primary tumor breakoff, enter the bloodstream and travel through the body to a new location, forming a new tumor. Many times, patients are asked to undergo invasive biopsy procedures for the purpose of monitoring disease progression and determining prognosis and treatment. During the process of metastasis, the circulating tumor cells (CTCs), which have made it to the bloodstream from the original tumors, provide a noninvasive window into the status of the disease progression. CTCs have been directly correlated with disease progression and outcome. Recent advances in single-cell technologies, like single-cell RNA sequencing, offer the opportunity to explore CTCs more fully at the molecular level and revolutionize the way we approach personalized medicine. CTCs are rare and difficult to capture, but this population of tumor cells could offer a unique window of opportunity that enables the monitoring of disease progression in real-time and importantly, in a noninvasive manner.

Our research team hopes to use innovative technologies to better understand CTCs, especially those of TNBC. To achieve this goal, we have established a highly reliable and sensitive methodology to identify and isolate CTCs from mouse models of breast cancer. We will utilize the cutting-edge technology, single-cell RNA sequencing, to determine the molecular characteristics of CTCs as well as the tumors that generate them.

Establishing this platform will enable liquid biopsy to inform how changes in CTCs affect tumor progression and potential metastasis. More detailed information about the cells that become metastatic tumors will allow more targeted treatment of cancer and improve survival of metastatic TNBC patients. These results will offer enormous opportunities to follow patient disease progression in a noninvasive manner and will contribute to groundbreaking discoveries of novel therapies targeting CTCs, the seeds of metastasis.

Proposal Title:	Re-Engineering the Intratumoral Myeloid-Macrophage Compartment to Enhance the Response to Radiation and Immunotherapy in Triple-Negative Breast Cancer
Log Number:	BC221288
Current PI Name:	Stephen Shiao
Award Number:	HT9425-23-1-0652
Current Contracting Organization:	Cedars-Sinai Medical Center
Current Performing Organization:	Cedars-Sinai Medical Center
Web Approval Date:	10-02-2023

Breast cancer remains the most common cancer in North America and the second leading cause of cancer death in women. Cytotoxic therapies like radiation therapy (RT) or chemotherapy play an integral part in the treatment of breast cancer, with the majority of triple-negative breast cancer (TNBC) patients receiving cytotoxic therapy sometime during the course of their treatment. The conventional view of cytotoxic therapies has largely focused on the effect of these therapies on the tumor cells themselves. However, recent studies have demonstrated a critical role for the immune system in determining the response of tumors to cytotoxic therapy and multiple studies, including our own, have demonstrated synergy between RT /chemotherapy and immunotherapy. However, despite the improvements in response rates with immunotherapy, more still do not respond, highlighting the need for better understanding and therapies to address immune-resistant tumors. One source of this immune resistance in tumors is thought to be mediated by macrophages, one of the major innate immune cells responsible for regulating inflammation. The objective of this research proposal is to study the impact of restricting the ability of macrophages to activate programming that supports tumor growth by deleting a master transcription factor called GATA-3 in macrophages that regulate pro-tumor programming. The proposal tests the hypothesis that blocking GATA-3 mediated programming in macrophages will enhance anti-tumor immune responses. We test this hypothesis by first looking at the efficacy of chemotherapy, RT, and immunotherapy in mice with GATA-3-deficient macrophages (Aim 1). We then try to understand why there is (or is not) enhanced efficacy of chemotherapy, RT, or immunotherapy by testing whether the effect of treatment is due to different immune cells using agents to remove specific immune cells individually (Aim 2); and testing various key macrophage behaviors to see if there are any differences in inflammatory behavior when the macrophages lack GATA-3. Finally, we focus on testing the idea that macrophages can be used as live cell therapy and that macrophages can be engineered to be deficient in GATA-3 and that these macrophages could enhance the response to therapy similar to the genetically ablated model (Aim 3). We will employ a combination of single-cell sequencing, flow cytometry, and ELISA to follow the changes in the immune profile of tumors following treatment in mice with normal macrophages and mice with macrophages missing GATA-3. The significance of this research is that it will provide insights into the macrophage regulation of tumor immune responses to combined therapy that will lead to new macrophage-based treatments to enhance the efficacy of treatment in breast cancer, and multiple other solid tumors, in which cytotoxic therapies and checkpoint blockade play an integral therapeutic role.

Proposal Title:	Re-Engineering the Intratumoral Myeloid-Macrophage Compartment to Enhance the Response to Radiation and Immunotherapy in Triple-Negative Breast Cancer
Log Number:	BC221288P1
Current PI Name:	Simon Knott
Award Number:	HT9425-23-1-0741
Current Contracting Organization:	Cedars-Sinai Medical Center
Current Performing Organization:	Cedars-Sinai Medical Center
Web Approval Date:	10-02-2023

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Proposal Title:	Using Multi-Modal Data Integration Across Breast Cancer Molecular Subtypes to Develop Predictors of Treatment Response, Early Relapse, and Survival and to Explore Early Signals of Later Metastatic Recurrence
Log Number:	BC221353
Current PI Name:	Jean Abraham
Award Number:	HT9425-23-1-0767
Current Contracting Organization:	Cambridge, University of
Current Performing Organization:	Cambridge, University of
Web Approval Date:	10-02-2023

Some of the critical questions that women and men with breast cancer ask during their treatment journey are: "How will I know if I am responding to treatment;" "When will I know if I am responding to treatment;" and "How will I know if my disease will come back." It is our opinion that, in order to answer these questions, we need to use a more cohesive approach by bringing together all the information and data generated about each individual patient. Specific information from each type of investigation, such as genetic tests, mammograms, or pathology specimens, can give us clues as to why some are likely to respond to treatment or progress to metastatic disease. However, currently we look at all this information in a disconnected and siloed manner. Thanks to the generosity of >6500 patients, who have provided samples and data through participating in clinical trials, we are proposing to build a unique data resource that links up the information from multiple types of investigations in each individual patient. By doing this with thousands of patient data and samples, we will create a platform with sufficient power to develop specific tools that help predict whether someone might respond to treatment or be at risk of their disease spreading.

This study will address three of the overarching challenges that we face: (1) revolutionize treatment regimens by replacing them with ones that are more effective and less toxic; (2) distinguish deadly from non-deadly breast cancers; and (3) identify why some breast cancers become metastatic while others do not. In addition to tests done in the clinic, such as mammograms, etc., we will also begin to look in fine detail, down at the level of single cells, to find out if specific factors or characteristics can help with predicting the response to treatment or metastatic spread. To do this, we will use cutting edge technologies that allow us to look at the breast cancer cells in minute detail and assess their relationships with cells around them and within the environment in which the tumor developed. The team is composed of scientists with world-class expertise in this area and clinicians with over 20 years of clinical trials experience. They have a range of experts with knowledge of all the different data types to be explored. The team is led by a clinician advised by two consumer patient advocates who will ensure that all prediction tools are developed with the patient and clinical implementation in mind.

This study aims to: (1) identify factors that predict for treatment response and early metastatic spread; (2) develop predictive tests using and combining these factors; and (3) create a unique breast cancer research resource that is comprehensive, that links together data from different investigations, and that can used by the breast cancer community to ask and answer many of the other challenges in breast cancer. This type of resource will undoubtedly speed up finding a path to ending breast cancer as a lethal disease.

Our team is split into working groups who focus on specific types of data, e.g., data from mammograms or other radiological images, which are called radiomics. Each working group will select key factors they believe influence prediction of response to treatment or risk of metastatic spread. The selected factors from each working group are then carefully combined using special mathematical models and tests, called machine learning and artificial intelligence. We will then understand what factors are truly important for these predictions. These assumptions will then be re-tested in an independent set of data.

We intend to complete this process in several different types of breast cancers starting with triple-negative breast cancer (TNBC). Here, some of the fine detail work at the cellular level has already been established through allied projects. This allows us to use TNBC as our prototype model within which to develop our predictors. We would hope to complete the TNBC treatment response predictor development within 24 months of the project starting. This process will then be used as a template for other types of breast cancer, e. g., HER2 positive. These two types of breast cancer are particularly at risk of early relapse and metastatic spread, so early predictions of response to treatment or risk of metastatic spread will be especially impactful.

We can only deliver this project because many thousands of patients who participated in our clinical trials and gave samples and data of many different types. Developing tools that help improve how long future patients survive or indicate how effective and accurate their treatment is likely to be, is the very reason that patients participate in research. The knowledge that these advances which benefit current patients have occurred, is because of the generosity of previous patients, and as such creates a virtuous circle and will encourage more patients to participate in research.

Proposal Title:	Using Multi-Modal Data Integration Across Breast Cancer Molecular Subtypes to Develop Predictors of Treatment Response, Early Relapse, and Survival and to Explore Early Signals of Later Metastatic Recurrence
Log Number:	BC221353P1
Current PI Name:	Gregory Hannon
Award Number:	HT9425-23-1-0768
Current Contracting Organization:	Cambridge, University of
Current Performing Organization:	Cambridge, University of
Web Approval Date:	10-02-2023

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Proposal Title:	Spatial Reprogramming of Tumor Immune Microenvironments by Preoperative Radiotherapy and Immune Checkpoint Inhibition in HER2-Negative Breast Cancer
Log Number:	BC221360
Current PI Name:	Gaorav Gupta
Award Number:	HT9425-23-1-0961
Current Contracting Organization:	North Carolina at Chapel Hill, University of
Current Performing Organization:	North Carolina at Chapel Hill, University of
Web Approval Date:	10-03-2023

Patients diagnosed with triple-negative breast cancer (TNBC) in the past had few options for effective treatment. More recently, the FDA approved a type of immunotherapy, referred to as anti-PD1 (aPD1) therapy, that extends the lives of patients with TNBC by stimulating the body's natural defense system. However, in these patients the current aPD1 immunotherapy is given in combination with four chemotherapy drugs, which together may cause serious side effects. In this project, we will investigate whether combining aPD1 immunotherapy with radiotherapy (RT) might allow us to eradicate metastatic disease in more breast cancer patients and improve their long-term quality of life as breast cancer survivors, while using less (or possibly eliminating the need for) chemotherapy. The major goal of this project is to identify a biomarker panel, i.e., a series of molecular changes within a patient's breast cancer, that can help us identify the breast cancer patients who are most likely to benefit from aPD1 and RT combination therapy. To achieve this goal, we will study cancer biopsy tissue from triple-negative and estrogen receptor-positive breast cancer patients taken before and after treatment with aPD1 therapy with or without RT in two clinical trials involving nearly 200 participants and representing a diverse patient population. We will analyze these samples using a cutting-edge technology called spatial transcriptomics, which will allow us to evaluate the interactions between tumor cells, immune cells, and other cell types within the breast tumor in detail and determine how these interactions changed in patients who responded to therapy. We will test the idea that uncovering the interactions between these different cell types in TNBC, and potentially also in the more common type of breast cancer that is estrogen receptor positive, will allow us to predict which patients would benefit from aPD1 combined with RT. The biomarkers identified in this project would then be used in future clinical trials to investigate whether, for some breast cancer patients, the combination of radiation and immunotherapy would be a safer and more effective alternative to the current standard of chemotherapy plus immunotherapy.

Proposal Title:	Develop a Preclinical Model for Gene Therapy of Fanconi Anemia Group C
Log Number:	BM220005
Current PI Name:	Ngoc Tung Tran
Award Number:	HT9425-23-1-0371
Current Contracting Organization:	Indiana University
Current Performing Organization:	Indiana University
Web Approval Date:	06-09-2023

Bone marrow failure (BMF) happens when the bone marrow cannot produce enough normal blood cells, including red blood cells, white blood cells, or platelets. Thus, a patient without treatment cannot distribute oxygen throughout the body's tissue, cannot fight infection, and are unable to stop bleeding. BMF is the leading cause of early death in patients with Fanconi Anemia (FA). FA is the most common inherited BMF disorder and is also associated with congenital abnormalities and cancer predisposition. FA is caused by mutations in any member of the FA gene family, which is crucial for removing DNA damage and maintaining genome integrity. About 90% of patients have mutations in FANCA, FANCG, or FANCC genes. FANCC-mediated FA (group C) patients show typical clinical symptoms of FA. There is no cure for this disease. Current treatment strategies focus on mitigating symptoms of BMF, treating secondary cancer, and improving the life quality of patients. BMF can be treated by allogeneic stem cell transplantation from a matched donor. However, besides the frequent unavailability of matched donors, transplanted FA patients have an enhanced risk of developing graft-versus-host disease. A personalized approach that overcomes the limitations of allogeneic stem cell transplantation is to use gene therapy that corrects mutations in patient-derived stem cells, and then transplant the corrected stem cells back into the patient. Indeed, lentivirus-based delivery of functional FA genes into patient-derived stem cells can restore functions of patient stem cells. The corrected stem cells can be engrafted into a patient without conditioning, thus mitigating the BMF symptom. However, random integration of the viral genome into the host genome and the inability to control the expression of the transgene are major safety concerns. Thus, precise mutation correction in patient-derived stem cells is crucial for safe gene therapy of FA. CRISPR/Cas9 is the state-of-the-art technology that allows modifying the genome seamlessly. Scientists have used this technology to precisely correct mutations in blood stem cells that can be applied for the treatment of blood genetic diseases (sickle cell, beta-thalassemia, and primary immune deficiency). This approach specifically introduces double-stranded breaks (DSB) into the genome and precisely repairs it via a DNA repair pathway called Homology-Directed Repair. However, DSBs may cause extensive genetic abnormalities. FA patient-derived cells have reduced ability to repair DNA damage; thus, CRISPR/Cas9-induced DSBs might harm the cell functionality. Hence, an approach that introduces few to no DSBs is ideal for gene therapy of FA disease.

We have recently developed a Spacer-Nick system that can efficiently correct mutations in blood stem cells while mitigating all the adverse effects of DSB. Here, we will exploit the Spacer-Nick system to correct mutations in FA group C patients. We will place a functional copy of the FANCC gene into the original site of the FANCC gene in patient-derived blood stem cells. We hypothesize that corrected stem cells will express a normal level of the FANCC gene product and restore the blood cell production and function both in laboratory tests and in living animals.

We have developed and shown that the Spacer-Nick system works efficiently in healthy blood stem cells. In this proposal, we will apply that system to correct FANCC mutations in two systems: blood stem cells from mice that don't express the Fancc gene, and blood stem cells isolated from FA patients. We will validate the functions of corrected stem cells in these two preclinical models. This proposal fits well with the award focus area to find effective BMF treatments and cures for FA-related BMF disease.

Our proposal is innovative because our new gene-editing system will provide a universal gene therapy that benefits all patients with FANCC mutations. In addition, our innovative approach that minimizes DSB at the targeted site will efficiently correct FANCC mutations while circumventing all the adverse genetic effects of DBS. Of note, the high specificity of the system will abrogate all the off-target effects that are associated with the conventional CRISPR/Cas9 system.

In the short term, results from our proposed work will establish a comprehensive preclinical model that evaluates the efficacy and safety of the Spacer-Nick system in both mouse and human experimental systems. In the long term, it will be the groundwork for developing a safe and efficient gene therapy to treat BMF and improve the quality of life for all FA patients with FANCC mutations.

Proposal Title:	Investigating Clonal Evolution in Fanconi Anemia
Log Number:	BM220015
Current PI Name:	Robert Rowe
Award Number:	HT9425-23-1-0433
Current Contracting Organization:	Children's Hospital, Boston
Current Performing Organization:	Children's Hospital, Boston
Web Approval Date:	06-09-2023

Bone marrow failure (BMF) disorders affect both adults and children. BMF results in inadequate production of mature blood cells rendering patients susceptible to infection and dependent on blood transfusions. Many BMF disorders are also predisposed to the development of leukemia, which is often resistant to treatment. Fanconi anemia (FA) is a specific BMF disorder that is associated with a very high risk of leukemia. Since bone marrow transplantation (BMT) can cure the BMF and leukemia of FA but is also a high-risk procedure, identification of the patients who are committed to the earliest stages of leukemia development is crucial so that these patients can receive timely BMT. Therefore, the critical overarching problem addressed in this proposal is how to recognize the earliest stages of leukemia development in BMF to identify patients who would benefit from pre-emptive BMT. The FY22 BMFRP Idea Development Award Focus Area addressed in this work is understanding the causes and progression of BMF diseases.

We have developed a new model system of human FA that captures the key hallmarks of this disease, in particular the blood stem cell failure that underlies BMF. In the preliminary work forming the basis of this proposal, by performing targeted modification of specific genes, we can also model FA-associated leukemia. Here, we aim to apply this system to understand the process of development of leukemia in FA. Our hypothesis is that FA patients acquire gene mutations in their blood stem cells that trigger the process of leukemia development before they start to show signs of symptoms of leukemia, providing an opportunity for early intervention. We will therefore sequence all of the genes in a group of FA patients to identify potential gene mutations that trigger the process of leukemia development. Once we identify candidate gene changes, we will test the effect of these mutations in causing leukemia-like outcomes in our human model system. When we have validated the effects of these gene changes, in the future, we could then screen for them in FA patients to pre-emptively find those at highest risk of leukemia. We believe that successful completion of these studies will form a new paradigm of research that can be broadly applied to many other BMF diseases.

We believe that this project is innovative in that we combine information gained from gene sequencing in FA patients with our new cell model of human FA to identify and validate gene changes that initiate the cascade toward development of leukemia. Successful completion of this project will have a direct positive impact on FA patients by providing new approaches monitoring and screening for leukemia. We expect that these studies will be broadly impactful on the field of BMF research, as this general approach can be applied to the study of other forms of BMF that are also associated with a risk of leukemia.

Proposal Title:	Beyond VEXAS and UBA1: Identification of Novel Drivers of MDS Autoimmune Syndromes
Log Number:	BM220023
Current PI Name:	David Beck
Award Number:	HT9425-23-1-0507
Current Contracting Organization:	New York University School of Medicine
Current Performing Organization:	New York University School of Medicine
Web Approval Date:	07-11-2023

Background: Myelodysplastic syndrome (MDS) is a common form of bone marrow failure that causes increased morbidity and mortality. Both the underlying mechanism and prognosis of MDS have been attributed to specific driver gene mutations that have led to distinct clinical subgroups and differential treatments. MDS frequently cooccurs with autoimmune diseases (rheumatoid arthritis, vasculitis) and this subset has not been evaluated for genetic causes. We recently identified a new genetic cause of MDS with co-occurring inflammation, namely UBA1 and VEXAS syndrome, which has led to changes in treatment, prognosis, and screening. We propose here to expand beyond UBA1 and characterize the genetics, clinical manifestations, and mechanism of such MDS-autoimmune diseases to help find additional subgroups. Together our work will provide insights not only into UBA1 but also general mechanisms found broadly in MDS.

Critical Questions: Are there distinct genetic causes and mechanisms underlying MDS-Autoimmune syndromes? Do these genetic alterations lead to unique clinical manifestations and treatment responses?

Specific BMF Disease: We will study a subtype of MDS with co-occurring autoimmune diagnoses. These patients encompass 30% of total patients with MDS and have not previously been systematically evaluated.

Bone Marrow Failure Research Program (BMFRP) Objective: The work proposed here fulfills the BMFRP objective to understand acquired bone marrow failure syndromes, particularly MDS-autoimmune overlap syndromes such as VEXAS and VEXAS-like diseases.

Innovation: Current genetic studies in MDS have primarily focused on sequencing a small subset of genes previously implicated in blood cancers (about 40-80 genes of the 20,000 total genes) for mutations. In addition, in some cases, MDS studies have excluded patients with overlapping rheumatologic diagnoses due to potential confounding causes for blood abnormalities (since rheumatologic diseases can lead to low blood counts on their own), thus potentially missing patients with MDS-autoimmune overlap syndromes such as VEXAS syndrome. These limitations have led to (1) a failure to identify new genetic drivers of MDS since efforts have focused on known genes, and (2) a lack of characterization of MDS-autoimmune overlap diseases. My lab will systematically overcome these limitations to uncover and evaluate somatic and germline variants in MDS-autoimmune overlap syndrome patients. We will compare the clinical profile of patients with specific mutations and try to determine if there are unique clinical manifestations that can be used to help provide improved care for patients. In addition, I plan to extend beyond just finding somatic mutations by determining why these mutant cells lead to bone marrow disease in patient cells and in vitro. For these studies we will use cutting edge approaches to determine the signaling in mutant versus wildtype cells and compare profiles between MDS, MDS-Autoimmune diseases, and healthy controls.

Impact: Identification and detailed characterization of genetic drivers of the autoimmune subtype of MDS, in particular UBA1 and VEXAS syndrome, has already led to improved clinical care and new insights into acquired bone marrow failure. Identification of UBA1 has provided diagnoses for thousands of individuals

within a short period of time and has led to curative bone marrow transplants and prospective clinical trials. We expect that further genetic studies in this previously overlooked cohort will uncover clinically important associations, have direct benefits for patients in terms of diagnosis and treatment, and will provide the basis for a molecular understanding of disease. Our in vitro and in vivo studies of the role of specific cell types and mutations into disease will help provide key insights into disease pathogenesis and provide potential targets for treatment approaches.

Proposal Title:	Harnessing RvE1 to Treat Bone Marrow Failure
Log Number:	BM220031
Current PI Name:	Katherine MacNamara
Award Number:	HT9425-23-1-0435
Current Contracting Organization:	Albany Medical College
Current Performing Organization:	Albany Medical College
Web Approval Date:	06-09-2023

Bone marrow failure (BMF) syndromes can be inherited or acquired and result in the failure to generate blood and immune cells. They are fatal if left untreated and the current therapeutic approach revolves around immunosuppressive therapies (IST) and hematopoietic stem cell (HSC) transplantation. There have been many successes in treating BMF in recent years, particularly for young patients where therapies are most effective. Unfortunately, older patients have reduced success with HSC transplantation and poorer responses to IST. Alternative therapies are needed because immune suppression is not tolerated well and carries increased risk of infection. Using a mouse model of severe aplastic anemia (AA) that mirrors many of the features of human disease, we identified a novel role for macrophages in the interferon-gamma-dependent loss of HSCs. Macrophages are equipped with machinery to induce and limit inflammation, and therefore have the capacity to restore homeostasis after acute inflammatory stress. In healthy tissues macrophages play an essential role in resolving inflammation by removing dead cells and releasing factors that aid in tissue repair. Our preliminary studies demonstrate macrophage dysfunction impairs resolution processes in AA, and reduced resolution correlates with an imbalance in pro-inflammatory and pro-resolving lipid mediators. Administration of pro- resolving lipid mediators was able to mitigate disease, demonstrating therapeutic efficacy of targeting resolution. We hypothesize that enhancing resolution ameliorates BMF and will improve IST by modulating macrophage function and lymphocyte activity in the BM and result in durable improvement of HSC function. The rationale for the proposed work is that rather than blunting inflammatory responses, promoting resolution and reparative processes may improve HSC function and normal blood production without impacting immunity and host defense. We will investigate how exogenous RvE1 impacts inflammation resolution, T cell function, and HSC function in an established mouse model of disease. The proposed studies are rooted in the conceptually innovative idea that resolution processes are delayed or absent in BMF, and the results of the proposed work may provide a novel approach to treat BMF without the use of immune suppression. We will also examine the ability of RvE1 to improve suboptimal IST treatments. These studies will be impactful by providing new therapeutic options for BMF patients, particularly older patients where IST has failed, and wherein host defenses are already weakened.

Proposal Title:	Mutational and Aging Risk Factors for Myelodysplastic Syndrome
Log Number:	BM220034
Current PI Name:	Pinkal Desai
Award Number:	HT9425-23-1-0431
Current Contracting Organization:	Joan and Sanford I Weill Medical College of Cornell University
Current Performing Organization:	Joan and Sanford I Weill Medical College of Cornell University
Web Approval Date:	06-12-2023

Myelodysplastic syndrome (MDS) is a bone marrow failure state that is acquired as people age and leads to low blood counts needing transfusion support, risk of infections, and sometimes conversion to Acute Myeloid Leukemia (AML). When MDS occurs after damage to bone marrow caused by chemotherapy or radiation as a result of cancer treatment, it is known as therapy-related myelodysplastic syndrome (t-MDS). This is a devastating complication of previous cancer therapy and is generally resistant to treatment with average cure rate of less than 10% in older patients. Most patients with MDS cannot go through bone transplant and even for those that do go through it, relapse rate is high. Large-scale gene sequencing studies in normal healthy populations have shown that people above 60 years have a high chance (30%) of having an age-related acquired mutation in peripheral blood. This condition has been termed as Clonal Hematopoiesis (CH). CH occurs as blood cells make mistakes dividing in an aging bone marrow. CH is associated with increased risk of MDS and AML as well as cardiovascular disease and it is estimated that although CH converts to blood cancer at the rate of 1% per year, majority of patients with CH don't progress to MDS. Understanding risk factors that put a patient with CH at high risk for transformation to MDS would be a major breakthrough in early detection, intervention, and prevention of MDS. We have identified that a "high risk" CH gene signature increases the future risk of AML by using samples collected a decade before diagnosis of AML in a population cohort, but this risk is not absolute.

The goal of this study is to determine the impact of aging risk factors in progression of CH to MDS. CH by itself is an aging-related phenomenon. It is well known that aging is linked to genetic instability and changes in gene function that can be measured by "methylation clocks." These methylation clocks can be used to estimate the biological age of individuals. Some individuals can be older as measured by the methylation clock compared to their actual age. This state is termed as epigenetic age acceleration (EAA). We found that people who have CH and EAA together are more likely have increased incidence and mortality from cardiovascular disease. In addition, CH can increase inflammation in our bodies and this in turn can help propagate the CH positive cells, making it a feedback loop. Our main hypothesis is that factors related to aging (Aim 1) and inflammation (Aim

2) can help determine the progression from CH to MDS and these factors can be identified so that risk can be better assessed.

To achieve these aims, we have identified two cohorts, the Women's Health Initiative and the Multiethnic Cohort that collected blood on healthy individuals and followed them for over 20 years for any cancer diagnoses. We have identified appx 600 participants who eventually developed MDS/t-MDS and will match these individuals with healthy normal controls that never developed any blood cancer. We will perform a custom CH gene sequencing developed at Weill Cornell Medicine to determine the presence of CH in blood collected many before diagnoses of MDS/t-MDS. We will also measure aging and inflammatory factors in peripheral blood collected at the same time as the DNA for the CH testing, all before the diagnosis of MDS. By comparing the mutational, aging, and inflammatory patterns to normal unaffected controls, we will be able to create "signatures" that are linked to a very high risk of progression to MDS/t-MDS. Through the study, we will be able to define the aging process that distinguishes participants with CH that are at highest risk of MDS/t-MDS, which can then allow the establishment of monitoring strategies as well as form the basis of inclusion in prevention-oriented clinical trials. Currently there are some trials of anti-inflammatory

drugs, vitamin C, and other targeted treatments that are being touted as agents for prevention of MDS but we do not know, to whom to deploy these treatments. Treating those that are not destined to progress to MDS is not ethical and exposing patients to unnecessary toxicities, while it is unfair if we do not intervene on individuals that are destined to progress. Our study would be able to distinguish these groups and have a major impact on prevention of MDS. In addition, many of the aging and inflammatory risk factors are modifiable and thus our study has the potential to provide a strategy for prevention. We are innovative in our idea as well as our solid population-based cohort with a large number of participants and decades of follow-up for any cancer outcomes.

Our preliminary data supports our hypothesis. We have already established that CH increases risk of blood cancers and that individuals with CH have a high prevalence of EAA and inflammatory cytokines and that these factors can increased risk of cardiovascular disease in individuals with CH. A similar strategy for MDS would be a breakthrough in early detection and prevention. Our proposed studies will address fiscal year 2022 Bone Marrow Failure Research Program IDEA Development Focus Area to Understand the causes and progression of BMF diseases. We have compiled an excellent, multidisciplinary team that has substantial expertise in CH, MDS, statistics, inflammation, and aging. We will leverage our combined expertise and experience to fulfill our aims.

Proposal Title:	Targeting Marrow Niche Dysregulation During Stem Cell Transplant for Bone Marrow Failure Associated with Monosomy 7 Myelodysplastic Syndromes
Log Number:	BM220037
Current PI Name:	Timothy Olson
Award Number:	HT9425-23-1-0569
Current Contracting Organization:	Children's Hospital, Philadelphia
Current Performing Organization:	Children's Hospital, Philadelphia
Web Approval Date:	09-14-2023

Background: Individuals born with gene mutations that cause bone marrow failBurMe 2sy2n0d0r3o7m: eTsim(BoMthFySO) alsreon at high risk for acquiring a pre-leukemia state in bone marrow (BM) known as myelodysplastic syndrome (MDS). The most severe bone marrow failure syndromes (BMFS) are associated with BM cells acquiring MDS defined by loss of chromosome 7 "monosomy 7") which initially improves impaired blood cell production. Unfortunately, monosomy 7 ultimately makes BM more likely to acquire more genetic changes that lead to leukemia. Patients with these BMFS require stem cell ("bone marrow") transplant to cure bone marrow failure or prevent/treat MDS or leukemia. At best, if Monosomy 7 is caught early, cure after transplant is seen in up to 60% of patients. Patients with advanced Monosomy 7 MDS/AML and additional high-risk features have cure rates as low as 10%.

During transplant, interactions between donor stem cells (the "seeds") and the patient’s own specialized bone marrow support cells known as niches (the "soil") are required for blood and immune recovery ("engraftment"). In prior studies, our research team has found that in BMFS associated with monosomy 7, the same genetic mutation that causes BM failure in the patient’s blood stem cells that we replace by stem cell transplant, also causes niche ("soil") dysfunction in cell types that are not replaced by conventional transplant. This poor niche function impairs donor engraftment, explaining why patients with BMFS associated with monosomy 7 are at high risk for transplant failure and even death caused by poor graft function and relapse.

The critical problem/question our proposal addresses is how genetic mutations that predispose to BMFS associated with monosomy 7 prevent normal niche ("soil") function during stem cell transplant. This knowledge will allow us to develop new therapies targeting restoration of niche function to improve outcomes of stem cell transplant for patients with severe BMFS.

Our primary hypothesis is that our novel mouse and patient-derived cell models of two severe BMFS associated with monosomy 7, Shwachman-Diamond Syndrome and SAMD9/SAMD9L syndromes, will prove that decreased production and survival of bone-forming cells ("osteoprogenitors") within bone marrow niches is the primary cause of impaired host ability to engraft donor cells efficiently and durably during transplant.

Our two primary objectives address both of the fiscal year 2022 Bone Marrow Failure Research Program Idea Development Award Focus Areas:

• Understand the causes and progression of BMF diseases: Our proposal seeks to define the cellular and molecular mechanisms that explain why, in severe BMFS associated with monosomy 7, the patient's own bone marrow environment is dysfunctional and fails to support baseline blood cell production and reliable donor engraftment after stem cell transplantation.

• Find effective BMF treatments and cures: Understanding how stem cell transplant outcomes for severe BMFS can be compromised by poor bone marrow niche ("soil") function is a critical first step to defining strategies to improve treatment outcomes. In our proposal, we will test several strategies to improve niche function during transplant in our models of Shwachman-Diamond and SAMD9/SAMD9L syndromes.

Innovation: Our proposal is innovative in that while most research devoted to improving stem cell transplant outcomes for BMFS focuses on preventing infections, immune rejection, and graft versus host disease, we are focused on targeting the patient's own bone marrow niches ("soil") to improve the quality of donor blood and immune production after transplant regardless of the type of donor available. Additional innovations include developing novel animal and patient-derived cell models of BMFS, use of cutting-edge gene expression techniques to precisely define the bone marrow environment during transplant, and development of a screening platform to identify strategies to improve transplant outcomes for BMFS.

Impacts:

• Patients helped by this research: Short-term impacts of this research are focused on patients with the many BMFS associated with Monosomy 7. Long-term, knowledge gained of pathways that can be targeted to improve engraftment could help any patient with a BMFS who requires stem cell transplant.

Benefit to the BMF Research Field: Short-term, our research will provide valuable insights to understand how the bone marrow environment that is not replaced by stem cell transplant adapts to support donor blood cell production during transplant, and how this support is affected by gene mutations that cause BMFS. It will also provide valuable new animal and patient-derived BMFS cell models to the BMFS research field. Long-term, results from our proposed translational strategies targeting bone-forming "osteoprogenitors" to improve niche support of blood cell production will provide fundamental knowledge and a framework for additional preclinical studies and clinical trials to improve BMFS outcomes.

Proposal Title:	Epigenetic Determinants of Corticosteroid Responsiveness in Diamond Blackfan Anemia Syndrome
Log Number:	BM220045
Current PI Name:	Jason Farrar
Award Number:	HT9425-23-1-0640
Current Contracting Organization:	Arkansas Children's Hospital Research Institute
Current Performing Organization:	Arkansas Children's Hospital Research Institute
Web Approval Date:	07-27-2023

Diamond Blackfan anemia syndrome (DBAS) is an inherited bone marrow failure syndrome (IBMFS) where inadequate red blood cell production is the major abnormal finding. Thus, many DBAS patients have life-long, severe anemia that requires treatment with chronic steroid medications, regular red blood cell transfusions, or bone marrow transplantation. Like other IBMFSs, DBAS patients are also more likely to have an array of specific physical developmental defects identified after birth and are predisposed to increased rates of development of certain cancers throughout their lives.

Intensive research over the past 20+ years has defined the genetic defect in the majority of patients. DBAS genetics are truly complicated, with mutations in over 27 genes identifiable in roughly 80% of DBAS patients. The common thread running among these gene mutations is that nearly all reduce production of the ribosome, which is the site of protein production in all cells. Since normal cell growth and function in all tissues requires adequate protein production, the reason that DBAS patients show mostly anemia is not well understood. This finding suggests that, at least in many non-bone marrow tissues, there are innate compensatory mechanisms that can overcome protein production defects.

In addition to defining the molecular site of the underlying defect in DBAS, these genetic studies have enabled detailed study of how DBAS gene mutations develop, pass, and are shared among family members. These studies have revealed that DBAS gene mutations are often present in family members without anemia, underscoring and expanding the clinical observation that DBAS disease manifestations can be quite variable.

One commonly observed variable feature lies in individual patient responses to medical therapies. Chronic steroid treatment has been used in DBA for over 60 years, but not all patients benefit from this treatment modality. Some patients do not respond at all, some respond but the high doses required to correct the anemia are too toxic, some can stay on very low dose steroids for very long periods with minimal or no anemia, and some who have been responsive to steroids over many years will ultimately lose that response in adulthood. The basis for this variability in steroid response specifically, and other, more generally observed features of DBAS such as clinically unaffected family members, is not understood. However, these observations have a very important implication with direct relevance to therapy for anemia in DBAS: A ribosomal gene mutation is not a mandatory sentence to severe anemia. In many patients, the developing red cells clearly employ intrinsic compensatory mechanisms that are conceptually if not mechanistically akin to compensation in non-bone marrow tissues.

Understanding the variable steroid response in DBAS will have direct application in the Bone Marrow Failure Research Program Idea Development Focus Area of finding effective BMF treatments and cures in several ways. Features that regulate this response could immediately be used to as biomarkers for diagnostic testing to predict steroid response, thus avoiding toxic steroid exposure in many DBAS patients. Identification of the key steroid regulator pathways would permit design and testing of novel therapeutic approaches to “switch on” steroid response, thus allowing combination therapies in previously nonresponsive patients. A similar strategy could augment steroid response, thus permitting reduced and less

toxic doses of steroids to be used broadly in DBAS. Finally, such red cell escape pathways would be ideal targets for gene therapy in DBA, where the high number of involved genes presently precludes direct therapy of the mutated DBAS gene in all but the most common types.

One of the primary limitations to understanding this response has been availability of appropriate scientific models to probe these differences. An important innovative aspect of this proposal is direct use of DBAS-derived red cells to probe the regulation of steroid response. Another innovative aspect lies in our hypothesis that changes in the regulatory code (epigenetic features) present in the developing red cells regulate this variability, rather than additional genetic changes, such as cooperating mutations. Recent studies show an interesting and likely related phenomenon in how red cell precursors from normal donors vary in their responsiveness to steroids by stem cell source from either adult or umbilical cord blood. This type of developmental switch is also highly likely to be regulated by epigenetic mechanisms. We will investigate the similarities and differences in this system compared to DBAS primary cells to determine relevance as a model that could be used to further test interventional strategies to engage (or re-engage) red cell compensatory mechanisms. Finally, this proposal is innovative in the incorporation of newly developed genome-wide epigenetics and systems biology approaches to interrogation of epigenetic changes in DBAS variability.

This work would provide both immediate and long-lasting impact in the field of DBAS, initially through development of clinical tests to predict steroid responsiveness in patients and subsequently through identification and testing of novel treatment strategies to engage erythroid escape pathways, to promote steroid response in nonresponders, to design steroid-sparing therapies in other DBAS patients, as well as novel targets for gene therapy with relevance to a broad array of genotypes.

Proposal Title:	Measurement and Impact of Body Composition Using CT Imaging in Patients with Germ Cell Tumors Undergoing Chemotherapy on the NCTN Phase 3 Study AGCT1531
Log Number:	CA220026
Current PI Name:	Tyler Ketterl
Award Number:	HT9425-23-1-0836
Current Contracting Organization:	Seattle Children's Research Institute
Current Performing Organization:	Seattle Children's Research Institute
Web Approval Date:	09-14-2023

Fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) Topic Area: Germ cell cancers

FY22 PRCRP Military Heath Focus Areas: (1) Mission readiness: Gaps in prognosis and treatment that may impact mission readiness and the health and well-being of military members, Veterans, their beneficiaries, and the general public. (2) Mission readiness: Gaps in quality of life and survivorship that may impact mission readiness and the health and well-being of military members, Veterans, their beneficiaries, and the general public.

FY22 PRCRP Overarching Challenges: (1) Transform cancer treatment through the identification of novel biomarkers and new targets, especially for advanced disease (metastatic), and eliminate the risks of therapy associated toxicity. (2) Develop strategies and biomarkers to predict cancer risk, treatment resistance, recurrence, and advanced disease to mitigate risk in target populations.

Scientific Objective and Rationale: Malignant germ cell tumors are the most common solid tumor in adolescents and young adults. They are highly curable, yet current treatment regimens often come with unacceptable rates of toxicities. The current treatment regimens, which include a medication called cisplatin, are known to have significant short- and long-term toxicities, including permanent irreversible hearing loss and damage to the kidneys, which, in recent studies, have been shown to continue to worsen after treatment has been completed.

The change in body composition (percentage of the body that is fat, skeletal muscle, and bone) over the course of treatment has not been previously studied in pediatric, adolescent, and young adult patients with germ cell tumors, and the potential role of body composition in predicting those who will develop adverse side effects from chemotherapy reactions remains a gap in knowledge. We hypothesize that body composition at diagnosis and changes in body composition during the course of chemotherapy, are predictive of the development of toxicity to chemotherapy. While it is known that different factors impact how our bodies break down chemotherapy drugs, one factor called body composition, which looks at the amount of muscle and fat an individual contains, has been overlooked. In two individuals with the same height and weight, but who differ in the components of muscle and fat tissue, there will likely be very significant differences in response to chemotherapy based on the impact of how different types of body tissue break down the drug. The importance of body composition and its role in chemotherapy toxicity has been demonstrated in adults with colon cancer treated with a drug called 5-Fluorouricil.

To date, there has been no evaluation of how skeletal muscle characteristics affect outcome or toxicity in patients receiving chemotherapy for malignant germ cell tumors. The ongoing study AGCT1531, offers a

diverse population with robust data collection including imaging scans, hearing exams, detailed toxicity, and outcome data to investigate this question. In this proposed imaging study, evaluation of skeletal muscle quality and quantity will be determined using baseline computed tomography (CT) scans collected for study purposes. Finally, we will use a new automated system to analyze body composition from CT and compare this to manual methods.

Impact: This study will deliver in the near-term important and new research into the treatment of germ cell tumors. By embedding this research within a large and already rapidly recruiting clinical trial will allow this work to be completed within the next 4 years and will provide unique insights into side effects from chemotherapy and survivorship after treatment is complete. This study has the opportunity of completely change how chemotherapy is dosed in the future for germ cell tumors, and potentially for many other tumors in the future. This may lead to further refinement of prognostic/stratification criteria, support development of targeted exercise interventions, and investigation of alternate dosing strategies. In addition, the use of CT software to quickly calculate body composition measures may have important clinical applications across many treatments and disease types affecting pediatric, adolescent, and young adult patients.

Relevance to Military Health: This work will have direct impact on the military health Focus Area of Mission Readiness, in that it can change how chemotherapy is dosed in the future in order to reduce late effects in military personnel, such as hearing loss and neuropathy due to platinum chemotherapy. Germ cell tumors are an important cancer in the young adult military-age population and prevention of hearing loss and neuropathy will allow for better quality of life and survivorship for military members, and a return to Mission Readiness post-therapy of their cancer.

Proposal Title:	Measurement and Impact of Body Composition Using CT Imaging in Patients with Germ Cell Tumors Undergoing Chemotherapy on the NCTN Phase 3 Study AGCT1531
Log Number:	CA220026P1
Current PI Name:	Christina Dieli-Conwright
Award Number:	HT9425-23-1-0837
Current Contracting Organization:	Dana-Farber Cancer Institute
Current Performing Organization:	Dana-Farber Cancer Institute
Web Approval Date:	09-14-2023

Fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) Topic Area: Germ cell cancers

FY22 PRCRP Military Heath Focus Areas: (1) Mission readiness: Gaps in prognosis and treatment that may impact mission readiness and the health and well-being of military members, Veterans, their beneficiaries, and the general public. (2) Mission readiness: Gaps in quality of life and survivorship that may impact mission readiness and the health and well-being of military members, Veterans, their beneficiaries, and the general public.

FY22 PRCRP Overarching Challenges: (1) Transform cancer treatment through the identification of novel biomarkers and new targets, especially for advanced disease (metastatic), and eliminate the risks of therapy associated toxicity. (2) Develop strategies and biomarkers to predict cancer risk, treatment resistance, recurrence, and advanced disease to mitigate risk in target populations.

Scientific Objective and Rationale: Malignant germ cell tumors are the most common solid tumor in adolescents and young adults. They are highly curable, yet current treatment regimens often come with unacceptable rates of toxicities. The current treatment regimens, which include a medication called cisplatin, are known to have significant short- and long-term toxicities, including permanent irreversible hearing loss and damage to the kidneys, which, in recent studies, have been shown to continue to worsen after treatment has been completed.

The change in body composition (percentage of the body that is fat, skeletal muscle, and bone) over the course of treatment has not been previously studied in pediatric, adolescent, and young adult patients with germ cell tumors, and the potential role of body composition in predicting those who will develop adverse side effects from chemotherapy reactions remains a gap in knowledge. We hypothesize that body composition at diagnosis and changes in body composition during the course of chemotherapy, are predictive of the development of toxicity to chemotherapy. While it is known that different factors impact how our bodies break down chemotherapy drugs, one factor called body composition, which looks at the amount of muscle and fat an individual contains, has been overlooked. In two individuals with the same height and weight, but who differ in the components of muscle and fat tissue, there will likely be very significant differences in response to chemotherapy based on the impact of how different types of body tissue break down the drug. The importance of body composition and its role in chemotherapy toxicity has been demonstrated in adults with colon cancer treated with a drug called 5-Fluorouricil.

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Impact: This study will deliver in the near-term important and new research into the treatment of germ cell tumors. By embedding this research within a large and already rapidly recruiting clinical trial will allow this work to be completed within the next 4 years and will provide unique insights into side effects from chemotherapy and survivorship after treatment is complete. This study has the opportunity of completely change how chemotherapy is dosed in the future for germ cell tumors, and potentially for many other tumors in the future. This may lead to further refinement of prognostic/stratification criteria, support development of targeted exercise interventions, and investigation of alternate dosing strategies. In addition, the use of CT software to quickly calculate body composition measures may have important clinical applications across many treatments and disease types affecting pediatric, adolescent, and young adult patients.

Relevance to Military Health: This work will have direct impact on the military health Focus Area of Mission Readiness, in that it can change how chemotherapy is dosed in the future in order to reduce late effects in military personnel, such as hearing loss and neuropathy due to platinum chemotherapy. Germ cell tumors are an important cancer in the young adult military-age population and prevention of hearing loss and neuropathy will allow for better quality of life and survivorship for military members, and a return to Mission Readiness post-therapy of their cancer.

Proposal Title:	Measurement and Impact of Body Composition Using CT Imaging in Patients with Germ Cell Tumors Undergoing Chemotherapy on the NCTN Phase 3 Study AGCT1531
Log Number:	CA220026P2
Current PI Name:	Shahrad Rassekh
Award Number:	HT9425-23-1-0838
Current Contracting Organization:	British Columbia, University of
Current Performing Organization:	British Columbia, University of
Web Approval Date:	09-14-2023

Fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) Topic Area: Germ cell cancers

FY22 PRCRP Military Heath Focus Areas: (1) Mission readiness: Gaps in prognosis and treatment that may impact mission readiness and the health and well-being of military members, Veterans, their beneficiaries, and the general public. (2) Mission readiness: Gaps in quality of life and survivorship that may impact mission readiness and the health and well-being of military members, Veterans, their beneficiaries, and the general public.

FY22 PRCRP Overarching Challenges: (1) Transform cancer treatment through the identification of novel biomarkers and new targets, especially for advanced disease (metastatic), and eliminate the risks of therapy associated toxicity. (2) Develop strategies and biomarkers to predict cancer risk, treatment resistance, recurrence, and advanced disease to mitigate risk in target populations.

Scientific Objective and Rationale: Malignant germ cell tumors are the most common solid tumor in adolescents and young adults. They are highly curable, yet current treatment regimens often come with unacceptable rates of toxicities. The current treatment regimens, which include a medication called cisplatin, are known to have significant short- and long-term toxicities, including permanent irreversible hearing loss and damage to the kidneys, which, in recent studies, have been shown to continue to worsen after treatment has been completed.

The change in body composition (percentage of the body that is fat, skeletal muscle, and bone) over the course of treatment has not been previously studied in pediatric, adolescent, and young adult patients with germ cell tumors, and the potential role of body composition in predicting those who will develop adverse side effects from chemotherapy reactions remains a gap in knowledge. We hypothesize that body composition at diagnosis and changes in body composition during the course of chemotherapy, are predictive of the development of toxicity to chemotherapy. While it is known that different factors impact how our bodies break down chemotherapy drugs, one factor called body composition, which looks at the amount of muscle and fat an individual contains, has been overlooked. In two individuals with the same height and weight, but who differ in the components of muscle and fat tissue, there will likely be very significant differences in response to chemotherapy based on the impact of how different types of body tissue break down the drug. The importance of body composition and its role in chemotherapy toxicity has been demonstrated in adults with colon cancer treated with a drug called 5-Fluorouricil.

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diverse population with robust data collection including imaging scans, hearing exams, detailed toxicity, and outcome data to investigate this question. In this proposed imaging study, evaluation of skeletal muscle quality and quantity will be determined using baseline computed tomography (CT) scans collected for study purposes. Finally, we will use a new automated system to analyze body composition from CT and compare this to manual methods.

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Relevance to Military Health: This work will have direct impact on the military health Focus Area of Mission Readiness, in that it can change how chemotherapy is dosed in the future in order to reduce late effects in military personnel, such as hearing loss and neuropathy due to platinum chemotherapy. Germ cell tumors are an important cancer in the young adult military-age population and prevention of hearing loss and neuropathy will allow for better quality of life and survivorship for military members, and a return to Mission Readiness post-therapy of their cancer.

Proposal Title:	Neoplasia-Derived Extracellular Vesicle microRNAs Cause DNA Damage to Promote Carcinogenesis in Barrett Esophagus: Novel Targets for Chemical Ablation
Log Number:	CA220069
Current PI Name:	Ajay Bansal
Award Number:	HT9425-23-1-0385
Current Contracting Organization:	Kansas, University of, Medical Center Research Institute, Inc.
Current Performing Organization:	Kansas, University of, Medical Center Research Institute, Inc.
Web Approval Date:	09-14-2023

Our proposal addresses the fiscal year 2021 Peer Reviewed Cancer Research Program Topic Area of esophageal cancer focusing on esophageal adenocarcinoma (EAC). This lethal malignancy has affected approximately 8,000 Veterans over the last decade, 85% of whom died within 2 years of the diagnosis. EAC develops from Barrett's esophagus (BE), the condition in which the normal squamous lining of the esophagus is replaced by a metaplastic intestinal-type lining. Other risk factors for EAC are gastroesophageal reflux disease (GERD), obesity, and smoking, all of which are inordinately common conditions in Veteran patients. The prognosis for patients with EAC is strongly related to cancer stage at the time of diagnosis. The overall mean 5-year survival rate for EAC is a dismal 18%, but exceeds 80% for those with early cancers. Early cancers arising from BE can be treated with an endoscopic procedure that resects the cancer followed by radiofrequency ablation (RFA), an endoscopic procedure that burns away the cancer-prone metaplastic BE lining. However, RFA is fraught with complications, and early cancer recurs in 15% of patients. Thus, a better way to destroy Barrett's cells surrounding an early cancer is needed.

Our proposal will test a new idea that exploits the presence of DNA damage within BE cells surrounding the cancer. Intact DNA is necessary for healthy cells, but when DNA is damaged cells can progress to cancer.

However, this DNA damage makes the cells more vulnerable to treatments that selectively attack those cells. Our preliminary experiments demonstrate that EAC cancer cell lines release small vesicles termed extracellular vesicles (EV) that contain molecular cargo that signals to surrounding BE cells, causing DNA damage through a process called minority mitochondrial outer membrane permeabilization (MOMP). Ordinarily, cells repair DNA damage via homologous recombination (HR), but this pathway is also disrupted in the BE cells exposed to EAC EV. Long-term accumulation of such DNA damage can eventually result in cancer development of those BE cells. We found that the molecular cargo of the EAC EVs might be microRNAs, small fragments of genomic material that inhibit the expression of genes involved in MOMP and HR. Using blood from Veteran patients with EAC, we isolated EV from the blood and found that they contain a number of microRNAs that are similar to those found in the EAC cancer cell EVs, and that they caused DNA damage in BE cells while inhibiting its repair. To stop BE cells with DNA damage from becoming cancer, we can use a new cancer drug that inhibits a molecule called PARP, which BE cells with DNA damage need to survive. If we inhibit PARP, then DNA damage in the BE cell worsens to a point that kills them. Our proposal aims to understand the molecular events of how DNA damage occurs in BE cells exposed to EAC EV and if inhibiting PARP can selectively kill those BE cells at the highest risk of developing cancer. Thus, we hypothesize that microRNAs within neoplasia-derived EV initiate minority MOMP and inhibit HR, mechanisms that promote neoplastic progression of benign BE cells by causing DNA damage while blocking its repair. The aims of this study are to elucidate how BE neoplasia-derived EV cause DNA damage and impede DNA damage repair of benign BE cells and promote their neoplastic progression and to assess the potential of inhibitors of PARP to ablate BE surrounding a nidus of neoplasia.

Instead of using RFA, which indiscriminately kills BE cells as well as normal cells in the esophagus and causes a number of complications, our results will help determine if PARP inhibition is a viable therapeutic approach. Our ultimate goal is to develop tailored treatments to selectively kill BE cells at high risk for cancer progression, thus stemming the rising incidence of EAC in our Veterans and preventing EAC-related deaths among Veterans and non-Veterans with BE.

Proposal Title:	Combining mRNA Nanotherapy with Standard Antiangiogenic/Immuno Therapy for HCC
Log Number:	CA220079
Current PI Name:	Jinjun Shi
Award Number:	HT9425-23-1-0846
Current Contracting Organization:	Brigham and Women's Hospital, Inc.
Current Performing Organization:	Brigham and Women's Hospital, Inc.
Web Approval Date:	09-14-2023

The fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) Topic Area addressed by our research project is Liver Cancer.

The objective of this Impact Award proposal is to develop a transformative treatment strategy for hepatocellular carcinoma (HCC), which is the most common type of liver cancer. Targeting the immune system of cancer patients with the so called "immune checkpoint blockers" has revolutionized cancer treatment, but the benefits for patients with advanced HCC have been limited so far. In part, this is because existing treatments do not directly target the cancer cells themselves, but rather target the tumor vasculature and the immune system. Consequently, the majority of HCCs still do not respond to or are likely to recur after these therapies. Our proposal specifically aims to improve the existing treatment by developing a new approach to target a widely mutated tumor suppressor gene p53 using nanoparticles to more effectively deliver the therapy to the liver cancer cells.

The loss/mutation of p53 gene has a causal role in the development and progression of a wide range of cancers. Recent clinical data reveal the mutation of p53 in approximately one-third of HCC patients, ranking as the most common among the altered genes. Interestingly, increasing evidence also suggests a key role of p53 in regulating the antitumor immune responses. Our latest research work supported by Department of Defense PRCRP Idea Award with Special Focus (2019-2021) has shown that: (i) nanoparticle-mediated delivery of mRNA for p53 is feasible and restores p53 protein expression in HCC cells; and (ii) combining p53 mRNA nanotherapy with immunotherapy results in markedly extending the duration of survival as well as reduced morbidity of the disease, without eliciting any detectable toxicities in state-of-the-art models of HCC in mice.

In this project, we will develop a translatable lipid nanoparticle (LNP) platform for p53 mRNA delivery to HCC and evaluate its combination with the new standard of care therapy, which targets the tumor vasculature and the immune system. The goal is to prove that this new strategy is feasible and can achieve more durable responses to treatment in HCC models. Of note, this LNP platform is similar to the LNP clinically approved for short inhibitory RNA therapy or COVID-19 mRNA vaccination. Importantly, our strategy will be more selective for HCC tissue and have much higher accumulation and retention in the liver cancer. We expect the unique combination strategy proposed herein to be rapidly advanced to clinical development upon successful completion of this project, and to have a major impact on the survival and quality of life of HCC patients with tumors driven by p53 gene mutation over the next 3 years through our network of clinical collaborators and patient advocates.

A recent study demonstrates that male U.S. military Veterans had an increased frequency of HCC at autopsy compared with the general population, likely due to more exposure to different risk factors, such as transfusion of blood products, intravenous drug use, hemodialysis, ear piercing in men, and organ

transplants. We expect the proposed research to benefit HCC patients in general but to have a particularly profound impact on the health of U.S. military Veterans who are impacted by the environmental exposure risk factors associated with cancer – one of the FY22 PRCRP Military Health Focus Areas.

This proposal is therefore also in accordance with the following two FY22 PRCRP Overarching Challenges: (i) improve treatment and outcomes for patients in underserved or under-recognized populations, and (ii) advance immunotherapy.

Proposal Title:	Targeting the SRPK1-AKT Signaling Axis in Endometrial Cancer
Log Number:	CA220096
Current PI Name:	James Duncan
Award Number:	HT9425-23-1-0751
Current Contracting Organization:	Institute for Cancer Research
Current Performing Organization:	Institute for Cancer Research
Web Approval Date:	09-14-2023

Endometrial carcinoma (EC), a cancer of the endometrium (the lining of the uterus), is the most common gynecologic malignancy in the United States, with 65,950 new cases and 12, 550 deaths expected in 2022. Strikingly, there has been an approximate 25 % increase in deaths due to endometrial cancer over the last 6 years (10,470 deaths in 2016), highlighting the importance of studying this deadly disease. Uterine serous carcinoma (USC), is the deadliest form of EC that accounts for approximately 80% of all EC-associated deaths. The current treatment for advanced USC includes debulking surgery and paclitaxel/platinum-based chemotherapies. Despite high initial response, the majority of patients relapse within 3 years, for which there are no follow-up treatments. The high mortality rate of USC patients is due to frequent drug resistance and metastasis, which is the spreading of cancer cells from the primary tumor site to other regions of the body. Thus, there is an urgent need to identify new drug targets and to develop new drug treatments that can be rapidly translated into clinical trials for the treatment of USC.

Targeted therapies are cancer treatments that use drugs to selectively block the function of one or more cancer genes and are often associated with personalized medicine. One of the most promising classes of targeted therapies are protein kinase inhibitors – drugs that block the function of a specific class of enzymes that help cancers cells grow, survive, and metastasize. These kinases are highly amenable to drug development, making the design and translation of kinase inhibitors for cancer therapies clearly attainable. Protein kinase inhibitors have shown dramatic therapeutic responses in cancer patients, causing complete regression of tumors that were previously untreatable, including cancers that have spread throughout the body. There are over 500 protein kinases in cancer cells, and many of these kinases have already been shown to be effective anticancer targets. Importantly, more than 60 kinase inhibitors are currently U.S. Food and Drug Administration-approved for the treatment of cancer, with several hundred in clinical trials for a variety of cancers. Our laboratory has expertise in the study of protein kinases in cancer and possesses unique technologies that allow us to identify new kinase targets for the treatment of cancer. Using these technologies, our lab recently discovered a new kinase target for USC called SRPK1, which we believe represents a very promising new drug target for the treatment of USC. Following a detailed characterization of this kinase in endometrial cancer cell models, we discovered it was important for many key cancer-related processes, and if we blocked its function using kinase inhibitors, we could prevent cancer growth and survival. Importantly, in preliminary in vivo studies using USC mouse models, we showed treatment of mice with drugs targeting SRPK1 was safe, tolerable, and had tumor inhibiting properties, highlighting SRPK1 as a promising new therapeutic avenue for USC. Additionally, our lab discovered that combining SRPK1 inhibitors with another kinase inhibitor that targets the protein kinase AKT, improved growth repression and killing of EC cells. Notably, AKT is overactivated in the majority of EC; however, a recent clinical trial evaluating AKT inhibitors in USC patients showed blocking AKT function in tumors had minimal clinical benefit in USC patients. Importantly, our preliminary findings suggest that the addition of SRPK1 inhibitors could improve the antitumor responses elicited by AKT inhibitors for the treatment of USC patients, which could be rapidly translated into clinical trials.

Based on our preliminary studies detailed above, we hypothesize SRPK1 represents a promising drug candidate in combination with AKT inhibitors for the treatment of USC that could have dual tumor inhibiting and anti metastatic benefits for EC patients. In the proposed studies, we will test these hypotheses by defining how exactly SRPK1 and AKT controls growth and metastasis of EC cells, and use technologies

to establish the scientific rationale for pursing SRPK1-AKT combination therapies in EC patients. We will then test the safety and efficacy of SRPK1-AKT combination therapies to inhibit tumor growth and metastasis in vivo using animal models of EC.

Finally, we will determine the effect of SRPK1-AKT combination therapies on the tumor microenvironment and immunotherapy response using a new technology, whereby EC patient tumors and tumor-associated cells are grown ex vivo in microfluidic chambers. If successful, our studies will make a significant impact on EC treatment by identifying a novel kinase inhibitor therapy that could reduce tumor growth and metastasis, improving overall quality of life and survival of military Service Members, Veterans, and their beneficiaries. Moreover, our comprehensive characterization of the functional relationship amongst SRPK1 and AKT function in EC cells could provide valuable new insights into the cellular mechanisms and biological processes controlling metastasis that could lead to new discoveries and therapies for EC, as well as other cancers. Once we've established the safety and efficacy of SRPK1-AKT inhibitor combination therapies to block tumor growth and metastasis in preclinical tumor models of EC, we will actively pursue an investigational new drug (IND) using SRPK1 inhibitor SPHINX31 in combination with MK-2206 in a phase 1 trial for EC patients at Fox Chase Cancer Center that will be led by Co-Investigator Dr. Gina Mantia-Smaldone.

Proposal Title:	Determination of Immunotoxin GB13 Intratumoral Drug Distribution for the Treatment of Adult and Pediatric Brain Cancers
Log Number:	CA220110
Current PI Name:	Randy Schrecengost
Award Number:	HT9425-23-1-0681
Current Contracting Organization:	Targepeutics, Inc.
Current Performing Organization:	Mayo Clinic
Web Approval Date:	09-14-2023

Brain cancer is a deadly diagnosis that affects adults and children. We are focused on how to improve the outcomes for adult glioblastoma (GBM) and pediatric diffuse midline glioma (DMG) patients, who have very few treatment options and a poor survival prognosis. These cancer types are of interest because nearly 80% express a receptor called the interleukin 13 receptor alpha 2 (IL13Ra2). This receptor is not found on noncancerous cells of the body and, as such, we have developed a drug that binds this receptor. GB13 is an immunotoxin therapy that specifically targets IL13Ra2 on cancer cells and combines a toxin component that provides very sensitive cancer killing capability. Based on preliminary studies, GB13 has been granted Orphan Drug designation for malignant glioma brain cancers and is ready to be moved into clinical trials.

One of the big issues with brain cancer treatments is how to get the drug to the tumor, because most drugs given intravenously cannot reach the brain. Therefore, we are developing GB13 to be administered directly into the brain tumor by a process called convection enhanced delivery (CED), which infuses a therapy over many hours with catheters. CED has been studied for many years, is safe for this kind of application, and limits toxicity compared to giving a drug through the blood stream. One of the limitations of CED is that many studies do not evaluate tumor distribution during infusion, which negatively impacts trial results and hinders clinical success. This is one recognized reason why CED-based therapies have failed despite there being preclinical steps that can be taken to answer this question. We are committed to performing the studies required to determine GB13 distribution to perform the most successful clinical trial possible.

This proposal will address three main objectives. First, Targepeutics' biologic therapy, GB13, will be labeled with a radioactive molecule to enable positron emission tomography (PET) imaging. Second, labeled GB13 will be administered to adult GBM and pediatric DMG animal tumors to determine the co-distribution with clinically validated magnetic resonance imaging (MRI) molecules. Finally, to address requirements for clinical trial approval, GB13 systemic distribution, and maximum dosing will be analyzed in animal models.

Overall, successful completion of these studies will result in a phase 1 clinical trial application for GB13 treatment of adult GBM and pediatric DMG. We will learn how to visualize the distribution of GB13 that will be used in the clinic and is critical for understanding trial data. Although there are many clinical trials that use CED, none have been approved by the U.S. Food and Drug Administration. One of the main reasons is because the distribution is unclear. We hypothesize that the answers gained from this Department of Defense Impact Grant will positively impact thousands of brain cancer patients each year. Because we are incorporating studies needed for clinical trial approval, we will be prepared to enter clinical trials no later than the conclusion of this grant. This grant represents some of the last remaining tests before we can submit the phase 1 clinical trial design.

The clinical applications of this proposal will directly impact the Peer Reviewed Cancer Research Program (PRCRP) Topic Areas of "brain cancer" and "pediatric brain tumors" by directly providing information that is critical for successful delivery of GB13 to the brain. This application will address the Overarching Challenge category of "Therapeutics" and the critical gap of "advancing immunotherapy across the different

PRCRP Topic Areas." These studies will address the military health focus of "environmental exposure risk factor associated with cancer," as there is a correlation identified in Veterans of the Gulf War, who are found to be at an increased risk for brain cancer and brain cancer related death due to nerve gas exposure in Iraq.

Together with the comprehensive research team from the Mayo Clinic that is united on performing these tests to support clinical development, Targepeutics is confident in the research strategy, the feasibility of completion, and the direct impact to improving the lives of adult and pediatric brain cancer patients.

Proposal Title:	Using Genetics to Identify New Endometrial Cancer Therapeutics
Log Number:	CA220131
Current PI Name:	Tracy O'Mara
Award Number:	HT9425-23-1-0764
Current Contracting Organization:	Queensland Institute of Medical Research
Current Performing Organization:	Queensland Institute of Medical Research
Web Approval Date:	09-14-2023

Endometrial cancer (fiscal year 2022 [FY22] Peer Reviewed Cancer Research Program [PRCRP] Topic Area) is cancer of the uterus lining and the fourth most commonly diagnosed cancer amongst American women, with around 60,000 people expected to be diagnosed in 2022. The number of people diagnosed with endometrial cancer each year is projected to double by 2030, thus representing a major health issue. Despite rising numbers of people being diagnosed and dying of endometrial cancer, it remains underfunded compared with other cancer types. This is reflected in the lack of new endometrial cancer drugs, with only three drugs approved for use for endometrial cancer treatment in the last 50 years.

It is clear that there is a need for new and innovative approaches for endometrial cancer drug discovery, especially as survival rates have not improved for decades and there is a lack of treatment options. We hypothesize that using large-scale genetic information we will find genes that cause endometrial cancer and provide opportunities for development of effective drug therapies. This approach to the identification of drug targets is supported by the recent observation that targets with genetic evidence are up to four times more likely to lead to a drug that is approved for clinical use.

Our project will use state-of-the-art endometrial models called organoids (miniaturized 3D structures resembling tissue) to study the role of genetics in endometrial cancer development. These organoids, also known as "organs in a dish," more accurately represent human endometrial tissue and tumors than commonly used models. Our study will be performed using organoids representing normal endometrium, a noncancerous endometrial cancer precursor and different types of endometrial tumors (including those associated with more aggressive disease). We will perform large-scale genetic experiments on these organoids to identify genetic features that affect the regulation of genes (Aim 1). We will then target genes that are at regions associated with endometrial risk and identify those that affect the development and growth of organoids (Aim 2). Lastly, the information from Aim 1 and Aim 2 will be integrated together with genetic information derived from endometrial cancer patients to identify which genes likely cause endometrial cancer and may provide effective targets for drug therapy (Aim 3).

This project will study the FY22 PRCRP Overarching Challenge: Therapeutics Category, Identify and elucidate the mechanisms behind cancer epigenetics/genetics and cancer development to improve treatment methods. Through the identification of drug targets that underlie endometrial cancer genetics, the short-term impact of this research will be to set in motion the development of new treatments. The first step will be to assess the targets uncovered by our research for therapeutic opportunities from repurposing existing drugs that are already used to treat people or new drugs identified from screening large libraries of compounds. These future studies will provide lead drug candidates for clinical trials to identify effective treatments for endometrial cancer patients.

Current treatment options available to endometrial cancer patients are limited, usually hysterectomy followed by additional treatment (e.g., chemotherapy or radiotherapy) for those patients with aggressive or advanced disease. Increasing the number of treatment options through the development of new drugs will significantly improve patient outcomes for those who do not respond to such additional treatments. Notably, this group of patients proportionally includes more Black patients, who are more frequently diagnosed with aggressive disease and twice more likely to die from endometrial cancer than white patients.

This project addresses the FY22 PRCRP Military Health Focus Area: Gaps in cancer research that may affect mission readiness. Rates of endometrial cancer are expected to mirror those in the general population and increase in active-duty Service Members, Veterans, and their beneficiaries. The standard treatment, total hysterectomy, requires time for recovery and consequently affects mission readiness of active-duty Service Members. Indeed, a U.S. study found one in five of cancer patients who had a hysterectomy experienced a change in employment status following diagnosis. Furthermore, force vulnerabilities are created following cancer diagnosis within a Service Member's Family unit and can lead to transfer or separation. Our research thus aims to address these impacts, by improving patient health and outcomes through the development of new effective treatments.

Proposal Title:	Screening and Early Detection of Testicular Germ Cell Tumor Using Serum miRNAs
Log Number:	CA220138
Current PI Name:	Aditya Bagrodia
Award Number:	HT9425-23-2-0040
Current Contracting Organization:	California, University of, San Diego
Current Performing Organization:	California, University of, San Diego
Web Approval Date:	09-14-2023

Testicular cancer is the most common cancer found in men between the ages of 15-34 years and the most common cancer among active-duty military personnel. Our proposal will directly benefit these patients. Testicular cancer (most commonly testicular germ cell tumor) is curable at any stage, including once it has spread (metastasized). When testicular cancer is confined to the testicle, it is cured with removal of the testicle (orchiectomy) in about 70% of cases. Detecting cancer earlier, prior to its spread beyond the testis, leads to a greater chance of cure and decreases the need for aggressive chemotherapy, radiation therapy, or extensive surgery to remove metastatic disease. This is critical to avoid long-term side effects in these young cancer survivors that directly impact mission readiness. Currently no screening tests exist for testicular cancer.

Recently, our group and other scientists have discovered that the vast majority of testicular cancers express small noncoding RNAs known as microRNAs. Importantly, one particular microRNA, miR-371a-3p, can be detected both in the testicular tissue and in the blood (or serum) of patients with GCT. These microRNAs can be detected in blood tests before testicular cancers become clinically evident and diagnosed and can potentially be used to screen for and accurately diagnose GCTs.

We improve mission readiness in the FY22 Topic Area of germ cell cancer with the overarching goal of transforming GCT care by detecting cancer initiation and detection. Our objective is to establish a novel means of screening for testicular cancer and dictating treatment in early-stage disease. The rationale for the study we propose is the discovery of a highly sensitive and specific blood-based biomarkers for GCTs.

We directly examine the utility of serum miR-371a-3p test as a screening test for GCT. The United States Preventative Services Task Force gives a grade D (does not recommend) for screening of GCTs due to the lack of specificity and sensitivity of self-examination or ultrasound. There is a precancerous lesion, germ cell neoplasia in situ (GCNIS), that is present along with more than 90% of invasive testicular cancers. It is thought that GCNIS is present for a long time prior to development of invasive GCT. Our group has identified measurable miR-371a-3p in patients with GCNIS only. Additionally, our group and others have demonstrated that tumors smaller than 0.5 cm (before clinical detection) have measurable amounts of miR-371a-3p in the serum. The DOD (Department of Defense) Serum Repository is perhaps the only resource in the world that has biannually banked serum for all men in the military. Essentially, all military personnel have serum stored and banked at 2-year intervals since their time of enrollment. We can investigate historical serum samples from patients that ultimately developed testicular cancer to see if there is evidence in their pre-diagnosis blood tests of testicular cancer being present. Our collaboration of urologic oncologists from two of the largest military hospitals in the United States (Walter Reed and Madigan Army Medical Center) allows us to create a clinical database and linked biobank of patients that developed GCT during active military service along with blood samples collected prior to clinical detection of cancer. We will leverage the Bagrodia Lab expertise in microRNAs to investigate if and when patients developed a positive serum miR-371a-3p test prior to clinical detection of testicular cancer. This could directly lead to implementation of screening protocols that would (1) increase disease-free and overall survival rates, (2)

minimize burden of treatment including cardiovascular disease, secondary cancers, infertility, and need for major operations. There are no risks associated with this aim.

Our work has a tremendous impact on mission readiness for military personnel, including early detection and diagnosis, improved survivorship, and overall well-being of military members and their Families.

Proposal Title:	Noninvasive, Saliva-Based Assay for Screening and Surveillance of Patients with Oral Cavity Squamous Cell Carcinoma (OCSCC)
Log Number:	CA220164
Current PI Name:	Evgeny Izumchenko
Award Number:	HT9425-23-1-0614
Current Contracting Organization:	Chicago, University of
Current Performing Organization:	Chicago, University of
Web Approval Date:	09-14-2023

This project addresses the fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) PRCRP Topic Area – Head and Neck Cancers. Scientific objective and rationale: Oral cavity squamous cell carcinoma (OCSCC) is the most common head and neck cancer worldwide. Finding OCSCC early, when it's small and hasn't spread, allows for more successful treatment and increases patients' survival. However, oral cancer (especially in early stages) can be clinically deceptive, leading to misdiagnosis and treatment delay. Visual and tactile examination (current methods used for OCSCC detection) may often fail to discriminate between noncancerous and malignant lesions, ultimately requiring tissue biopsy evaluation (cutting of the lesion for laboratory testing) to confirm diagnosis and initiate treatment. However, these procedures are invasive, costly, and depend on examiner experience. Given that about 10% of the U.S. population have some type of oral mucosal abnormality (many of which are not cancerous), there is a pressing need for a non-invasive, accessible, and cost-effective method for screening patients with suspicious oral lesions, allowing early OCSCC detection while sparing unnecessary biopsies for patients with benign oral disorders. As OCSCC grows, it accumulates mutations in genes known to play a role in cancer progression. Our group and others have reported that such mutations can be detected in saliva of patients with OCSCC. However, no saliva-based screening method for early detection of OCSCC are currently available. Recently we have developed a method based on the targeted sequencing technology specifically designed to detect OCSCC-associated mutations (tumor DNA) in saliva, and validated this assay using matched primary tumors and saliva specimens collected in India (a county with high incidence of OCSCC). While these findings provide the foundation for using this ultra-sensitive and cost-efficient assay in clinical settings, frequency of cancer-driving mutations may vary in patients from different ethnical backgrounds. Given the racially and genetically heterogeneous population of the U.S. Armed Forces, it is critical to further assess the performance of this assay in demographically and epidemiologically diverse patient's cohort. Our proposal will leverage the unique geographic location of the University of Chicago to evaluate the true potential and limitations of this test across heterogeneous patient populations, as well as across diverse therapeutic approaches for treatment of OCSCC.

In Aim 1, we will assess the potential of this assay for noninvasive screening of patients with suspicious oral lesions and ruling out cancer. For this Aim we have prepared three groups of oral rinse specimens: (i) 300 samples collected from untreated patients with OCSCC (representing ethnically heterogeneous patient population); (ii) 100 samples from patients with noncancerous oral lesions; and (iii) 100 samples from healthy volunteers. A well-validated, saliva-based cancer detection assay with optimal performance would represent a significant clinical advancement in cancer care by reducing mortality, while lowering the socio-economic burden of OCSCC.

In Aim 2, we will evaluate the feasibility of using this saliva-based method to detect early recurrent disease after OCSCC diagnosis and treatment. There are 25,000 patients treated in the U.S. alone that require routine monitoring (follow-up visits and radiologic imaging for at least 5 years after diagnosis), to ensure that their

cancer hasn’t returned. Our highly sensitive assay could be performed more frequently than imaging, allowing identification of early recurrence or residual disease noninvasively, leading to increased chances of a curative salvage treatment.

For this Aim, longitudinal saliva samples are already being collected from 120 patients with OCSCC: at baseline and 3, 6, 9, and 12 months following completion of treatment. If high correlation between tumor DNA clearance and patients’ recurrence exists, this assay would represent a significant clinical advancement in cancer care, by monitoring for recurrence prior to radiologic imaging.

FY22 PRCRP Overarching Challenge: This proposal directly addresses the overarching challenges in (i) prevention – by developing innovative early detection methods to decrease cancer burden in diverse different populations; (ii) diagnostics/prognostics – by identifying strategies to predict recurrence and the development of advanced disease and developing minimally invasive method to detect cancer initiation, progression; and (iii) disparity – by developing strategies to improve accessibility to care and to address survivorship.

FY22 PRCRP Military Health Focus Area: Veterans and active-duty service personnel have disproportionately high rates of tobacco and alcohol consumption (major risk factors for oral cancer), as these habits are ingrained in military culture, increasing their risk for developing OCSCC. This project addresses FY22 PRCRP Military Health Focus Area on mission readiness by (i) allowing sensitive and rapid screening of individuals with suspicious oral lesions, so that Service Members with negative result could quickly return to full duty; (ii) reducing the need for in-person evaluation by a sub-specialized medical expert, allowing reliable diagnosis for hard-to-reach military personnel during deployment; (iii) identifying early recurrence noninvasively could lead to improved treatment responses and survival of Service Members, Veterans, and their Families.

Proposal Title:	Next-Generation Tethered Capsule Endomicroscopy Platform for Clinically and Commercially Viable Esophageal Cancer Screening
Log Number:	CA220230
Current PI Name:	Guillermo Tearney
Award Number:	HT9425-23-1-0694
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	10-03-2023

The fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) Topic Area to be addressed by the proposed research is Esophageal Cancer. The FY22 PRCRP Military Health Focus Area(s) in Section II.A.2 to be addressed include environmental/exposure risk factors associated with cancer as well as gaps in cancer prevention, early detection/diagnosis, prognosis, and/or treatment.

The scientific objective of this proposed project is to develop a low-cost disposable diagnostic tool that can be readily accessible at a primary care physician office and used as part of a routine physical exam. The technology currently exists as a reusable device that is light-based and could replace standard endoscopy as a diagnostic tool for identification of Esophageal Cancer and its precursor, Barrett's Esophagus. The light-based device is a swallowable capsule, approximately the size of a Tylenol capsule, but with a string-like attachment or tether from the capsule to the instrument running the diagnostic scanning. Currently, more than 550 people have participated in diagnostic studies with the tethered capsule; however, the swallowability rate of the current capsule is about 75%. Through development of a more flexible tether attachment and a simpler battery powered device, the expectation is that swallowability will increase to 95%. Increasing swallowability and decreasing cost will make this system more readily available to clinics and primary care offices, and will allow nurses, physician assistants, or doctors to conduct the procedure in their office as part of a routine physical exam. Because the capsule-based procedure does not require sedation, this becomes a more accessible procedure with limited patient inconvenience or preparation.

This research is conducted in partnership with MGH Home Base, a program that has served more than 30,000 Veterans and their Family Members and trained over 85,000 clinicians across the nation. Home Base’ s integrated team of professionals work together to ensure that Veterans, Service Members, and their Families receive or are connected to appropriate and uniquely tailored care. From our collaboration with Home Base, their current patient base includes Veterans from the Vietnam War and current Service Members whose diagnosis aligns with environmental factors that are precursors to Barrett's Esophagus. Home Base has seen an increase in cancer rates in their Veteran and active-duty patients and are interested in this partnership to develop a suite of screening tools that could be included as part of a primary care visit to identify precursors of cancer, such as Barrett's Esophagus, without requiring a more invasive, cumbersome, and costly upper endoscopy procedure. Pre-diagnostic capability gives active-duty Service Members the opportunity for treatment and avoidance of advancement to cancer, allowing a swifter return to work.

The swallowability study is the first step to realizing a lower cost version of the current reusable OCT capsule. It is expected that, should this proposal be funded and successfully achieve the goals, the next steps would be transfer to industry that would commercialize the product and conduct larger scale pivotal clinical trials.

The FY22 PRCRP Overarching Challenges to be studied in this proposal include Prevention, Diagnostics /Prognostics, and Disparity. Through Prevention, this disposable OCT device can be used in a primary care setting with a patient who does not need to be sedated or prepared clinically, allowing early identification of

Barrett's Esophagus. Those who exhibit Barrett's Esophagus can be clinically evaluated for treatment with the goal of prevention of Esophageal Cancer. If indications are that Barrett's Esophagus is present, this swallowable device can also monitor further progression of the disease. Through Diagnostics/Prognostics, where primary care screenings will be conducted with this swallowable capsule diagnostic tool, treatment resistance, recurrence, and the development of advanced disease can be recognized and predicted.

Sedated endoscopy, the current standard of care, is inadequate for Barrett's Esophagus screening because it is too expensive and inconvenient. The proposed inexpensive, swallowable capsule can become a practical alternative to Barrett's Esophagus screening. By detecting and treating this cancer precursor before it has progressed to Esophageal Cancer, this device can significantly benefit the life, treatment, and outcomes for Veterans, active military, and their Families.

Proposal Title:	Optimizing PD-1 Immunotherapy Through Increased T Cell Regeneration and Reduced Antigen Load
Log Number:	CA220233
Current PI Name:	Sam Yoon
Award Number:	HT9425-23-1-0635
Current Contracting Organization:	Columbia University Medical Center
Current Performing Organization:	Columbia University Medical Center
Web Approval Date:	09-14-2023

The fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) Topic Areas to be studied are: (1) Metastatic Cancers and (2) Stomach Cancer.

The proposal addresses the FY22 PRCRP Overarching Challenge to advance immunotherapy across the different PRCRP Topic Areas.

Stomach cancer is a devastating disease. There are approximately 1 million new gastric cancer cases and 700,000 gastric cancer deaths worldwide per year, accounting for almost 10% of all cancer deaths. The majority of gastric cancer patients present with locally advanced or metastatic disease. Chemotherapy is the standard first line treatment resulting in a median survival of only 10-12 months. The addition of immunotherapy to chemotherapy has modestly increased this survival.

Immunotherapies seek to activate the patient’s immune system to fight cancer. One type of immunotherapy, immune checkpoint blockade, takes the brakes off the immune system and has the potential to revolutionize cancer care. However, immune checkpoint blockade using anti-PD-1 antibodies is limited by substantial resistance, with many if not most patients not achieving durable anti-tumor responses. There is limited mechanistic understanding of resistance to anti-PD-1. It was originally thought that anti-PD-1 restores potency to anti-tumor T cells, which express the highest levels of PD-1. Recent breakthroughs demonstrate that anti-PD-1 instead, induces proliferation and differentiation of precursors to anti-tumor T cells that that express intermediate or low levels of PD-1. These precursor T cells express a protein called PI3K-delta.

This proposal tests the primary hypothesis that anti-PD-1 immunotherapy for gastric adenocarcinoma will be most effective when BOTH (1) the availability of precursor T cells is optimized by inhibition of PI3K-delta and (2) tumor antigen load is reduced by targeted anti-tumor therapy.

Current biomarkers cannot adequately predict patients' sensitivity and resistance to immunotherapy owing to incomplete understanding of how anti-PD-1 impacts T cell biology. In our mouse studies, anti-PD1 and PI3K-delta inhibition was associated with a higher ratio of TCF1+ to TCF- T cells (T cell regenerative index) in tumor and blood compared to anti-PD-1 alone. The secondary hypothesis is that the T cell regenerative index and tumor burden in patients with advanced gastric cancer can predict responsiveness to anti-PD-1 therapy and chemotherapy.

This proposal has two Specific Aims.

Aim 1: Determine whether PI3K-delta inhibition and tumor burden reduction improve anti-PD-1 immunotherapy in mouse models of gastric cancer.

Aim 2: Assess quantitation of T cell regenerative capacity and tumor burden as predictive biomarkers of patient response to anti-PD-1 immunotherapy and chemotherapy for advanced gastric cancer.

Proposal Title:	Building a Rapid Drug Discovery Pipeline Specific for Pediatric Acute Myelogenous Leukemia with Machine Learning/In Vitro Feedback Loops
Log Number:	CA220262
Current PI Name:	Kolja Eppert
Award Number:	HT9425-23-1-0706
Current Contracting Organization:	McGill University Health Centre Research Institute
Current Performing Organization:	McGill University Health Centre Research Institute
Web Approval Date:	10-03-2023

Fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) Topic Areas: This research proposal addresses the two Topic Areas of blood cancer and pediatric, adolescent, and young adult cancers.

Scientific Objective: The overarching objective of this proposal is to use artificial intelligence to develop new and safer drugs to treat a deadly type of childhood blood cancer: acute myeloid leukemia (AML).

Scientific Rationale: The need for safe and effective ways to treat children with AML. Pediatric AML is a deadly form of cancer and is responsible for nearly half of all deaths among children with leukemia. Indeed, 4 out of every 10 children with AML die within 5 years of being diagnosed with this devastating disease. Chemotherapy for children with AML involves extremely intense and very toxic treatments that cause heart and fertility problems and can even cause other forms of cancer later in life. Since currently available treatments for AML are so toxic, doctors have little ability to adjust therapies to make them more aggressive to treat the most high-risk patients. This illustrates a critical need for safer and more effective options to extend and improve the lives of children with AML.

Eliminating cancer-causing fusion oncoproteins to treat AML: Our team predicts that the key to creating safer and more effective treatments for AML lies in the ability to eliminate fusion oncoproteins from the body. Fusion oncoproteins are a unique type of protein only found in cancer cells that form when portions of two otherwise separate proteins combine (fuse) to form a new, cancer-causing protein. One of the key reasons that children develop AML is because of these fusion oncoproteins; therefore, fusion oncoproteins are ideal drug targets because they transform healthy cells into cancerous cells. However, fusion oncoproteins are nearly impossible to eliminate with drugs using current drug-development approaches because of their unique shape and activity. Thus, new approaches are needed for scientists to develop drugs that can bind to and eliminate fusion oncoproteins.

Our plan to develop new drugs that target and eliminate fusion oncoproteins: To develop new drugs capable of eliminating fusion oncoproteins from the body, there are three important steps that need to be overcome that we will address in our proposed project. Firstly, we need to determine the shape of each fusion oncoprotein (Aim 1); secondly, we need to identify compounds that can bind to the unique shape of each fusion oncoprotein (Aim 2); and lastly, we need to test how well these identified compounds can bind to and eliminate fusion oncoproteins from cancer cells (Aim 3). Since traditional approaches to determine protein shape and identify binding partners are highly inefficient, we will harness the power of artificial intelligence to produce a new algorithm that can predict the shape and potential binding partners of fusion oncoproteins. We will then test how effectively the compounds that we identified using our algorithm can bind to and eliminate fusion oncoproteins from special leukemia cell models developed by our team.

FY22 PRCRP Overarching Challenges: Our proposed project addresses the overarching challenge of Therapeutics. The current drug development process in AML involves a trial-and-error approach that tests one potential drug at a time; as such, this process is slow, inefficient, and has left drug discovery in AML stagnant for nearly 40 years. We propose that the development of a new and improved drug discovery pipeline, which combines the use of artificial intelligence to first predict novel drug candidates with experimental cell models to then test these candidates, will lead to the efficient and rapid design of safe and effective drugs that eliminate fusion oncoproteins from cancer cells. Ultimately, our work has the potential to transform AML treatment options by (1) developing the drugs able to eliminate fusion oncoproteins and (2) revolutionizing the drug development pipeline in AML by dramatically accelerating the drug discovery process.

FY22 PRCRP Military Health Focus Areas: Currently, available treatment options for pediatric AML are ineffective for nearly half of all patients. Of those patients fortunate enough to survive the toxic treatments, many suffer from side effects such as impaired development and other long-term complications. In the short-term, successful completion of our project will advance the field of cancer research by developing effective and safe drugs that target fusion oncoproteins to treat pediatric AML patients. In the long term, this project can improve the health and well-being of the general public and beneficiaries of military personnel by improving treatment effectiveness, lowering treatment toxicity, and increasing overall patient survival. Our project could provide a foundation upon which to improve mission readiness by increasing patient survival and minimizing relapse for the children of Service Members, thus allowing the Service Members to remain on active duty.

Proposal Title:	Targeted Antibody and Immunotoxin Combination Improves T-Cell Lymphoma Therapy
Log Number:	CA220309
Current PI Name:	Sanggu Kim
Award Number:	HT9425-23-1-0582
Current Contracting Organization:	Ohio State University, The
Current Performing Organization:	Ohio State University, The
Web Approval Date:	07-11-2023

This proposal aligns with the fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) Topic Area of “Lymphoma.” We propose developing a novel antibody and immunotoxin combination therapy for T-cell lymphomas. T cells are a type of blood cells that play a critical role in the body’s immune system. T-cell lymphomas are a diverse group of T-cell cancers that are usually fast-growing and difficult to treat with conventional cancer therapies. To date, there is no curative therapy for the vast majority of patients. CD3-immunotoxin (CD3-IT), a promising T-cell specific precision medicine agent, has been developed to treat T-cell lymphomas. CD3-IT showed great potential to cure T-cell lymphoma patients in a recent clinical test, but its effects were limited to certain T-cell lymphoma patients for unknown reasons. We have recently found that a special type of T cells that reside in a unique anatomic site (B-cell follicle) are difficult to kill with CD3-IT. It is likely that the local tissue environment surrounding these cells protects them from being killed by CD3-IT. Here, we propose to investigate the mechanisms underlying the resistance of these local “difficult-to-kill” T cells and to test whether a specific antibody agent (called costimulatory blockade) used in combination with CD3-IT will reduce such treatment resistance and in turn improve the overall effectiveness of CD3-IT treatment. We will also test whether newly engineered CD3-ITs with an improved safety profile would further enhance T-cell depletion in mice. To that end, we will use cutting-edge cell-tracking technologies and an advanced mouse T-cell lymphoma model to extensively evaluate the effects of our new treatment approaches on both circulating and local organ-resident T cells. If successfully completed, this study will identify an unexplored cancer treatment resistance mechanism and generate an innovative treatment approach that can significantly improve T-cell lymphoma therapy in clinic. Furthermore, the new resistance mechanisms and new treatment strategies we propose here will be applicable to other T-cell targeting biologics, including other anti-T-cell immunotoxins, antibodies, and antibody-drug conjugates.

This proposal aligns with the FY22 PRCRP Topic Area of “Lymphoma.” We propose to address the FY22 PRCRP Military Health Focus Area “Gaps in cancer research that may affect mission readiness” by identifying an unexplored treatment resistance and developing innovative therapeutic strategies for patients with T-cell lymphomas. Our novel treatment strategies can minimize the likelihood of cancer relapse and time spent in the hospital, thereby enabling a more expeditious return to duty for Service Members.

This proposal addresses the FY22 PRCRP Overarching Challenge “Therapeutics.” It identifies previously unknown treatment resistance mechanisms of T-cell lymphomas, especially in advanced stages, and proposes a therapeutic innovation to overcome a major barrier to successful T-cell lymphoma therapy. In addition, the knowledge gained through the proposed studies will also advance immunotherapy generally across the various PRCRP Topic Areas.

Proposal Title:	Combinatorial Targeting of High-Risk Neuroblastoma by Localized FLASH Proton Therapy and Systemic Immunotherapy
Log Number:	CA220310
Current PI Name:	Lai Man Wu
Award Number:	HT9425-23-1-0453
Current Contracting Organization:	Children's Hospital, Cincinnati
Current Performing Organization:	Children's Hospital, Cincinnati
Web Approval Date:	07-11-2023

Neuroblastoma (NB) is a deadly nervous system cancer in children age 5 or younger. About 50% of patients develop high-risk NB with poor outcome. Nearly half of the patients have the cancer spread to other parts of the body, making it very difficult to treat. Current treatments, including surgery, drugs, immunotherapy, and radiation, usually cause long-term side effects and still end in relapse, with tumors redeveloping. Thus, there is an urgent need to create new treatment approaches that are both better at destroying the tumors and less harmful to the body.

Radiation therapy (RT) can kill cancer cells and activate the immune system, but these benefits cannot be maximized due to collateral damage to surrounding healthy tissues. Proton RT is one of the most precise and advanced forms of RT used in NB treatment and can address the toxicity issue to a certain extent. The Proton Therapy Center at Cincinnati Children’s Hospital is currently the only center in the nation that includes a dedicated gantry for proton irradiation research in animal models. Recently, radiation delivered at extremely fast speed (completed in less than a second, compared with several minutes conventionally) known as FLASH, promises to be a groundbreaking strategy for killing cancer cells with minimal harm to surrounding healthy tissue. However, this strategy has not been tested in children with high-risk NB. To leverage our unique proton research facility, we will investigate the impact of innovative FLASH proton treatment on growth and progression of high-risk NB in preclinical mouse models, including mice engrafted with patient-derived NB. The tumors in these mice are very similar to those seen in high-risk NB patients. We therefore can use these mice to learn about NB biology in response to FLASH proton therapy.

Another major cause of death for patients with high-risk NB is that cancer often spread to other parts of the body. Although immunotherapy that help make cancer cells visible to immune cells, which act like “police” cells in the body, has shown success in adult cancer patients, immunotherapy as a monotherapy is not effective for children with high-risk NB because pediatric cancers use different evasion strategies to hide themselves from the immune system. Local radiation to tumors can activate the immune system in the body, allowing immune T cells to detect and kill metastatic tumors that have not been irradiated. This is a rare phenomenon called the abscopal effect. Combining radiation and immunotherapy, including immune checkpoint inhibitors should be a powerful strategy to take advantage of abscopal effect for elimination of local and metastatic cancers. We hereby propose an innovative NB treatment strategy combining the benefits of FLASH proton radiation to local tumors and systemic immunotherapy for distant, metastatic NB elimination. We will test this novel combination treatment in mouse models bearing high-risk NB. (Section II.A.2: Mission readiness-gaps in treatment that may impact the mission readiness and the health of the beneficiaries of military members and the general public).

Cancer behaviors, including how a tumor grows, spreads, and resists being killed by therapy critically depend on how tumor cells talk among themselves and to the surrounding cells through a “signal sending-receiving” relationship. To understand what signal is exchanged between individual cells within a tumor, we use single-cell transcriptomics and epigenomics that enable us to detect global gene expression patterns of individual cells and what gene networks control these patterns at very high resolution. Single-cell data can

tell us what cell populations are within a tumor and how they talk to one another to respond to therapy. One way tumor cells become more powerful is to escape from surveillance by immune T cells. We can develop drugs to make cancer cells more visible to the immune system. This will open new opportunities for finding out how immune cells interact with NB tumor cells and develop better tools to specifically attack the tumor. We will use this state-of-the-art technology to profile NB cells in response to FLASH proton therapy or in combination with immunotherapy.

Our study will address Fiscal Year 2022 Peer Reviewed Cancer Research Program Overarching Challenges including, (1) transform cancer treatment through the identification of new targets for advanced disease; improve immunotherapy; and eliminate the risks of therapy-associated toxicity by FLASH proton radiation, and (2) identify the mechanisms behind NB cancer epigenetics, biological development, and therapy response by single-cell technology.

This research emerges as a paradigm shift in pediatric NB therapy and will guide the clinical implementation of FLASH proton therapy, alone or in combination with immunotherapy, in NB patients. Specifically, the strong research program at CCHMC Proton Therapy Center provides a direct pathway to the clinical realization of the biological benefits of FLASH. Our data will establish proof-of-principle of novel therapeutic strategies for rapid translation to the clinic and improve the efficacy of tumor control by radiation therapy with less harm to the body and ultimately the outcome of NB patients, including the beneficiaries, such as the children, of active-duty Service Members.

Proposal Title:	Harnessing Artificial Intelligence to Overcome Liver Immune Tolerance
Log Number:	CA220311
Current PI Name:	Taran Gujral
Award Number:	HT9425-23-1-0542
Current Contracting Organization:	Fred Hutchinson Cancer Center
Current Performing Organization:	Fred Hutchinson Cancer Center
Web Approval Date:	07-11-2023

The proposed research project is important and directly relevant to the fiscal year 2022 Peer Reviewed Cancer Research Program (PRCRP) Overarching Challenge; “transform cancer treatment by identifying new targets, especially for advanced disease and metastasis,” and “advance immunotherapy across the different PRCRP Topic Areas.”

Metastases are responsible for as much as 90% of cancer-associated deaths. This is because we continue to struggle with how to develop drugs for metastatic cancers. Metastatic cancers occur when cancer cells spread from their organ of origin (called the primary cancer site) to another organ (called the secondary or metastatic site). Metastasis is exceptionally dangerous as it usually affects essential organs such as the liver. The destruction of these organs by metastases results in rapid decline and failure. Unfortunately, drugs that are effective in treating localized cancer – that is, cancer that is contained within the primary cancer site – usually show little efficacy in thwarting the same cancer at the secondary site. That is because the behavior of cancer cells and their response to therapy are influenced by the specific environment surrounding them, the tumor microenvironment (TME). The TME varies significantly between the primary and secondary sites. Consequently, the 5-year relative survival rate for localized colorectal cancer is 91%, while the survival rate for distant metastatic colorectal cancer is only 14%. Our goal is to change the statistics by inventing a new way to discover drugs for metastatic cancers.

The TME of cancer significantly impacts the drug response, including the response to immunotherapy. However, standard strategies for discovering, screening, and testing drug effects for novel cancer drugs use cancer cells in isolation, ignoring the important role of the TME. These “Petri-dish” models lack the complexities of the human tissue microenvironment and are not reliable predictors of the physiological drug response. As a result, about 95% of all oncology drugs currently being developed will not pass through the Food and Drug Administration (FDA) approval process. In preliminary data, we have taken a bold and risky approach past this hurdle: We developed a novel method combining human tissue microenvironment, artificial intelligence, and chemical tools to reliably predict candidate drugs and their combinations that significantly improve the response to immunotherapy. Using this innovative strategy, we identified cabozantinib, an FDA-approved drug that restores the response to immunotherapy in metastatic liver tissues. This Idea Development Award aims to build a robust preclinical package around cabozantinib and be backed by a solid experimental rationale. Specifically, we will confirm the efficacy of a combination of cabozantinib with immunotherapy in the liver metastases mouse model and in metastatic liver tissues from patients. The successful outcome of this proposal would accelerate the clinical development of cabozantinib and could provide new therapeutic and safer options for the treatment of metastatic liver cancer, offering unprecedented hope to the providers and patients who currently face this disease with severely limited alternatives.

Proposal Title:	Developing YAP/TEAD Inhibitors for Novel NASH-Associated Hepatocellular Carcinoma Therapeutics
Log Number:	CA220314
Current PI Name:	Chenglong Li
Award Number:	HT9425-23-1-0737
Current Contracting Organization:	Florida, University of
Current Performing Organization:	Florida, University of
Web Approval Date:	10-03-2023

This project is to propose a potential novel therapeutic option for liver cancer.

Hepatocytes maintain vital functions in our body, but they are prone to damages resulting from alcoholism, obesity, viral infection, and drug toxicity in chronic liver disease. Non-alcoholic fatty liver disease (NAFLD) is usually part of the metabolic syndrome found in patients who do not drink alcohol but develop fatty livers due to diabetes mellitus, hypertension, dyslipidemia, obesity, and insulin resistance. Nonalcoholic steatohepatitis (NASH) is advanced form of NAFLD with complications such as inflammation and liver scarring. This long- term hepatic damage ultimately activates oncogenic pathways that transform hepatocytes into tumor phenotypes known as hepatocellular carcinoma (HCC). Current immunotherapy shows promising effects on inhibiting HCC in patients who suffer hepatitis B or C. However, NASH limits anti-tumor surveillance in immunotherapy. Sorafenib is the first line option of drugs in anti-HCC targeted therapy and has been shown to improve patient's survival rate in the short term. However, resistance to this drug eventually develops leading to failure of the treatment. Drug resistance remains the principal cause of treatment failure during the use of targeted therapies.

Yes-associated protein (YAP) is an oncogenic protein and acts as a transcriptional coactivator for Tea domain transcription factors (TEAD). YAP activation through forming complexes with TEAD induces arrays of target genes for HCC survival and growth. The YAP/TEAD complex not only drives tumorigenesis but also confers to drug resistance. Activated YAP induces molecules that can allow HCC escape from death and gain resistance to drugs such as Sorafenib. Currently, there is no FDA-approved drug available to disrupt the YAP/TEAD complex.

YAP interacts with TEAD with three interfaces. Considering that TEAD molecules appear to be largely dispensable for normal functions of our body, its inhibition by our compounds should not result in major adverse toxicity effects. Our intention is to discover the first-in-class small molecule drugs that disrupt interface 3 in TEAD because our initial studies have demonstrated that disrupting interface 3 greatly weakens YAP binding while preserving tumor suppressor protein VGLL4 binding at interfaces 1 and 2. In order to design potent, specific, easy-to-make and drug-like molecules, we use computational intelligence to re-engineer a new set of compounds that are potent and efficient for inhibition of YAP/TEAD interaction. In addition, we have developed very sensitive reporter systems that allow us to assess the inhibitory activities of candidate compounds in cultured tumor cells and in animals with implanted HCCs. HCC development is a stepwise chronic liver disease process that may take several decades to evolve in human. However, it is difficult to follow changes of liver histology in patients due to the asymptomatic nature of NASH to HCC transition during early stages. Patients with liver function failure are often at advanced stages of the diseases, and therefore the only option for treatment is to carry out a liver transplantation. However, liver donors are limited every year. There is an urgent need to identify molecular targets and develop anti-HCC therapy for the treatment of NASH. We have also established a preclinical model that faithfully induces human liver pathologies of NASH, fibrosis, and HCC in the setting of YAP overexpression. This model enables us to determine whether our candidate compounds can synergize with Sorafenib and inhibit NASH progress to

HCC. Knowledge obtained in this proposal may open new avenues for therapeutic strategies by targeting the oncogenic YAP/TEAD pathway against liver pathologies such as NASH, fibrosis, cirrhosis, and HCC, benefitting the health and well-being of military members, Veterans, their beneficiaries, and the general public who are prone to NASH, alcoholism, hepatitis C, and chronic hepatitis B infection.

This proposal addresses FY22 Overarching Challenge of "Disparity: Improve prevention strategies, diagnosis, treatment, and outcomes for patients in underserved or under recognized populations."

It addresses military health focus of "Environmental exposure risk factors associated with cancer" and "Gaps in cancer prevention, early detection/diagnosis, prognosis, and/or treatment that may impact mission readiness and the health and well-being of military members, Veterans, their beneficiaries, and the general public."

Our multidisciplinary team is composed of translational and clinical investigators with complementary expertise including a drug designer/organic chemist (Chenglong Li, Ph.D.), a liver cancer and YAP/TEAD biologist (Liya Pi, Ph.D.), a liver cancer pathologist (Tong Wu, MD/Ph.D.), and a radiologist for in vivo evaluation of HCC development using MRI imaging (Joanna Long, Ph.D.). The research team offers a novel approach to suppress HCC growth, metastasis, and prevent tumor recurrence.

Proposal Title:	RNA Methyltransferase FTSJ3 Regulates Ribosome Biogenesis in Liver Cancer: Molecular Mechanisms and Therapeutic Potential
Log Number:	CA220315
Current PI Name:	Zeng-Quan Yang
Award Number:	HT9425-23-1-0620
Current Contracting Organization:	Wayne State University
Current Performing Organization:	Wayne State University
Web Approval Date:	07-12-2023

Liver cancer is a devastating disease that affects millions of men and women worldwide. The incidence of liver cancer is increasing in the United States, especially among military personnel and Veterans. Each year in the United States, about 24,500 men and 10,000 women get liver cancer, and about 18,600 men and 9,000 women die from this disease. Among the main risk factors for liver cancer are hepatitis B and C infection, alcohol consumption, obesity, and male gender, all of which are over-represented in the U.S. military and Veteran communities. Currently, less than 20% of patients with liver cancer are responsive to curative therapies, and most are treated with systemic and/or locoregional therapies that only extend survival by months, not years. Thus, there are significant needs to develop new classes of molecules that target its unexplored cellular mechanisms to fight this devastating disease.

Our research focuses on identifying key oncogenic factors that drive the progression of different subtypes of human cancers and then testing the effects of new anticancer agents. RNA serves as a connecting link by which genetic information passes from DNA to protein. There are many chemical changes to RNA nucleotides – known as RNA modifications – that regulate the structure, stability, translation, and function of almost every major class of human RNA. Notably, RNA modification enzymes have emerged as promising molecular targets for anti-cancer drug development. By integrated analysis of genomics/proteomics and clinical data of The Cancer Genome Atlas (TCGA) and the Clinical Proteomic Tumor Analysis Consortium (CPTAC), we discovered one of the RNA modification enzymes, called FTSJ3 (FtsJ RNA 2'-O-methyltransferase 3), is dramatically up-regulated in a set of liver cancer samples, and significantly associated with high-grade tumors and poor disease prognosis in liver cancer patients. Metagenomic analysis of TCGA dataset revealed that the FTSJ3 gene is gained/amplified in 35.2% of liver cancer. Our preliminary studies uncovered that knockdown of FTSJ3 inhibits liver cancer cell growth in vitro. Additionally, FTSJ3 enzymatic domain had specific motifs and showed inhibitory active site architecture. Furthermore, FTSJ3 protein is predominantly localized at the nucleolus, the primary site of rRNA processing and ribosome biogenesis. Ribosomes are the molecular machines that produce all cellular proteins through a complex and highly regulated biochemical process called translation. Strong upregulation of ribosome biogenesis is an important molecular alteration of rapidly dividing liver cancer cells, owing to the high demand for ribosomes and protein translation. All these findings make the FTSJ3 an attractive target for the treatment of liver cancer.

The objectives of this application are: (1) elucidate the biological roles and molecular mechanisms by which RNA modification enzyme FTSJ3 promotes liver tumorigenesis, and (2) explore the therapeutic potential of targeting FTSJ3 in a set of FTSJ3-amplified/overexpressed liver cancer. The first Specific Aim will define the functional significance of FTSJ3 and the therapeutic potential of targeting FTSJ3 in liver cancer in culture cells and animal models. We will use genetic approaches and/or small-molecule compounds to measure the effects of inhibiting FTSJ3 on blocking aggressive phenotypes of liver cancer cells in vitro. We will then determine the impact of inhibiting FTSJ3 function on the tumor growth of liver cancer in mouse models. In Specific Aim 2, we will determine the functional role and molecular mechanism by which FTSJ3 alters RNA modification patterns, ribosome biogenesis, and translational capacity, subsequently supporting

liver tumorigenesis. We will examine how FTSJ3 recognizes a combined sequence and structural feature of targeted RNA in liver cancer cells. We will define the functional roles of FTSJ3 on RNA modification abundance, Ras-like GTPase (GNL2: Nucleolar GTP-binding protein 2) activity and ribosome biogenesis in liver cancer cells. Upon completion of this study, we will: (1) establish FTSJ3 as a critical player in promoting liver cancer growth and progression; (2) fundamentally understand the molecular mechanism of FTSJ3 activation in liver cancer; and (3) demonstrate the therapeutic potential of FTSJ3 inhibitors for treating aggressive FTSJ3-upregulated liver cancer.

Incidence of liver cancer is significantly higher in military personnel compared to the general population due to their increased exposure to various risk factors for liver cancer. In this proposal, we will examine the cancer- causing roles, molecular mechanisms, and therapeutic potential of the novel RNA modifier FTSJ3 in liver cancer. Thus, our proposed studies directly address the Peer Reviewed Cancer Research Program (PRCRP) Challenge: “Identify and elucidate the mechanisms behind cancer epigenetics/genetics and cancer development to improve treatment methods.” Based on these factors, the fiscal year 2022 PRCRP Military Health Focus Area to be studied is mission readiness through addressing “gaps in cancer prevention, early detection/diagnosis, prognosis, and/or treatment that may impact mission readiness and the health and well-being of military members, Veterans, their beneficiaries, and the general public.”

Proposal Title:	Engaging the Immune System to Kill Sarcoma: A Comparative Oncology Approach
Log Number:	CA220317
Current PI Name:	Daniel Vallera
Award Number:	HT9425-23-1-0583
Current Contracting Organization:	Minnesota, University of, Twin Cities
Current Performing Organization:	Minnesota, University of, Twin Cities
Web Approval Date:	08-16-2023

Overarching Challenges Addressed: Improving the efficacy of Immunotherapy and Transforming cancer treatment for sarcoma patients.

Near-Term Impact and Applicability of the Research: For many years, the hope was to harness the patient's own immune system to fight cancer. There were many attempts that failed, so hopes dimmed along with these many failures. Only in the past few years, it was discovered that bioengineered drugs composed of antibody fragments that simultaneously bind cancer target cells and cells of our own immune system could successfully engage the immune system with remarkable anti-cancer responses in patients who failed chemotherapy. These drugs have mostly been effective against blood-born tumors such as leukemia. These discoveries launched a new direction of the cancer research field. It is now believed that we can harness this technology so we can treat very difficult solid tumors such as those we see in those individuals diagnosed with sarcoma. This will be a more difficult challenge.

In this proposal, we believe we are positioned to have major impacts on sarcoma immunotherapy. (1) We have improved the ability of genetically engineered drugs to bind by changing the antibody framework to that of llama antibody. (2) In the same drug, we incorporated a signal that dramatically expands the number of immune natural killer cells to kill the tumor. (3) Since the target that we intend to hit with antibody is extremely important in cancer, we have targeted a marker that is an extremely high-quality sarcoma target called B7-H3. B7-H3 is highly expressed on sarcoma cells and minimally expressed on non-target cells in the body. (4) In addition to the drug itself, the way we intend to study it is extremely important, and few centers have the means to accomplish it. We intend to combine our drug development program with our canine veterinary oncology program. We engineered our drug so it has the ability to recognize B7-H3 sarcoma in dogs and in humans. Dogs develop sarcoma far more frequently than humans, so we have many canine patients from our canine oncology clinic to study. By respectfully treating these canine patients, we will learn how best to dose animals to get the maximal anti-cancer response with the least amount of toxic side effects and what route of injection is best. (5) There is something else very special about our drug. It belongs to a new class of drugs that engage natural killer cells to kill cancer. Natural killer (NK) cells are white blood cells that have evolved to kill cancer cells. When people get cancer, it is because, in part, NK cells are blinded and are not performing their intended duties. Our drug is designed to assist NK cells and to engage them so they can better do their job as cancer killers. This proposal also will assess the effect of our drug on the dog's immune system so that we can better understand whether the drug is working as intended. Since we already have designed, manufactured, and vialed our drug, these studies will immediately benefit dogs and dog owners. It will provide critical information since what we learn in dogs is applicable to humans and a future human clinical trial.

Scientific Objective and Rationale: Our primary objective is to generate preclinical support for our NK cell-enhancing species, cross-reactive drug in a canine system that will benefit patients, most notably young adults. The potential benefit to dogs with sarcoma and their owners is not trivial, and is a secondary goal. Our rationale is multifaceted. (1) Since components of human and dog immunity are similar, companion dog

therapy can be used to better understand new immunotherapies in a valuable large animal model of sarcoma, which cannot be replicated in mouse models. (2) Our drug targets B7-H3, a high-quality sarcoma target. B7-H3 is expressed on sarcoma and minimally expressed on normal tissue. Our drug is species cross-reactive since the anti-B7-H3 nanobody fragment recognizes both dog and human B7-H3. (3) Our understanding of route and dosing of antibody fragment-based therapies is limited in humans. Based on molecular dog/human similarity, dogs are a relevant model for study. (4) Once the study begins, the work outlined in canines will determine what type of immune cells can be correlated to an objective anti-sarcoma response.

How Is the Proposed Research Relevant to active-duty Service Members, Veterans, and Other Military Beneficiaries? Sarcoma is a disease of adolescents and young adults that make up over 80% of our military so it will directly affect the military, their Families, and dependents. Sarcomas are not considered a highly occurring cancer. However, when diagnosed, they are debilitating and have an impact far beyond the individual. Briefly, through crucial studies in a highly relevant large animal model, we intend to expedite approval of new immunotherapeutic drugs, and thus, we intend to have a major impact on the health of military members, military Families, Veterans, Veteran Families that develop debilitating sarcomas. It is crucial in these trying times that our military be at a healthy peak. We will impact military health services by offering a new simple and cost-effective therapeutic approach that will have major impact on newly diagnosed or chemotherapy refractory sarcomas. Furthermore, greater clinical success means less hospital time in our over-taxed military hospitals, improved responses that will permit a more rapid return to active duty, and minimization of the trauma of Families. Also, canines play a major role in military duty and as Family members in thousands of military Families. This proposal not only benefits the many diagnosed with sarcoma, but will have a major impact on state-of-the-art veterinary medicine since dogs have a disproportionally high incidence of sarcoma occurrence.

Proposal Title:	Treatment-Induced Metastasis in Ewing Sarcoma
Log Number:	CA220355
Current PI Name:	David Loeb
Award Number:	HT9425-23-1-0421
Current Contracting Organization:	Albert Einstein College of Medicine
Current Performing Organization:	Albert Einstein College of Medicine
Web Approval Date:	06-09-2023

Topic Area: Ewing sarcoma is the second most common bone tumor in children, adolescents, and young adults, and our proposal aims to prevent metastatic spread. This project therefore falls under three PRCRP Topic Areas: pediatric, adolescent, and young adult cancers; sarcoma; and metastatic cancers.

Objective and Rationale: Ewing sarcoma is the second most common bone tumor in children, adolescents, and young adults. Patients who are diagnosed with a tumor that has not spread are usually cured. Those who are diagnosed with metastases (the tumor has spread from its initial location) are rarely cured despite decades of clinical trials and intensifying treatment regimens aimed at improving their survival. Our objective is to better understand how Ewing sarcoma cells spread from the initial tumor and then to develop new treatment strategies to prevent this from happening. Our work focuses on a group of cells termed "TMEM doorways," which we believe allow sarcoma cells to enter the bloodstream and spread to distant sites. We have made the surprising discovery that after exposure to chemotherapy, tumors have more TMEM doorways, suggesting that chemotherapy could increase tumor cell spread. We have also found that different ways of delivering radiation treatment can affect how quickly mice with Ewing sarcoma die from metastases. We will first directly test whether exposure to either chemotherapy or low doses of radiation directly cause an increase in the number of TMEM doorways and a corresponding increase in disseminated tumor cells, and then we will determine whether drugs that are either already in clinical use or are in development can reverse this effect.

What Types of Patients Will Be Helped and How? If our research is successful, our results will change the way patients with Ewing sarcoma are treated. Currently, the first step in treatment is the administration of chemotherapy, and radiation therapy, when used, is given in multiple low doses over 6 weeks. If these ways of treating Ewing sarcoma are found to increase the ability of tumor cells to spread, changing the treatment paradigm would be expected to reduce the risk of metastatic relapse. If there are drugs that can prevent the chemotherapy-mediated increase in TMEM doorways, then adding these drugs to the chemotherapy regimen might be beneficial. If not, patients may benefit from having surgery before chemotherapy is given. If a different way of giving radiation is associated with less tumor cell spread, then the way radiation is administered would certainly change. And if these changes reduce the risk of metastatic relapse, cure rates will improve and fewer patients will die from Ewing sarcoma.

Overarching Challenge Being Addressed and Impact: Our work addresses the "overarching challenge" of Therapeutics because our work seeks to transform cancer treatment through the identification of new targets, especially for advanced disease and metastasis. It will make an impact by altering the way Ewing sarcoma is treated, as described above, in ways that would be expected to decrease metastasis and improve cure rates.

Military Health Focus Area: This project addresses the Military Health Focus Area of "Gaps in cancer research that may affect mission readiness." Ewing sarcoma is a disease of young adults, who comprise the bulk of military Service Members. It also can affect younger children, who may be the children of Service Members. Ewing sarcoma, therefore, represents a threat to mission readiness by predominantly affecting young adults and their children. Thus, improved treatment, especially prevention of metastasis, will increase

military mission readiness by decreasing the chance of an active-duty Service Member being called home due to a relapse of a child's cancer and by decreasing the risk that an active-duty Service Member will be sent home due to a relapse of their cancer.

Proposal Title:	YAP1-Mediated Stromal Activation Leads to Gastric Adenocarcinoma Progression
Log Number:	CA220365
Current PI Name:	Jaffer Ajani
Award Number:	HT9425-23-1-0724
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	10-03-2023

Scientific Objectives and Rationale: Gastric adenocarcinoma (GAC) continues to be a major health burden on military personnel and their Families, mainly because they travel to areas with higher risk factors (Helocobactor pylori, radiation, atmospheric contamination, and high level of stress); thus they (and their Families) acquire risks by traveling to high-risk areas. In addition, GAC is rampant around the world with more than 1.1 million new cases and more than 750,000 deaths each year. Early detection strategies are not implemented (or implementable) in most countries. Therefore, GAC is diagnosed in advanced stage, and it is a fatal disease in most patients. Therapeutic armamentarium is limited (very slow and very modest progress). Many patients and their families suffer because after first line of therapy (which inevitably fails in most cases), we have little to offer them. More in-depth understanding of molecular biology of GAC is urgently needed to figure out new targets and develop more effective drugs. One expectation is to drive GAC therapy based on biomarker(s) so that therapy is not given to all patients to find out only few benefits while imposing side effects on every patient. This is easier stated than done. We have made several discoveries and we believe that with some additional experiments, we will be able to treat a group of GAC patients in a rationally designed trial. We are trying to address a special unmet need in GAC patients that is called peritoneal spread of GAC. Peritoneal space (is within the belly) contains large- and small-bowel, spleen, liver, and stomach. GAC tends to spread there in 45% of advanced GAC cases. This is called peritoneal carcinomatosis (PC). PC leads to rapid decline in the patient's condition. We have found that Yap1 is responsible for promoting PC. We also found that Yap1 is a very powerful protein that manipulates normal cells to protect GAC cells.

We are proposing a systematic research plan to understand Yap1-mediated damage done to the host (patient) and then figure out how new drugs can reduce PC and eventually, even prevent PC. We have excellent mouse models (four different types) and many GAC samples derived from patients. We believe that upon completion of this research, we will be able to have sufficient solid knowledge to start a novel clinical trial in GAC patients with PC.

Therefore, in the near term, we will complete all the steps needed to provide sufficient evidence to launch a clinical trial (biomarker-based). In the long term, we will begin such a trial. Drugs for all potential targets already exist. The potential benefit to patients will be immense, as PC is a unique unmet need and our therapy is specially designed to address PC. There is no PC-specific therapy at the moment. Benefits could be considerable; however, risks to the patients cannot be established until initial phases of such a trial are completed. It is possible that immense risk could arise, and the proposed combination will become impractical.

Our research is very relevant to military troops and their Families since they at higher risk for developing GAC and PC.

Proposal Title:	Investigate Extracellular Matrix Proteins' Local and Systemic Role in Promoting Soft Tissue Sarcoma
Log Number:	CA220366
Current PI Name:	Jlenia Guarnerio
Award Number:	HT9425-23-1-0775
Current Contracting Organization:	Cedars-Sinai Medical Center
Current Performing Organization:	Cedars-Sinai Medical Center
Web Approval Date:	10-03-2023

Sarcoma is one of the Peer Reviewed Cancer Research Program (PRCRP) Topic Areas for the fiscal year 2022 (FY22). Our proposed research addresses this critical topic by developing new and more effective therapies for different subtypes of soft tissue sarcomas (STS) with a high risk of recurrence and death. Surgical resection and chemotherapy are the mainstay treatments for STS; however, many sarcomas relapse after the treatment and often metastasize to the lungs. When this happens, the 5-year survival rate is below 20%. Thus, a major clinical challenge is understanding how to treat the primary lesions better and eradicate all the tumor cells to prevent their later relapse and metastasis.

In the past decade, our understanding of sarcoma biology has advanced. However, previous studies have failed to dissect the sarcoma composition at the single-cell scale necessary to provide a detailed portrait of the lesion. That is, previous approaches analyzed the properties of the entire bulk of the tumor mass without distinguishing between the different cells that compose it. In so doing, many details have been lost. For example, these methods could not distinguish between tumor cells and the non-malignant ones that infiltrate the tumor lesion, such as different types of immune cells. To overcome this limitation, our laboratory has employed cutting-edge technologies that allow the characterization of sarcoma mass at the single-cell level, such as single-cell RNA- sequencing. These new technologies enable novel studies of the tumor's immune "microenvironment," and will foster the discovery of new mechanisms used by the tumor cells to suppress their immune neighbors, which have been neglected in previous studies.

Additionally, the lack of adequate mouse models for preclinical testing has hindered the advancement of sarcoma therapies, in particular novel approaches of immunotherapy. Most of the sarcoma mouse models used in previous investigations could not recapitulate critical traits of the disease, such as the presence of immune cells in the tumor mass, which are known to play critical roles in tumor progression. Accordingly, these mouse models cannot be used to develop or evaluate novel immunotherapies, which aim to target the immune cells or the interaction mechanisms between tumor and immune cells instead of directly targeting tumor cells. Our laboratory has recently developed immunocompetent mouse models of soft tissue sarcoma to tackle this problem. Using these models, we have discovered new potential therapeutic targets, Periostin and Osteopontin, secreted by the sarcoma cells to dampen antitumor immune responses.

Our proposal intends to study the molecular involvement of the identified targets in sarcoma progression and, in parallel, test their blockade. Discoveries in this vein should bolster conventional treatments such as chemotherapy or enable the application of immunotherapies that have been previously unsuccessful. The proposed investigations have the potential to generate preclinical data that may directly impact future clinical trials of soft tissue sarcoma. This proposal will address two of the FY22 PRCRP Overarching Challenges: (1) Transform cancer treatment through the identification of new targets, especially for advanced disease and metastasis. (2) Advance immunotherapy across the different PRCRP Areas.

The proposed investigation will increase the number of military personnel who undergo curative resection of soft tissue sarcoma to facilitate their return to duty. Veterans, who have been exposed in the past years to

herbicides, will also benefit from this study. Achieving a cure for soft tissue sarcoma will profoundly impact the health and well-being of Service Members, their families, Veterans, and the general American population.

Proposal Title:	The Role of Diffuse Large B-Cell Lymphoma Genome Complexity in Shaping Immune Responses to Anti-CD19 Chimeric Antigen Receptor T-Cell Therapies
Log Number:	CA220385
Current PI Name:	Jonathan Schatz
Award Number:	HT9425-23-1-0750
Current Contracting Organization:	Miami, University of, Coral Gables
Current Performing Organization:	Miami, University of, Coral Gables
Web Approval Date:	10-03-2023

This proposal addresses the fiscal year 2022 Peer Reviewed Cancer Research Program (PRCRP) Topic Area: Lymphoma; and Military Health Focus Area: Gaps in Cancer Research That May Affect Mission Readiness. Lymphomas are cancers arising from B- and T-lymphocytes that are key components of the immune system. Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma, affecting 25,000 Americans annually, including many patients of active military age. This aggressive, rapidly progressive malignancy inherently prevents mission readiness for any affected Service Member. Cure is achieved for many DLBCL patients with aggressive frontline treatment combining multiple chemotherapy agents and monoclonal antibodies. Many other patients, however, require additional treatments for relapsed or refractory (rel/ref) disease. Unfortunately, a majority of rel/ref DLBCL patients are failed by currently available therapies and will die from their disease. A new kind of immunotherapy called chimeric antigen receptor (CAR) T cells is radically reshaping our approach to rel/ref DLBCL, able to produce long-term remissions in about 40% of those treated.

Reasons for CAR-T treatment failures, however, remain incompletely understood. CAR-T cells are derived from each patient's own immune system, and it is known that dysfunction of these cells is a reason for treatment failure in some patients. Many treatment failures are not explained by these factors, however, and in particular the ways by which lymphoma tumors themselves are able to avoid clearance by CAR-T cells is poorly understood. We recently undertook the first ever comprehensive look at genomic factors in DLBCL tumors treated with CAR-T cells. We employed whole-genome sequencing, which allowed us to investigate acquired genomic changes in tumors that are missed by other techniques. In particular, complex changes of tumor DNA such as rearrangements of chromosomes and fingerprints left behind in DNA by particular mutational processes were captured by our sophisticated techniques, fueled by the particular expertise of our investigative team. We found specific genomic changes associate very strongly with CAR-T treatment failures, demonstrating that tumor-intrinsic factors are key mediators of responses. Mechanisms by which this occurs, however, remain to be determined. In this proposal we will identify how specific mutational patterns in DLBCL genomes prevent CAR-T efficacy. Specifically, we will more clearly define genomic patterns associated with poor CAR-T responses and cross-compare their effects on additional treatment options available to these patients. We also will determine the way in which genomic factors lead to reprogramming of the immune cells that infiltrate DLBCL tumors, which are key drivers of both response and resistance to CAR-T therapy. Finally, we will employ sophisticated laboratory systems including accurate animal models to define the specific mechanisms that mediate outcomes so that new therapies able to overcome resistance can be developed on a more rational basis. Patients affected by this project are all those not cured by frontline DLBCL therapy, because CAR-T treatments are now available to increasing numbers of patients but need to be made to work long term for more than 40% of those treated. Immediate benefits to patients include bringing forward specific factors readily identifiable in tumors that allow clinicians to best identify those patients likely to be failed by CAR-T cells so that alternative approaches can

be pursued instead. Over a longer term, we expect to inform development of novel therapies able to overcome the resistance mechanisms we characterize.

The Overarching Challenge addressed by this proposal is Therapeutics: Advance Immunotherapy Across the Different PRCRP Topic Areas. CAR-T therapies are either available or under development for multiple additional malignancies. We expect the results of this project to be broadly applicable across multiple cancer types, both because of the unique approaches we will develop to address these research questions and the specific mechanism of disease resistance to therapy we reveal. Results are therefore directly relevant to thousands of active-duty and previous military Service Members. Our cancer center has more than 100 clinical encounters annually with lymphoma patients on active-duty military or U.S. Department of Veterans Affairs (VA) insurance plans. Because frontline treatment for lymphoma is provided by the VA itself, all VA patients we see are inherently those with rel/ref disease and therefore eligible for CAR-T and other salvage therapies

Proposal Title:	Prevalence and Biologic Impact of FGFR3 Genomic Alterations in Bladder Cancer
Log Number:	CA220407
Current PI Name:	Gopakumar Iyer
Award Number:	HT9425-23-1-0637
Current Contracting Organization:	Sloan Kettering Institute for Cancer Research
Current Performing Organization:	Sloan Kettering Institute for Cancer Research
Web Approval Date:	07-12-2023

The fiscal year 2022 Peer Reviewed Cancer Research Program (PRCRP) Topic Area to be addressed is "bladder cancer."

Scientific Objectives and Rationale: Metastatic bladder cancer remains incurable in most patients. Erdafitinib is an oral targeted therapy approved for treating patients with metastatic bladder cancer that harbors genetic changes (mutations or fusions) within the fibroblast growth factor receptor-3 (FGFR3) gene, present in about 20% of patients with metastatic disease. While the drug is effective at shrinking tumors and controlling disease, the median time that patients are on treatment before the disease progresses is less than 6 months. The reasons for this rapid onset of resistance are largely unknown, and the goal of this proposal is to identify these mechanisms. Additionally, bladder cancer is known to have a high degree of genomic heterogeneity, or differences in the profile of mutations, between different stages of tumors and even different portions of the same tumor in individual patients. We will leverage a largest-of-its-kind cohort of patients with bladder cancer who have undergone genetic sequencing of their tumors (including of FGFR3) to identify differences in FGFR3 mutation status between a primary tumor and metastatic biopsies within the same patients to gauge how often FGFR3 mutations are only found in a primary tumor or a metastasis. This work will help answer whether genetic sequencing of a metastatic site may be critical to the accurate identification of patients with FGFR3 mutations who may be eligible for erdafitinib therapy. Recognizing the fact that obtaining biopsies is simply not feasible for many patients, we will also explore the rate of FGFR3 alterations in circulating tumor DNA. Utilizing the same patient cohort with primary and metastatic biopsies available who are being managed at our institution, we will collect plasma for ctDNA isolation at the time of systemic treatment initiation (such as chemotherapy, immunotherapy, or erdafitinib). Following genetic sequencing of ctDNA, we will compare the FGFR3 alteration rate with tumor tissue. In some patients, we expect that FGFR3 alterations may only be detected in ctDNA due to underlying genomic heterogeneity that cannot be captured from single-site biopsies.

In a parallel effort, we will perform an in-depth evaluation of FGFR3 mutant mouse models derived from patient tumor biopsies. Specifically, mice injected with patient-derived tumors containing various FGFR3 mutations will be treated with erdafitinib to assess sensitivity to drug as a function of the FGFR3 mutation and the profile of co-alterations within the tumor. We will perform detailed analyses of tumors that grow or recur after initially shrinking to identify mechanisms of resistance to erdafitinib and we will generate erdafitinib- resistant models and study resistance mechanisms using genetic sequencing. We will validate novel mechanisms within the lab.

Finding ways to prolong treatment with approved drugs, such as erdafitinib, or combining erdafitinib with other drugs to delay or prevent resistance to therapy, represents an area of critical unmet need. Additionally, accurate identification of patients who are most likely to benefit from erdafitinib therapy is urgently needed. In the current era of precision medicine in which genetic sequencing has been incorporated into the routine management of several cancers, it is even more essential to define the optimal specimen to sequence given the spatial and temporal heterogeneity of therapeutically actionable genetic alterations. In the long-term,

several new FGFR inhibitors that are likely to be better tolerated than erdafitinib are about to begin clinical trial development. We plan to open two studies of FGFR3-specific inhibitors at MSKCC, and the proposed work will provide a head start to define resistance mechanisms to these novel agents.

This proposal seeks to address the PRCRP Overarching Challenge of identifying strategies to predict treatment resistance, recurrence, and the development of advanced disease. If successful, novel strategies to bypass these resistance mechanisms will be explored, including combination therapies with FGFR inhibition, to ultimately improve prognosis for patients with metastatic bladder cancer.

We seek to address the PRCRP Military Health Focus Area of gaps in cancer research that may affect mission readiness. By optimizing FGFR-targeted therapy strategies, we hope to minimize cancer relapse and improve survival outcomes in Service Members. Moreover, we will define the role of ctDNA as a noninvasive method to detect disease relapse and the onset of resistance to FGFR inhibition, thereby improving quality of life by minimizing the need for invasive biopsies.

Proposal Title:	CAR T-Cell Therapy to Target CD94 in T/NK Cell Malignancies
Log Number:	CA220435
Current PI Name:	Sattva Neelapu
Award Number:	HT9425-23-1-0606
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	07-12-2023

Genetically engineered T-cell therapy called chimeric antigen receptor (CAR) T-cell therapy has been shown to be potentially curative for patients with B-cell leukemias and lymphomas with four CAR T-cell therapy products approved in the last 5 years for the treatment of patients with various B-cell malignancies. However, the development of CAR T-cell therapy products for T/NK-cell leukemias and lymphomas has been challenging due to difficulty in identifying appropriate targets. We identified CD94 as a potential target for CAR T-cell therapy for certain T/NK-cell leukemias and lymphomas, which include extranodal NK/T-cell lymphoma, hepatosplenic T-cell lymphoma, aggressive NK-cell leukemia, T-large granular lymphocytic leukemia (T-LGL), and chronic lymphoproliferative disorder of NK cells (also known as NK-LGL). While these are rare malignancies, collectively, they represent a group with significant unmet clinical need and they fall into the fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) Topic Areas of blood cancers, lymphoma, and young adult cancers. Indeed, the median age of patients with aggressive NK-cell leukemia and hepatosplenic T-cell lymphoma is 30-40 years and their median survival is only 6-24 months. Similarly, in extranodal NK/T-cell lymphoma, which affects patients in their 40’s, outcomes are poor in those who have extensive disease or chemoresistant disease. T-LGL and NK-LGL have a median age of onset in the 60’s and have an indolent course, although a subset, exhibit aggressive disease and need novel therapies.

We developed a CAR molecule to target the above malignancies from a proprietary antibody we generated against CD94. CAR T cells targeting CD94 were very effective at killing CD94-expressing NK-cell leukemia cell lines but not B-cell lymphoma cell lines that did not express CD94. In this proposal, we will investigate strategies to optimize the CAR design and then test them in preclinical studies including in mouse models of T/NK-cell leukemias and lymphomas to determine the optimal CAR T-cell therapy product for eventual evaluation in patients. In addition, we will develop a novel safety switch, which could be activated to eliminate these CAR T cells in case of unexpected toxicity in patients. In the long term, this novel therapeutic product could significantly improve survival in these patients who have a major unmet clinical need. Furthermore, the novel safety switch could potentially be applied to other CAR T-cell products and cell therapies in the future.

Development of a novel and highly effective CAR T-cell therapy for such T/NK-cell leukemias and lymphomas that predominantly affect young adults would improve health and mission readiness of military personnel, Veterans, and their dependents, an FY22 PRCRP Military Health Focus Area, while also addressing the FY22 PRCRP Overarching Challenge through the identification of new target (CD94) and by advancing a novel immunotherapy strategy for treatment of five different cancers.

Proposal Title:	XPO1 as a Therapeutic Target in ZFTA-RELA-Driven Ependymoma
Log Number:	CA220510
Current PI Name:	Stephen Mack
Award Number:	HT9425-23-1-0624
Current Contracting Organization:	St. Jude Children's Research Hospital, Inc.
Current Performing Organization:	St. Jude Children's Research Hospital, Inc.
Web Approval Date:	07-12-2023

Our proposal aligns with the fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) Topic Area(s) on (i) brain cancer, and (ii) pediatric brain tumors and the FY22 PRCRP Military Health Focus Area “Gaps in cancer research that may affect mission readiness.”

The lead scientific investigators of this proposal, Dr. Mack and Dr. Roussel, study a type of aggressive pediatric brain tumor called ependymoma. Treatment for ependymoma has not changed in the last 20 years. In collaborative studies they have identified that ependymoma cells depend on two novel proteins to grow, called XPO1 and SMARCA4. Critically, drugs are available to target these two proteins, with XPO1 inhibitors in further clinical development and capable of crossing the blood brain barrier.

Our Overall Objective Is To: (1) Rigorously test drugs that block the Xpo1 and Smarca4 pathway across specific types of ependymoma in multiple animal models. (2) Identify drug combinations that may be rapidly transitioned to phase 1 clinical trials in ependymoma.

Our research will help children with a type of pediatric brain tumor called ependymoma. However, more broadly scientific findings from our research have the potential to inform treatment of other cancer types driven by the same pathway. Furthermore, the innovative framework we have developed to identify druggable targets using multiple technologies could readily extend to other pediatric and adult cancer types.

The ultimate goal of our research is to take findings from each of our objectives and directly translate these to a clinical trial in patients St. Jude. Critically, XPO1 inhibitors are currently being evaluated in clinical trials at St. Jude for another pediatric brain tumor type called ATRT. If findings from our proposal are successful, we seek to leverage this data to expand our St. Jude clinical trial to include ependymoma patients, making a near-term and immediate impact on patient care. We would anticipate translating these findings to clinical trials in patients in approximately 2 years. While this appears as an accelerated timeline, we have already defined clinical dosing in patients for XPO1 inhibitors along with safety and toxicity considerations.

This will provide patients and their families an option to enroll on a clinical trial that evaluates XPO1 therapy. This is particularly relevant since there few clinical trials open across the U.S. for this patient population and even fewer ependymoma-focused trials.

Our proposal will address the specific FY22 PRCRP overarching challenge to “Transform cancer treatment through the identification of new targets, especially for advanced disease and metastasis.” XPO1 and SMARCA4 pathway is a novel target that we have discovered in ependymoma using distinct methods. Our research proposal seeks to rigorously test XPO1 and SMARCA4 drugs and to identify rationale drug combinations so we can target drug-resistant cells with alternate therapies.

FY22 PRCRP Military Health Focus Area: Our research grant would directly affect “Gaps in cancer research that may affect mission readiness,” specifically the treatment and health of military members, Veterans, their

beneficiaries, and the general public. Our strategy to identify combination therapy using proteomics profiling and CRISPR-CAS9 KO screening has potential to broadly apply to the study of other cancer types and improving our understanding of cancer toward new therapeutic development.

Proposal Title:	Immunosuppressive Factors Contributing to Immunotherapy Resistance in Endometrial Cancer with Deficient Mismatch Repair System
Log Number:	CA220518
Current PI Name:	Haider Mahdi
Award Number:	HT9425-23-1-0807
Current Contracting Organization:	Magee-Womens Hospital
Current Performing Organization:	Magee-Womens Hospital
Web Approval Date:	10-03-2023

Identifying factors that contribute to immunotherapy resistance in endometrial cancer (EC) patients with deficient mismatch repair system (dMMR): Immunotherapy with immune checkpoint inhibition (ICI) has emerged as a promising therapy in several cancers. A subset of endometrial cancer (about 30%) are characterized by certain genetic alteration called "deficient mismatch repair" and are highly responsive to immunotherapy with ICI. However, only 30%-50% of these patients do benefit from immunotherapy. Also, treatment with immunotherapy can lead to development of secondary resistance. Therefore, effective strategies are warranted to improve the overall benefit and reverse resistance to immunotherapy in dMMR EC. Targeting immunosuppressive factors within the tumor immune microenvironment (TME) represents an attractive approach. Our focus in this proposal is on tumor-associated macrophages in dMMR EC.

Macrophages with a specific ’suppressor’ phenotype (M2 subtype) within TME play a significant role in promoting an immunosuppressive environment and in mediating therapy resistance. These cells are prominent in dMMR EC. However, another phonotype (M1 subtype) provides a favorable pro-inflammatory TME and enhance the immune response and synergize with ICI. Targeting macrophages and switching their phenotype from M2 to M1 is potentially promising approach that has not been investigated thoroughly before.

Identifying dMMR EC patients who will likely respond to immunotherapy with ICI combined with therapy targeting macrophages: Immunotherapy with ICI is very attractive given the longstanding activity, durable response, and lower risks. Other predictive markers have been investigated but failed to identify subsets of patients who likely benefit from this approach. We have developed novel molecular signatures that correlate with resistance to ICI. The potential promise of these signatures is to identify patients who will not respond to ICI alone, but will benefit from them in combination with targeting immunosuppressive macrophages.

Given the very high cost of these molecules (greater than $200,000/patient/year), there is also the real risk of financial toxicity for patients. Therefore, there is a clear unmet need for biomarkers to identify which dMMR EC patients harbor tumor with prominent immunosuppressive macrophages that will likely benefit from combination immunotherapy approach. Having such biomarkers will have significant impact, as it will allow us to identify the subset of these patients who will benefit from immunotherapy with durable response. At the same time, it will allow us to spare those who likely will not respond and spare them the time, effort, and side effects associated with the therapy, while also sparing them financial toxicity.

We have shown that tumor associated macrophages, immune signatures related to CD47 and transforming growth factor-beta (TGF-beta) pathways are associated with lower survival outcome in dMMR EC and lower response to immunotherapy.

The scientific premise of this project is to identify immune related pathways that contribute to immunotherapy resistance and can be reversed by targeted inhibition of these pathways. Further, molecular signature based on our 6-genes TGF-beta score and CD47 expression will predict resistance to ICI alone and identify patients who will benefit from targeted inhibition of TGF-beta or CD47 in combination with ICI.

In this study, we propose to investigate the role of macrophages and immune pathways related to it in mediating resistance to immunotherapy. This can provide the basis for future studies to target these pathways. TGF-beta is known to mediate resistance to immunotherapy and to induce M2 subtype of macrophage. Furthermore, CD47 is utilized by tumor cells to escape ingestion by macrophage and CD47 thereby impairs the innate immune response. We hypothesize that immunotherapy resistance in dMMR EC may be mediated by immunosuppressive tumor-associated macrophages, through upregulation of the TGF-beta and/or CD47 pathways. We also hypothesize that TGF-beta and CD47 are increased at baseline or recurrent setting and consequently mediate immunotherapy resistance. In our approach, we will utilize preclinical animal models of endometrial cancer. We also will utilize samples of patients before and during treatment with immunotherapy. We will use an 'omics' approach to identify specific genes and proteins expressed on both immune cells and tumor cells. Also, we aim to demonstrate the macrophage 'phenotype switch' in patients who develop resistance to immunotherapy with ICI and the relationship of this resistance to the TGF-beta and CD47 pathways.

If successful, the potential for clinical impact is significant, as TGF-beta inhibition and anti-CD47 targeted therapies are currently in early-phase clinical trials, and successful execution of these studies will provide a rationale for advancing these molecules into trials focused on combination with immune-based therapies in patients with dMMR EC in a biomarker-focused approach. The proposed project is novel as it focuses on the role of tumor-associated macrophages which are prominent in the dMMR EC microenvironment. The proposed research effort will address the problem of therapy resistance and advance the development of a novel therapy with potential durable response.

Proposal Title:	The Role of Bitter Taste Receptor TAS2R38 in Esophageal Cancer
Log Number:	CA220560
Current PI Name:	Prasad Dandawate
Award Number:	HT9425-23-1-0391
Current Contracting Organization:	Kansas, University of, Medical Center Research Institute, Inc.
Current Performing Organization:	Kansas, University of, Medical Center Research Institute, Inc.
Web Approval Date:	08-17-2023

Scientific Objective and Rationale: Esophageal Cancer (EC) is the seventh leading cause of cancer-related deaths in the U.S. and are projected to increase 35% by 2025, with a poor survival rate of less than 20%. Esophageal adenocarcinoma (EAC) is less studied compared to other cancers, and it typically shows extremely aggressive clinical features if diagnosed late, making it an extremely difficult cancer to treat. Three main causes for EAC increases are excess body weight, a high-fat diet, and an increased number of cases of Barrett's esophagus (BE), a precancerous condition for EAC. While studying the taste changes in patients with BE and gastrointestinal acid reflux disease (GERD), we unexpectedly discovered one of the taste receptors responsible for recognizing bitter taste is highly expressed in EAC patients.

Patient Investigators with Voices Together (PIVOT) is an organization within the cancer center where individuals with lived cancer experience (as a survivor or caregiver) periodically connect with investigators to discuss various bedside observations. It was during one of these meetings at which the Principal Investigator (PI) heard from a patient experiencing bitter taste prior to their cancer diagnosis. This led the drive to understand the phenomena. Although the taste would be something that relates to the tongue, interestingly, the PI made a unique observation that bitter taste receptor type 2 member 38 (TAS2R38) is being upregulated (44-fold) in EC when mining The Cancer Genome Atlas database. Our research confirmed TAS2R38 is highly expressed in EAC tissues and cell lines compared to normal tissues. Further, we treated EAC cell lines with a specific compound N-(3-oxododecanoyl)-L-homoserine lactone that selectively binds to TAS2R38, and we observed a calcium release in the cells suggesting the TAS2R38 receptor is functional in EAC. The objective of our study is to understand the role of TAS2R38 in EAC progression and as an early marker for EAC.

What Types of Patients Will the Research Help and How Will It Help Them? EAC biology is not extensively studied, and diagnosis and treatment strategies have not been developed. There are differences in EAC incidence and survival outcomes based on race and gender. African American (AA) population is reported to have a higher death rate as compared to Caucasians. Esophageal squamous cell carcinoma has been commonly diagnosed in AA and Caucasian females, whereas esophageal adenocarcinoma is more common among Caucasian males in the U.S. We found that TAS2R38 expression is high in the AA population compared to Caucasians and high in males compared to females. Hence, we believe TAS2R38 might serve as a biomarker to explain these racial and gender differences. Also, Kansas City hospitals provide treatment for patients living in rural areas. Traveling far to receive treatment acts as a barrier and is an under-recognized problem. We will include BE and EAC patient populations from different geographical locations, races, gender to establish TAS2R38 as a novel biomarker for early detection. Further, we will study the role of TAS2R38 in EAC progression, which will serve as a foundation for developing future studies for prevention and therapy of EAC in Veterans, their Families, underserved, underrecognized and the general population.

FY22 PRCRP Overarching Challenge(s): The research proposed in this proposal will establish TAS2R38 is a novel biomarker to predict EAC risk and advancement of the disease in active-duty Service Members, Veterans, and the American public. We will use a larger cohort of Veteran patients from different races, gender, and geographical locations to study the TAS2R38 expression. We will use patient-derived cell lines and cutting-edge technologies such as single-cell RNA sequencing and proteomics to help us study the role

of TAS2R38 signaling in EAC in an unbiased manner. We will also delete TAS2R38 from cell lines to study the effect of TAS2R38 loss on tumor growth in cell lines and animals. This will help us in establishing the role of TAS2R38 in EAC progression. This study will help prevention strategies, diagnosis, treatment, and outcomes for patients in underserved or under-recognized populations (e.g., military, minorities, and rural populated communities).

FY22 PRCRP Military Health Focus Area: EAC, along with BE is a major health problem among the active-duty Service Members and Veterans. Veterans with a history of GERD, tobacco, alcohol use, and obesity are at greater risk of EAC. It is estimated that 20%-30% of Veterans suffer from GERD and that 1% of them can develop BE.

U.S. Marine Corps Base Camps military members and their Families exposed to contaminated drinking water with industrial solvents, benzene, and other chemicals are at high risk for EAC. The data generated from these studies will be used as a foundation for developing future research studies aiming toward treatment of this debilitating disease and improve the overall health of active-duty Service Members and Veterans. This research will also help to close the gap between BE and EAC detection, improve prognosis, and serve as a target for future drug discovery to improve the EAC treatment paradigm of active-duty members, Veterans, and the American Public.

Proposal Title:	Novel Combination Therapy Targeting Growth Factor Receptor Cross Talk with Epitranscriptomic Regulation in Glioblastoma
Log Number:	CA220598
Current PI Name:	Jeremy Rich
Award Number:	HT9425-23-1-0689
Current Contracting Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Current Performing Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Web Approval Date:	08-17-2023

Brain tumors represent severe challenges in clinical oncology. Among all brain tumors, glioblastomas are the most prevalent malignant brain tumor that originates in the brain itself. Despite decades of research, glioblastomas remain universally fatal, with current treatments offering only palliation. Veterans and members of the armed services with brain cancer suffer worse outcomes than the general population. We are dedicated to improving the outcomes of patients afflicted with brain tumors using novel scientific techniques, directions, and therapies. Precision Medicine, or Personalized Medicine, combines genetic testing with other patient and cancer data to direct optimal care. Our studies will inform the development of improved Precision Medicine approaches for glioblastoma patients: the right patient, the right drug, the right dose, the right route, and the right time. These efforts will provide the basis for the development of clinical trials to which patients, including members of the Armed Forces, which may improve the performance status and survival of brain tumor patients. We anticipate that these studies will also identify biomarkers, which allow for patients to be selected for specific therapies and monitor their efficacy. Biomarkers are essential for improved patient selection to avoid treating patients with ineffective drugs and identification of early treatment failure. Eventual downstream deliverables of our studies could be applied to noninvasive imaging and metabolic monitoring. Our proposed studies will focus on laying the foundation for clinical trials based on novel paradigms. Thus, the goals of this project are aligned with the fiscal year 2022 Peer Reviewed Cancer Research Program Overarching Challenges to: (1) Transform cancer treatment through the identification of new targets, especially for advanced disease. (2) Identify and elucidate the mechanisms behind cancer epigenetics/genetics and cancer development to improve treatment methods.

Glioblastomas contain highly malignant tumor cells that replicate features of normal stem cells. These cancer stem cells contribute to the aggressiveness of brain tumors through their resistance to radiation and chemotherapy, ability to promote growth of new blood vessels to feed the tumor, evasion of the antitumor immune responses, and invasion into normal brain to prevent surgical removal. Designing new treatments that attack cancer stem cells, but not normal stem cells, could allow patients to survive longer and better. We generate cancer stem cells from the surgical biopsy samples from patients afflicted with glioblastoma. We are studying how the mechanisms by which these cancer stem cells are maintained. Cancer cells receive signals from their environment called growth factors. These growth factors and their receptors have served as targets for cancer therapies, but glioblastomas have been relatively resistant to these treatments. We find that these growth factor pathways regulate tumor cells through modifying RNA, which bridges DNA and proteins. As a result, cancer cells regulate their metabolism to survive stressful conditions. Using combinations of drugs against each of these targets, we predict that we will be able to specifically kill the cancer stem cells. Based on this background, we will extensively study these pathways and the dependency of cancer and normal cells to lay the foundation for clinical trials with brain cancer patients.

Proposal Title:	Potentiating T Cell Activity Against Multiple Myeloma Through SUMOylation Inhibition
Log Number:	CA220620
Current PI Name:	Steven Rosen
Award Number:	HT9425-23-1-0658
Current Contracting Organization:	City of Hope Beckman Research Institute
Current Performing Organization:	City of Hope Beckman Research Institute
Web Approval Date:	10-03-2023

This proposal addresses the fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) Topic Area of Myeloma and the FY22 PRCRP Military Health Focus Areas of Environmental exposure risk factors associated with cancer and Gaps in cancer research that may affect mission readiness. Multiple myeloma (MM), the second most common blood cancer, is characterized by abnormal growth of a type of white blood cells, called plasma cells, in the bone marrow. In the United States, more than 34,470 new patients are expected to be diagnosed with MM in 2022, and more than 12,640 patients will die of the disease. MM is currently incurable, and the survival rate is low: around 55% of patients survive for 5 years after the cancer is found. For patients for whom all other treatments have failed, chimeric antigen receptor (CAR) T cell therapy remains a last promising option that may offer hope. CAR T cells are manufactured by harvesting T cells from a patient, inserting a gene for a specific receptor into their T cells, which reprograms the cells to recognize and kill cancer cells, and infusing them back into the patient like a blood transfusion. CAR T cell therapy has shown impressive results for treating MM. We have tested two types of CAR T cells that have been reprogrammed to target proteins called B-cell maturation antigen (BCMA) or CS1 on the surface of MM cancer cells. Although CAR T therapy is quite effective, less than half of treated patients achieve long-term responses. Thus, there is an urgent need to improve CAR T cell therapy.

We have shown that inhibiting SUMOylation, a process of protein modification inside cells, is a novel therapy with the potential to address this need. We found a Small Ubiquitin-like MOdifier (SUMO) inhibitor drug called TAK-981, which effectively killed MM in mouse models of the disease. TAK-981 could kill MM cancer cells directly and could kill MM cancer cells indirectly by promoting immune cells to kill MM. Specifically, TAK-981 helped normal T cells function better and prevented T cell "exhaustion." T cell exhaustion is a loss of T cell immune function, which is a major barrier for improving CAR T cell therapy. Our data also showed that TAK-981 treatment enhanced the ability of BCMA-CAR T and CS1-CAR T cells to kill MM cells grown in the laboratory. More importantly, TAK-981 strengthened CS1-CAR T cell MM killing in a mouse model of MM, and mice treated with TAK-981 + CS-1 CAR T cells lived significantly longer than mice treated with CS1-CAR T cells alone.

Based on our promising preliminary data, we hypothesize that inhibiting SUMOylation improves the ability of CAR T cells to kill MM cancer cells by enhancing CAR T function and preventing exhaustion. We will: (1) Measure the ability of TAK-981 + CAR T cells to kill MM cells grown in the laboratory and in a mouse model made with human tumor cells from a patient with MM, and assess safety of the drug in the mice; (2) Inhibit SUMOylation during CAR T cell manufacturing and measure how much it improves the function of CAR T cells. We expect our study to show that SUMOylation inhibition improves CAR T cell killing of MM and to reveal the cellular mechanisms that cause this effect.

BCMA-CAR T cells are a U.S. Food and Drug Administration-approved therapy for MM treatment. CS1-CAR T cells were developed by our team and are currently in a phase 1 trial, and TAK-981 is in a phase 1/2 clinical trial for MM patients; thus, all three treatments have sufficient safety and efficacy clinical information. Our study will build new combination therapies (TAK-981+BCMA-CAR T and TAK-

981+CS1-CAR T) with the potential for rapid translation into MM clinical in the near-term. We expect to achieve a patient-related outcome in 2-3 years. Our new combination therapy will be used for patients with relapsing MM who have failed all other treatments. Because SUMOylation inhibition can prevent CAR T exhaustion and strengthen CAR T function, patients who receive TAK-981 after CAR T cell infusion will have more functional CAR T cells in their body to more efficiently kill cancer cells and keep CAR T cells lasting longer to prevent cancer relapse, resulting in better response and complete remission.

Active-duty Service Members and Veterans who were exposed to Agent Orange or other herbicides during service may have certain cancers related to their exposure. MM is one of these presumptive diseases and CAR T therapy remains the last promising option when all other treatments fail. Our study presents TAK-981 as a new potent regimen to enhance CAR T therapy efficacy as an effective way to minimize cancer relapse for Service Members or their families. Our proposed combination therapy will improve outcomes for patients with MM, with better quality of life and improved survivorship, that may improve mission readiness and the health and well-being of military members, Veterans, their beneficiaries, and the general public.

FY22 PRCRP Overarching Challenge - Therapeutics: Our study will support clinical translation of TAK-981, which inhibits a novel target (SUMOylation), to improve current CAR T cell therapy in MM, with great promise to advance immunotherapy in other blood cancers and solid tumors.

Proposal Title:	Improving the Oncolytic Immunovirotherapy of Malignant Glioma by Targeting the Glioma Secretome
Log Number:	CA220624
Current PI Name:	Jianmei Leavenworth
Award Number:	HT9425-23-1-0792
Current Contracting Organization:	Alabama, University of, at Birmingham
Current Performing Organization:	Alabama, University of, at Birmingham
Web Approval Date:	10-03-2023

This proposal is focused on the Topic Area of "Brain Cancer." Malignant brain tumor remains a fatal disease. With standard therapy, prognosis is still unfavorable, with a median survival of less than 2 years. We developed oncolytic herpes simplex virus (oHSV) to treat both adult and pediatric brain tumor patients, given its potential to selectively kill tumor cells while sparing normal brain cells. Although the therapy shows some promise based on the results from our phase 1 trials, all patients die of tumor relapse and growth. We studied the factors that may comprise the oHSV therapeutic efficacy, and found that a factor, called acyl-CoA-binding protein (ACBP), was secreted from the virus-killed tumor cells, inducing the aggressive behavior of remaining tumor cells and impairing immune system in fighting tumor, and subsequently leading to impaired mice survival. To prevent the ACBP-induced secondary effects and to improve the oHSV therapeutic efficacy while bypassing the hurdles from directly targeting soluble ACBP, we found that inhibition of Bmi1, a tumor-promoting factor, in glioma cells could suppress glioma growth, reduce ACBP expression/secretion, and promote anti-tumor immune response. Here we propose to define the underlying mechanisms for the ACBP’s pro-tumoral and immunosuppressive activity, and how inhibition of Bmi1 modulates the immune cells within the tumor beyond its conventional role in tumor cells. This comprehensive understanding of the pros and cons of oHSV and Bmi1 inhibition benefited us to develop a new strategy by timely combining these agents to treat brain cancer and to evaluate its therapeutic efficacy in preclinical and patient-derived xenograft models, which we anticipate should be readily translatable to the clinic and create a near-term impact, since both oHSV and Bmi1 inhibitor are currently evaluated in clinical trials.

We believe that the proposed research will uncover new mechanisms for promoting the malignancy of brain tumor, and have the potential long-term outcome to develop a biomarker (i.e., ACBP) to predict treatment resistance and recurrence, which can be exploited to strategically direct a combined therapeutic approach to mitigate adverse effects in brain tumor patients post-oHSV therapy; thereby, transforming cancer treatment in the field of oncolytic virus therapy of brain tumor toward a new direction (fiscal year 2022 [FY22] Peer Reviewed Cancer Research Program [PRCRP] Overarching Challenges). Military personnel, particularly Gulf War Veterans, are significantly associated with high risk of brain tumors. This study will address the gaps in cancer prognosis and treatment that may impact mission readiness and lead to improvements in survival while minimizing late effects and cancer relapse for the military personnel and the general population with brain cancer (FY22 PRCRP Military Health Focus Areas).

Proposal Title:	Effect of CD47 on the Tumor Microenvironment in Diffuse Midline Glioma, a Fatal Pediatric Brain Tumor
Log Number:	CA220651
Current PI Name:	Robyn Gartrell
Award Number:	HT9425-23-1-0570
Current Contracting Organization:	Columbia University Medical Center
Current Performing Organization:	Columbia University Medical Center
Web Approval Date:	07-11-2023

Project Narrative: Brain tumors (gliomas) are responsible for more childhood deaths and the greatest number of years of potential life lost than any other type of cancer. Despite advances in surgery, radiation, and chemotherapy, diffuse midline gliomas (DMG), are among the most aggressive of these tumors and are 100% fatal. New treatment methods (therapeutics) using the immune system (which is composed of cells that are involved in fighting infection), called immunotherapies, offer promise for treating brain tumors in children. However, in order to use immunotherapy in childhood glioma, it is important that we understand how the immune system responds to these tumors, also known as the tumor immune microenvironment. As radiation is the mainstay therapy for DMG, understanding how radiation affects the immune microenvironment – and especially microglia, the most abundant immune cell in the brain – is critical. The results of the proposed studies will bring us closer to successful treatment strategies and combination therapeutics using radiation and immunotherapy for pediatric brain tumors. Specifically, we find that radiation increases CD47, a “don’t eat me” signal given off by the tumors that allows them to escape immune attack. We can use an immunotherapy against CD47 during and after radiation to improve outcomes in these devastating diseases.

Career Development: The Principal Investigator (PI) for this study is currently an Assistant Professor of Pediatrics in the Division of Pediatric Hematology/Oncology at Columbia University Irving Medical Center. She completed her clinical training in Pediatric Oncology and also completed research training as a postdoctoral fellow in Immuno-oncology/Immunotherapy and Precision Medicine. During her training she successfully completed multiple projects leading to six first-author publications evaluating the tumor immune microenvironment in adult tumors, including brain cancer. She also completed an advanced degree, Master of Science in Patient Oriented Research in May 2020. Her training thus far has provided a strong base in clinical trial development, biomedical statistics, epidemiology, and grant writing while also improving her approach to scientific study.

This proposal presents a research and career development program focused on evaluating the CD47/SIRPa pathway, especially in response DMG, a fatal pediatric brain tumor. The outlined proposal builds on the candidate’s previous research in immune microenvironment and immunotherapy and integrates two new domains of expertise represented by her mentor team with career guide, Dr. Peter Canoll and co-mentor Dr. Raul Rabadán including mechanistic evaluation of preclinical models and computational analysis of immune cells. To pursue her long-term goal of researching pediatric brain tumors as an independent physician scientist, she has identified several gaps in her training and put together a strong Career Development plan that will address these gaps with the help of her mentors and advisory committee.

Impact: The median survival of a child with DMG is 9-15 months. Thus, a diagnosis of this cancer in the child of a military member will certainly impact mission readiness as there is not much time to spare once the tumor has been identified. Further, DMG, being one of the most devastating of pediatric brain tumors, will sacrifice the health and well-being not only of the child but also their military family. This Department of Defense – Career Development Award will improve the use of therapeutics, specifically advancing the

use of immunotherapy for DMG. The PI is already engaged directly with cutting edge pediatric cancer and immuno-oncology researchers in committees and working groups which will help to make the findings from this proposal directly available to patients as soon as the research is complete.

Proposal Title:	Dissecting the Role of PPM1D in Leukemia Development and Treatment Resistance
Log Number:	CA220652
Current PI Name:	Peter Miller
Award Number:	HT9425-23-1-0646
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	09-14-2023

Over the past few decades, we have made great strides in our ability to prevent, diagnose, and treat a wide array of cancers. As a result, outcomes for individuals with cancer have significantly improved, resulting in fewer side-effects from treatment, longer survival, and more cures. In parallel, our scientific understanding of how cancers develop, grow, and spread have accelerated tremendously, in large part due to advances in the technologies we utilize to study these diseases. However, there are many cancers in which neither treatment options nor patient outcomes have meaningfully improved, and patient outcomes remain very poor.

Blood cancers vary by the type of blood cell from which they arise, the types of treatments utilized, and the expected success of these therapies. Two types of blood cancers, myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), remain highly lethal diseases. MDS and AML that occur in individuals who have been previously exposed to chemotherapy or radiation for the treatment of another cancer are referred to as "therapy related." MDS or AML can also arise from environmental exposures such as ionizing radiation. Unfortunately, therapy-related MDS and AML are particularly resistant to treatment and carry an average life expectancy of less than one year. Thus, new prevention and treatment options are desperately needed.

We have previously shown that a gene called PPM1D is often altered in the blood cells of people who have been exposed to chemotherapy and radiation. As a result, PPM1D is often altered in individuals with therapy-related MDS and AML. In our prior and preliminary work, we found that this alteration in PPM1D causes it to protect cancer cells from chemotherapy and radiation, thereby allowing cancer cells to become more resistant to subsequent treatments. The goal of this proposal is to better understand how PPM1D influences the ability of normal blood cells to become cancerous and how PPM1D causes these cancer cells to become resistant to therapy. We seek to utilize these biological insights to improve the outcomes of patients with therapy-related MDS and AML by identifying treatments that may be more effective and by discovering new approaches to prevent and treat these diseases. To achieve these goals, we have proposed three main experimental approaches. In Aim 1, we will utilize two new mouse models that we have developed, which allow us to control the levels of PPM1D in blood cells. Using these mice, we will determine how PPM1D impacts the ability of normal blood cells to become cancerous and study differences between cancer cells that have a normal version of PPM1D from those that have an altered version. In Aim 2, we will assess the ability of various classes of chemotherapies to kill leukemia cells, with the goal of finding the therapies rendered least effective by PPM1D.

We will then test whether blocking PPM1D with an inhibitor drug reverses the protective effect of PPM1D and causes leukemia cells to become more sensitive to these chemotherapies. Finally, in Aim 3 we will use genetic tools to study exactly how PPM1D causes cells to be resistant to chemotherapies and investigate how a therapeutic strategy to inhibit PPM1D could be influenced by other genes in the leukemia cells. This work is highly aligned with the Topic Areas (blood cancers), health Focus Areas (environmental factors that cause cancer and mission readiness), and Overarching Challenges (prevention and therapeutics) of the Department

of Defense' Peer Reviewed Cancer Research Program (PRCRP). Our study of blood cancers seeks to understand how they develop (new prevention strategies) and become treatment resistant (new therapeutic approaches). Achieving both could improve mission readiness by limiting time in hospital and decreasing relapses. Moreover, active members of the Armed Forces and Veterans are at an increased risk of developing therapy-related AML and MDS for two reasons. First, this population, particularly the aging Veteran population, have higher rates of many cancers that often require treatment with chemotherapy or radiation. Second, current and former members of the military are exposed to environmental, factors including ionizing radiation and chemicals such as Agent Orange or building debris that have been associated with increased rates of developing precancerous blood cells, MDS, and AML. While the immediate application of these studies is to patients with therapy-related MDS and AML, our preliminary data suggests that these insights could also directly be applied to non-therapy-related blood cancers. Furthermore, alterations in PPM1D are also found in many non-blood cancers. Thus, an understanding of the biology of PPM1D and ways to target it could have broad implications across oncology.

I am a scientist and physician who is passionate about using our biological understanding of blood cancers to develop new ways to prevent and treat these diseases. I run a blood cancer research laboratory and in my clinical role, I care for patients admitted to the hospital with leukemia. At this early stage of my independent scientific career, this Department of Defense award would have a transformative effect on my career development and ability to become a leader in my field. Not only would the financial support from this grant initiate an entirely new line of scientific inquiry in my lab, resulting in publications, additional funding, and an enhanced ability to recruit talented scientists, but it would also allow me to join the PRCRP's Virtual Cancer Center. The Virtual Cancer Center includes a rich network of scientists and individuals with a shared goal of advancing the care for patients with cancer. These individuals from outside my own institution can serve as mentors, peers, and collaborators, and in so doing, provide unique opportunities for improving myself as a scientist, principal investigator, and leader.

Proposal Title:	P300/CBP Inactivation: A Novel Epigenetic Therapeutic Approach for Ewing Sarcomas
Log Number:	CA220653
Current PI Name:	Darko Bosnakovski
Award Number:	HT9425-23-1-0456
Current Contracting Organization:	Minnesota, University of, Twin Cities
Current Performing Organization:	Minnesota, University of, Twin Cities
Web Approval Date:	07-12-2023

Ewing sarcoma is an aggressive cancer of the bone and soft tissues that typically occurs in children and young adults. Around 2% of all cancers in teens aged 15 to 19 are Ewing sarcoma. Despite aggressive treatment, the cancer relapses in 75% of the metastatic cases, with a 5-year survival rate of 20%. Therefore, a novel and effective therapy is greatly needed.

The most common underlying genetic cause for Ewing sarcoma is EWS-FLI1, an oncogene that arose from the fusion of two genes, EWS RNA binding protein 1 (EWSR1) and Friend leukemia integration 1 (FLI1). EWS- FLI1 is a transcription factor that controls the activity of many other genes crucial for the initiation, proliferation, and metastasis of Ewing sarcoma. Transcriptional factors are considered “undruggable” targets, thus, a specific therapy for Ewing sarcoma based on direct inactivation of EWS-FLI1 is less likely.

It was recently reported that the transcription activity of EWS-FLI1 depends on its interaction with two very similar proteins, E1A binding protein P300 (P300) and Cyclic adenosine monophosphate response element binding protein (CBP). P300/CBP are histone acetyltransferases that induce epigenetic changes to facilitate gene activation. Therefore, we hypothesized that the inactivation of P300/CBP could be an appropriate approach for suppressing EWS-FLI1 activity and an effective target for treating Ewing sarcoma.

For this reason, we aim to better understand the epigenetic mechanism of EWS-FLI1/CBP/P300 axis-driven carcinogenesis and to explore P300/CBP inactivation/degradation as a targeted therapy for Ewing sarcoma.

Our final goal is to develop a novel effective and specific therapy for Ewing sarcoma based on the inactivation of P300/CBP that will replace the current, in most cases, ineffective chemotherapy.

Our proposal for the fiscal year 2022 Peer Reviewed Cancer Research Program addresses two Topic Areas: “Cancer in children, adolescents, and young adults” and “Sarcoma,” with the objectives of bridging the gap of an effective treatment options for Ewing sarcoma that affects military members, Veterans, their beneficiaries, and the general public alike and improving mission readiness of Service Members by minimizing patients’ time in the hospital, reducing cancer relapse, and increasing the survival rate.

Proposal Title:	Elucidating Mechanisms of CAR-T Cell Immunotherapy Resistance and Sensitivity to Improve Treatment of Myeloma
Log Number:	CA220676
Current PI Name:	Erin Meermeier
Award Number:	HT9425-23-1-0298
Current Contracting Organization:	Mayo Clinic and Foundation, Scottsdale
Current Performing Organization:	Mayo Clinic and Foundation, Scottsdale
Web Approval Date:	06-09-2023

Scientific Objective and Rationale: Multiple myeloma is a type of blood cancer that affects plasma cells and is generally incurable. In multiple myeloma, malignant plasma cells accumulate in the bone marrow and crowd out normal tissue, which can lead to impaired immune function, bone loss, and kidney damage. Conventional cancer treatments, such as chemotherapy, are effective against multiple myeloma in the short-term, but can lower quality of life due to side effects and need to change frequently when tumors become insensitive. New approaches to treating cancer include the use of immune therapies, which train a patient's immune system to fight cancer. There has been remarkable success using immune therapies to treat multiple myeloma as evidenced by the U.S. Food and Drug Administration's recent approval of two types of chimeric-antigen-receptor (CAR) T cell therapies. One drawback to CAR-T cell therapy, which seems to be unique to treating multiple myeloma, is that while it is highly effective and safe, nearly all patients relapse on average 1 year post treatment. Our research will focus on understanding (i) biological phenomena underpinning this lack of more durable cancer control by CAR-T therapy, and (ii) test an alternative way of creating CAR-T therapy from immune cells that we hypothesize could be more effective specifically in the fight against multiple myeloma. To achieve these goals, we will employ the internationally recognized and clinically faithful mouse model of multiple myeloma for controlled experimentation. Our overall goal is to improve upon the highly effective immune therapies for multiple myeloma, with a vision for a future where safe, effective, durable treatment that utilizes a patient's own immune system can be employed early in the disease and may ultimately eliminate multiple myeloma.

Career Goals in Cancer Research: Dr. Meermeier aspires to be a leader in understanding how to use the immune system to fight multiple myeloma and other cancers. She is a Ph.D.-trained research immunologist who enjoys collaborating on multidisciplinary teams with physicians and basic scientists, who aims to translate her discoveries in the laboratory into new treatments for patients with multiple myeloma. This award will expand Dr. Meermeier's research program, foster new collaborations and projects, and allow her to pursue an ambitious set of experiments that could identify ways to advance immune therapies for multiple myeloma in the future. Dr. Meermeier will interact regularly with Drs. Bergsagel, M.D. and Chesi, Ph.D., world renowned myeloma specialists to ensure career and research milestones are accomplished.

Applicability of the Proposed Research: Overall, successful completion of this translational project may help many patients with multiple myeloma by identifying ways to make immune therapy more durable by subverting drug resistance. We hope that, by the end of the award period, the research will provide the rationale for further CAR-T cell development and correlative studies in multiple myeloma patients undergoing CAR-T therapy. We anticipate that this research will contribute to engineering next-generation CAR-T cell therapy more tailored to fight multiple myeloma and inform possible combinations of existing immune therapies that could give patients much longer periods of cancer-free life.

Overarching Challenge: In this proposal we aim to advance immunotherapy, specifically for multiple myeloma but also in ways that may apply to other cancers in the future. We expect our investigations will advance our understanding of CAR-T treatment resistance and recurrence that develops often in patients

with multiple myeloma. If successful, we expect our findings will contribute to making CAR-T cell therapy more effective and applicable to more patients, in time, reducing the need for conventional chemotherapies with more substantial treatment side effects.

Relevance to Military Health: Given the increased risk of developing MM from environmental exposures associated with military service and age, active Service Members, Veterans, and their families will benefit from research toward improving the efficacy and durability of safe CAR-T immunotherapy. CAR-T immunotherapy provides improvement in survival while eliminating the need for continual conventional cancer treatment that often involves chemotherapy with debilitating side effects. Advancing the efficacy and tolerability of cancer treatment with modern immune therapies will maximize the performance of active-duty Service Members afflicted by cancer, which will support mission readiness.

Proposal Title:	Targeting Inflammatory Signals as Therapeutic Strategies for Peripheral T-Cell Lymphomas
Log Number:	CA220704
Current PI Name:	Wen-Hsuan Lin
Award Number:	HT9425-23-1-0518
Current Contracting Organization:	Columbia University Medical Center
Current Performing Organization:	Columbia University Medical Center
Web Approval Date:	07-11-2023

Peripheral T-cell lymphomas (PTCLs) comprise a group of aggressive blood cancers that originate from normal T-cells. PTCLs affect middle-aged to older adults and show poor responses to conventional chemotherapy (5 year overall survival rate of about 40%). Therefore, there is an unmet need to develop treatments targeting PTCL- specific changes.

One well-known phenomenon is that PTCL cells attract many normal immune cells to their surroundings and form an environment for the lymphoma cells to live and grow; this environment is called the tumor microenvironment. Thus, the tumor microenvironment in PTCLs presents an attractive potential avenue for developing new PTCL-specific treatments. However, how the tumor microenvironment supports PTCLs' growth is not well understood. Inflammation is a complex cascade of immune reactions involving a network of many cell types. By studying patient samples, we found evidence of increased inflammatory proteins and gene expression in the cancer cells and the tumor microenvironment. Furthermore, while elevated inflammation predicts poor outcomes in PTCL patients, dampening inflammation via a drug called TTI-101 showed an anti- cancer effect in mouse models of PTCL. Thus, the goal of our proposal is to find out if increased inflammation supports the growth of PTCLs and if reducing inflammation in cancer cells and the tumor microenvironment could be a new strategy to treat PTCL.

To test our ideas, we will use cutting-edge technologies, including single-cell and spatial transcriptomics, molecular profiling methods that allow scientists to measure all the gene activity in a tissue sample and map where the activity is occurring to understand the composition of cells and inflammation in the tumor microenvironment. We will study tumor samples from patients with PTCL and two mouse models of PTCLs generated in our lab. These mouse models are great tools for our studies, because they carry genetic mutations identical to some PTCL patients and develop tumors mimicking human disease. Moreover, we will study precisely how TTI-101 works to shrink PTCL and determine whether blocking two key inflammatory proteins, IL-6 and TNF-alpha, can reduce PTCL. TT1-101 is a drug now being tested in clinical trials for patients with advanced solid tumors, and there are many available drugs targeting IL-6 and TNF-alpha for the treatment of rheumatic diseases. If these drugs are proven to have a robust anti-cancer effect in our preclinical PTCL mouse models, the findings will be instrumental in developing these drugs into novel treatments for PTCL patients.

This work is important and impactful because of the urgent need to improve our knowledge of PTCL and to identify novel targeted therapies for this aggressive disease lacking effective treatment options. Furthermore, our study could improve military readiness by findings new therapeutic agents, which may reduce time in the hospital and minimize PTCL relapse for Service Members, their families, and Veterans.

The Principal Investigator of this proposal, Dr. Wen-Hsuan Wendy Lin is an MD-Ph.D. physician-scientists and board-certified hematopathologist at Columbia University Medical Center (CUMC). She has extensive research experience in T-cell lymphoma, immunology, vaccine development, and immunity to viral diseases. Her interdisciplinary background makes her an ideal researcher to lead this project, which requires expertise

in the malignant T-cells and immune system that may help to control PTCL. Dr. Lin will work with her career guide, Teresa Palomero Ph.D., a Professor at CUMC and an expert in the mutations that make T-cells cancerous, who has characterized several PTCL mouse models that are valuable to study the disease and evaluate new treatments. Dr. Lin's long-term career goal is to lead an independent research group in translational lymphoma research, focusing on the disease mechanisms and finding cures for peripheral T cell lymphomas. Receiving the DOD Career Development Award would allow her to gain additional skills and expertise in the mouse models of PTCL, high throughput sequencing experiments, and computational analysis and achieve her goal of becoming a pillar in the lymphoma research community.

Proposal Title:	CAR T Cell Strategies to Counter Solid Tumor Antigen Immune Escape
Log Number:	CA220705
Current PI Name:	Leonid Cherkassky
Award Number:	HT9425-23-1-0439
Current Contracting Organization:	Health Research Inc., Roswell Park Division
Current Performing Organization:	Health Research Inc., Roswell Park Division
Web Approval Date:	07-11-2023

Scientific Objective/Rationale: Colorectal cancer often spreads to other organs (metastasize). Even though we are able to help extend survival by combining chemotherapies, targeted therapies, and surgically removing colon cancer that has spread to the liver, recurrence rates (chances the cancer comes back) are 50%. As a surgical oncologist who removes these tumors, I also see patients who have more advanced unresectable disease who have often had their tumors grow despite being on therapy. These observations underscore the need for more effective therapies that treat the whole body (called “systemic therapy”). Immunotherapies are a powerful systemic therapy that harness a patient’s immune system to attack the cancer. The clinical success of immune checkpoint blockade therapies that “release the brakes” on tumor-targeted T cells demonstrates how powerful T cells can be as an anti-cancer agent, yet this therapy is not effective in most colorectal cancers. We instead use gene-engineered T cell therapies, a type of immunotherapy where we take T cells and “teach” them to recognize and attack cancer cells. This is done by putting genetic material into T cells (gene-engineer) so they can express a receptor that allows them to attack and kill cancer cells (we use a receptor called a chimeric antigen receptor or CAR). Since T cells can persist for a long time (form “memory”) and can circulate throughout the blood, they have particular promise for treating even metastatic tumors and achieving long-term cancer survivors. However, while CAR T cell therapy has success in treating liquid malignancies (such as leukemias), treating solid tumors has not been successful. A major obstacle leading to treatment failure in solid tumors is antigen immune escape (AIE) by cells negative for the antigen targeted by T cells. Most CAR T cell formulations target just a single cancer antigen, allowing antigen-negative tumors to “escape” from attack and cause cancer recurrence. We have developed strategies that increase the number of antigens that are targeted by T cells, which we hypothesize will prevent AIE. These two strategies are to: (1) use CAR T cells programmed to recruit and activate new T cell immune responses by non-CAR T cells that are specific for additional mutated or non-mutated cancer antigens or (2) use CAR T cells that are able to target two antigens simultaneously. We perform experiments in clinically relevant immune competent mouse models and in mouse models using patient-derived tissues.

Career Goals: As a surgeon, I have seen firsthand many patients whose colon cancers came back following surgical resection, highlighting the need for effective systemic treatments to increase the number of long-term survivors. The one goal that I am most committed to achieving as a clinician-scientist is to develop T cell therapies that can increase long-term survival in patients with metastatic colon cancer and other solid malignancies. The strategies we develop in this proposal can be applied to multiple other solid cancers/Topic Areas. Also, the cancer antigens we target with CAR T cells are shared by multiple other solid cancers. Receiving this award would provide me with the resources to publish high-impact manuscripts and generate preliminary data that can be used to obtain independent funding to support my laboratory. I am fortunate to have Dr. Renier Brentjens serve as career guide. He is a world leader in CAR T cell therapy who has led 12 CAR T cell clinical trials and has extensive experience mentoring junior faculty. He is an ideal mentor to guide me in establishing a nationally recognized and respected research program and become a leader in advancing immunotherapy to help patients.

Applicability of Research: Solid cancers cause a majority of cancer-related deaths in active Service Members, Veterans, their families, and in the general population. At the conclusion of this award, we will have developed a more effective CAR T cell therapy for metastatic colorectal cancers and other solid cancers that we will apply in clinical trials. After the conclusion of this award, we will have a multidisciplinary discussion to initiate a clinical trial at Roswell Park. We envision that the initial trial population will be unresectable metastatic colorectal cancer. We have a Food and Drug Administration (FDA)-approved system to collect, transduce, and expand patient T cells; personnel and hospital resources to execute a T cell therapy trial; and a quality control team to review data for new technology submissions to the FDA, resulting in an outstanding track record for bringing T cell therapies to the clinic. Roswell Park is therefore an ideal place to develop a CAR T cell therapy addressing a critical need in solid tumor treatment and to then translate this to patients.

Benefit to Active-Duty Service Members, Veterans, and Other Military Beneficiaries: As I have previously practiced at a Veteran Affairs Medical Center and as a surgical oncologist who routinely cares for Veterans, I have witnessed firsthand the impact of metastatic colorectal cancer on patients' lives and their caregivers. T cell therapies have the potential to transform outcomes for these patients, preventing the cancer from returning after surgical removal, and treating those patients who have advanced disease.

Proposal Title:	Validation of Novel Drivers of Juvenile Polyposis Syndrome
Log Number:	CA220720
Current PI Name:	Suzanne MacFarland
Award Number:	HT9425-23-1-0785
Current Contracting Organization:	Children's Hospital, Philadelphia
Current Performing Organization:	Children's Hospital, Philadelphia
Web Approval Date:	10-03-2023

This application will address the Peer Reviewed Cancer Research Program (PRCRP) Topic Areas: Colorectal Cancer and Pediatric/Adolescent/Young Adult Cancer. It will also address the PRCRP Military Health Focus Area: Gaps in Cancer Research That May Affect Mission Readiness.

Juvenile Polyposis Syndrome (JPS) is a cancer predisposition syndrome that is usually diagnosed in childhood, with polyps developing in the gastrointestinal tract (stomach and colon). These polyps are also associated with up to 50% risk of cancer lifelong. That cancer risk can be decreased by close surveillance with endoscopy and colonoscopy and removal of polyps.

In some patients with JPS, there is a genetic cause of the polyps that is passed down in families (this is called mutation-positive JPS). The two genes that are known to cause JPS are BMPR1A and SMAD4. In the majority of patients, the genetic cause is not known (mutation-negative JPS). Our group has shown that for patients with mutation-negative JPS, there is a clear difference in features of the disease (phenotype). This includes fewer polyps as patients get older, a younger age at diagnosis of JPS, and polyps mainly in the colon (not in the stomach). This tells us that there is a difference in these patients, which suggests that there is a different reason that they develop JPS. However, without a genetic cause, it is difficult to understand their cancer risk, and it requires surveillance of all of their Family Members, including parents, siblings, and children.

We have investigated the cause of polyps in these patients and have found that in a little under half (40%), there is a mutation that might cause their polyps; however, to prove that those mutations are related, we will need to conduct studies in colon organoids, which are three-dimensional cell structures that mimic the human colon. Dr. Lengner will develop these organoids in his lab and knock down (remove) or introduce specific mutations into the genes that we suspect cause JPS. We will then study the phenotype of gene-edited organoids relative to isogenic normal organoids and those carrying known JPS mutations. These studies will determine whether newly identified candidate genes contribute to polyp growth and may be causative of JPS.

At the same time, Dr. MacFarland will use her large bank of patient samples, that have been collected in conjunction with her polyposis clinic to understand what other genetic changes might lead to JPS. This includes changes in the genetics of cells that are just in the colon (called mosaicism), or changes in the factors that tell cells to express those genes (called epigenetic changes). This will involve specialized genetic studies on a large number of samples, including comparing samples from both the colon and the polyps for each individual.

Relevance to Intent: Through this work, we hope to improve our ability to treat patients with mutation-negative JPS - improving our ability to conduct cancer surveillance, and to know how to treat their Family Members, and which of their Family Members are at risk for cancer. Additionally, understanding the genetic cause of JPS will have implications for all patients with gastric and colorectal cancer, because the genetic changes in JPS are the first step in the development of cancer. Thus, this may suggest the cellular pathways that might be targeted in treatment of both polyposis and cancer.

This will address the fiscal year 2022 PRCRP Overarching Challenge in Diagnostics/Prognostics: distinguish unique features driving cancer occurrence across the spectrum of ages. For both pediatric and adult JPS patients, as well as their Family Members, a better understanding of JPS genetics will help us to understand their cancer risk and potentially suggest new strategies of cancer prevention and treatment. This will be relevant to active-duty Service Members and their Families who are affected by JPS, helping them to understand their cancer risk and the need for close follow up, which may affect their ability to serve in certain roles.

Proposal Title:	Validation of Novel Drivers of Juvenile Polyposis Syndrome
Log Number:	CA220720P1
Current PI Name:	Christopher Lengner
Award Number:	HT9425-23-1-0786
Current Contracting Organization:	Pennsylvania, University of
Current Performing Organization:	Pennsylvania, University of
Web Approval Date:	10-03-2023

This application will address the Peer Reviewed Cancer Research Program (PRCRP) Topic Areas: Colorectal Cancer and Pediatric/Adolescent/Young Adult Cancer. It will also address the PRCRP Military Health Focus Area: Gaps in Cancer Research That May Affect Mission Readiness.

Juvenile Polyposis Syndrome (JPS) is a cancer predisposition syndrome that is usually diagnosed in childhood, with polyps developing in the gastrointestinal tract (stomach and colon). These polyps are also associated with up to 50% risk of cancer lifelong. That cancer risk can be decreased by close surveillance with endoscopy and colonoscopy and removal of polyps.

In some patients with JPS, there is a genetic cause of the polyps that is passed down in families (this is called mutation-positive JPS). The two genes that are known to cause JPS are BMPR1A and SMAD4. In the majority of patients, the genetic cause is not known (mutation-negative JPS). Our group has shown that for patients with mutation-negative JPS, there is a clear difference in features of the disease (phenotype). This includes fewer polyps as patients get older, a younger age at diagnosis of JPS, and polyps mainly in the colon (not in the stomach). This tells us that there is a difference in these patients, which suggests that there is a different reason that they develop JPS. However, without a genetic cause, it is difficult to understand their cancer risk, and it requires surveillance of all of their Family Members, including parents, siblings, and children.

We have investigated the cause of polyps in these patients and have found that in a little under half (40%), there is a mutation that might cause their polyps; however, to prove that those mutations are related, we will need to conduct studies in colon organoids, which are three-dimensional cell structures that mimic the human colon. Dr. Lengner will develop these organoids in his lab and knock down (remove) or introduce specific mutations into the genes that we suspect cause JPS. We will then study the phenotype of gene-edited organoids relative to isogenic normal organoids and those carrying known JPS mutations. These studies will determine whether newly identified candidate genes contribute to polyp growth and may be causative of JPS.

At the same time, Dr. MacFarland will use her large bank of patient samples, that have been collected in conjunction with her polyposis clinic to understand what other genetic changes might lead to JPS. This includes changes in the genetics of cells that are just in the colon (called mosaicism), or changes in the factors that tell cells to express those genes (called epigenetic changes). This will involve specialized genetic studies on a large number of samples, including comparing samples from both the colon and the polyps for each individual.

Relevance to Intent: Through this work, we hope to improve our ability to treat patients with mutation-negative JPS - improving our ability to conduct cancer surveillance, and to know how to treat their Family Members, and which of their Family Members are at risk for cancer. Additionally, understanding the genetic cause of JPS will have implications for all patients with gastric and colorectal cancer, because the genetic changes in JPS are the first step in the development of cancer. Thus, this may suggest the cellular pathways that might be targeted in treatment of both polyposis and cancer.

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Proposal Title:	Dissecting the Role of DNA Damage Repair Deficiency in Ewing Sarcoma Pathogenesis Through Integrative Computational Biology
Log Number:	CA220721
Current PI Name:	Riaz Gillani
Award Number:	HT9425-23-1-0471
Current Contracting Organization:	Dana-Farber Cancer Institute
Current Performing Organization:	Dana-Farber Cancer Institute
Web Approval Date:	07-11-2023

Scientific Objective and Rationale: Ewing sarcoma is the second most common bone and soft tissue cancer impacting children and adolescents worldwide. It is an aggressive malignancy requiring multimodal treatment that confers significant morbidity, and cure rates for metastatic and relapsed disease remain poor. The inherited genetic events that contribute to the tumor features of Ewing sarcoma and how different patterns of tumor features can impact treatment approaches remain largely unknown. I led a study of genetic predisposition in a large cohort of pediatric sarcomas and found that inherited changes in DNA damage repair genes occur more than would be expected by chance in patients with Ewing sarcoma. Work is still needed to understand the full extent to which defects in DNA damage repair increase risk for Ewing sarcoma, as well as how these changes impact the features of Ewing sarcoma tumors. The primary objectives of this research proposal are to (1) identify the inherited genetic changes in DNA damage repair genes that increase risk for Ewing sarcoma to improve approaches to prevention and (2) analyze the tumor features related to DNA damage to improve risk stratification, predict treatment resistance, and lay the foundation for more informed treatment in Ewing sarcoma.

Career Goals: I am committed to a career as a pediatric oncology physician-scientist. In my scientific career, I will develop and apply novel computational approaches to strive toward a better biological and clinical understanding of pediatric and young adult cancers more broadly, and sarcomas specifically. The proposed research will move the field toward better patient care for two PRCRP Overarching Challenge Categories:

(1) Identify and elucidate the mechanisms behind cancer epigenetics/ genetics and cancer development to improve prevention methods

(2) Identify strategies to predict treatment resistance, recurrence, and the development of advanced disease

This research program will provide me with the skills I need to develop more fully as a translational researcher who can carry out computational work with clinical relevance and to establish myself as an expert in the germline (normal tissue) and tumor genomics of pediatric sarcomas. Following the completion of this research program, I will have the experience to apply for advanced funding to screen for and validate DNA damage biomarkers among patients with Ewing sarcoma and further study the impact of germline variants on tumor features through embedded biology aims in future Ewing sarcoma clinical trials.

Clinical Applicability: Our proposal bridges the genetic and mechanistic underpinnings of Ewing sarcoma to clinically relevant biomarkers with prognostic and potential therapeutic implications in the treatment of this aggressive pediatric cancer. Through a better understanding of the underlying biology contributing to Ewing sarcoma, the pediatric oncology community will be able to develop more informed and less toxic treatment regimens, as well as better screen children at risk for disease, opening the door to opportunities for earlier detection and even prevention. In the short-term, we anticipate that this research will lead to better genetic screening and risk stratification for Ewing sarcoma. In the long-term, we anticipate that the insights into DNA damage repair mechanisms will lead to more tailored treatment approaches for subsets of patients with

Ewing sarcoma. Our work will inform clinical trial priorities for patients with Ewing sarcoma, and spur innovation in research and clinical care extending to other pediatric and young adult cancers.

Proposed Impact on Military Health: The proposed research to dissect the role of DNA damage repair deficiency in Ewing sarcoma pathogenesis through integrative computational biology is of high relevance to the health of military Service Members and their families. In a study conducted over 2 years in the early 2000s, over 500 cases of bone and soft tissue sarcomas were treated within the Military Health System.

Moreover, Ewing sarcoma is often seen in child and adolescent populations, contributing significantly to the overall burden of childhood cancer, which remains the number one cause of death by disease among children and indiscriminately impacts families including those of military Service Members. A better understanding of the causes of Ewing sarcoma and biomarkers associated with treatment resistance and response would have direct applications and extensions to the management of other sarcomas and childhood cancer. Thus, the proposed research on Ewing sarcoma would significantly impact the well-being of military Service Members and their families through alleviating the burden of cancer.

Proposal Title:	Deciphering the Mechanisms Underlying Dietary Protection from Colorectal Cancer
Log Number:	CA220764
Current PI Name:	Maayan Levy
Award Number:	HT9425-23-1-0151
Current Contracting Organization:	Pennsylvania, University of
Current Performing Organization:	Pennsylvania, University of
Web Approval Date:	07-10-2023

I am an early-career scientist in cancer research. As an Assistant Professor at the University of Pennsylvania' s Perelman School of Medicine, I specialize in investigating colorectal cancer, one of the most common forms of cancer and one of the most frequent causes of cancer-associated mortality in the United States. My team has recently made a breakthrough discovery in the field of colorectal cancer. This finding described a new molecular pathway by which diets inhibit tumor growth in the gut. Specifically, my research group found that ketogenic diets, which are diets largely devoid of carbohydrates and rich in dietary fats, prevent the development of colorectal cancer in animal models and in human cancer cells.

We now aim at understanding the mechanisms underlying this protective effect, in order to bring this discovery closer to application in patients. To this end, I have two major goals: (1) understanding the role of gut bacteria and immune cells in the tumors that mediate the impact of diet on intestinal tumor growth, and (2) build a network of collaborators, mentors, and peers who can support the next phase of research in this exciting field. Our goal is to involve patient communities in order to initiate an active exchange about the possibilities of using ketogenic diets and its metabolites in the prevention and treatment of colorectal cancer.

I hope to become part of PRCRP's Virtual Cancer Center in order to make actionable progress toward both goals.

This proposal specifically addresses the following topic areas and challenges:

FY22 PRCRP Topic Area: Colorectal Cancer

FY22 PRCRP Military Health Focus Area: Environmental Exposure Risk Factors Associated with Cancer

FY22 PRCRP Overarching Challenges: Prevention, Therapeutics

Up to 2 million individuals are diagnosed with colorectal cancer each year. Screening for colorectal cancer remains the most important and cost-effective strategy in reducing the incidence and mortality of this disease, as treatment typically includes local excision by surgery, removal of nearby lymph nodes, chemotherapy, and radiation therapy – all of which have significant side effects. A new strategy that aides in the prevention and treatment of colorectal cancer development is thus urgently needed.

We believe that diet is a powerful yet underutilized lifestyle element that can potentially support currently existing tools for screening and therapy. The subject of this research proposal is thus a central question in the prevention and treatment of cancer: What should we eat in order to minimize our risk of developing tumors?

Our research will aim at identifying dietary patterns, metabolite supplementations, and the resultant signaling pathways in the body that have tumor-inhibitory effects in the gastrointestinal tract. This research needs to be carried out in a step-wise manner: (1) Identification of dietary patterns that are associated with

inhibition of colorectal cancer in animal models and human cells. This part of the research program has been completed and recently published. (2) Deciphering of the mechanisms by which specific diets and diet-derived molecules prevent cancer. This research is subject of the current proposal. (3) Initiation of clinical trials to determine the safety and efficacy of dietary interventions or metabolite supplementation in at-risk populations. This step will depend on the results of the proposed project.

This research will benefit everybody at risk of developing colorectal cancer. This encompasses essentially the entire population above the age of 50, since the risk for developing colorectal tumors increases with advanced age. There are also specific patient populations with an elevated risk for developing colorectal cancer. Among these populations are patients with Lynch Syndrome, an inherited disorder characterized by mutations in DNA repair genes. Patients with Lynch syndrome are predisposed to the development of cancer, including an increased risk for colorectal cancer. Active-duty Service Members and Veterans will likewise benefit from research aimed at identifying the optimal dietary composition that prevents cancer development.

In summary, this research will benefit the entire U.S. population above 50, including Veterans, and will be particularly valuable for at-risk populations, such as individuals with Lynch Syndrome.

Proposal Title:	Defining the Genetic Determinants of Head and Neck Cancer Progression and Immune Evasion Through Computational and Functional Investigation
Log Number:	CA220766
Current PI Name:	Luc Morris
Award Number:	HT9425-23-1-0556
Current Contracting Organization:	Sloan Kettering Institute for Cancer Research
Current Performing Organization:	Sloan Kettering Institute for Cancer Research
Web Approval Date:	10-03-2023

Our research proposal, “Defining the Genetic Determinants of Head and Neck Cancer Progression and Immune Evasion Through Computational and Functional Investigation,” seeks to improve our understanding and treatment of head and neck squamous cell carcinoma (HNSCC). Head and neck cancer is one of the fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) Topic Areas, and has particular impact on U.S. military Service Members and Veterans, due to its markedly higher incidence in these communities, and because its high symptom burden has led to elevated risks of suicidal self-directed violence in Veterans diagnosed with HNSCC.

HNSCC arises from the lining of the mouth and throat. It is the seventh leading cause of cancer death around the world. The most aggressive types of HNSCCs are non-virus associated tumors, for which tobacco and alcohol exposure are major risk factors. These tumors, and the intense surgical, radiation, and chemotherapy treatment that we use to treat them, profoundly debilitate our patients' speech, swallowing, breathing, and physical appearance. Unfortunately, there have been few new therapies for HNSCC over the past 3 decades; as a result, rates of recurrence have remained high and survival rates have not improved during this time. Because of the disproportionate impact of HNSCC on Service Members and Veterans, improvements in how we treat this cancer, and reduce risk of relapse, will have substantial impact on these patients and overall mission readiness - a PRCRP Military Health Focus Area.

The major unmet clinical need we face in the field of head and neck oncology is that we have identified many genetic alterations in these tumors, but we do not yet have the necessary biological knowledge to understand what many of these genetic alterations mean for tumor behavior or how to target them with effective therapies. There is currently a daunting list of potential new targets and new drugs, and we need better data to prioritize the most promising clinical trials for this cancer type.

To tackle this significant challenge, we have formed a collaborative multi-institutional team. Our team brings together scientific experts in HNSCC tumor genetics and animal modeling as well as immunology and immunotherapy, developers of new techniques to study the functions of genes in cancer models at scale, developers of new artificial intelligence-based analyses of large cancer genetics datasets, and clinical oncology experts in HNSCC in special populations such as Veterans.

In Aim 1, we will use a novel artificial intelligence-based learning approach to glean new insights from a large set of clinical and tumor genomic profiling data for over 2,200 patients with head and neck cancer. This analysis will allow us to find new genetic alterations that predict aggressive tumors and poor survival in HNSCC patients, and to provide clinicians with an accurate prediction tool to help guide treatment decisions.

In Aim 2, we will study the functions of these genes, and a set of genes suggested by prior research, by modeling what happens when they are altered in models of HNSCC that we have developed. These new models represent the human disease better than many existing models. We will determine how these genes affect tumor growth and metastasis, and whether these alterations we see in tumors confer resistance to chemotherapy or radiation therapy. Such treatment resistance poses major limitations in our current ability to treat patients.

In Aim 3, we will examine a new dataset of over 300 HNSCC patients treated with immunotherapy, to identify genetic alterations that allow tumors to be resistant to these treatments. We will then perform experiments in mouse models of cancer to examine how these genes, and a list of genes we have selected from prior research studies, affect the ability of tumors to evade the immune system or to develop resistance to immunotherapies. Because most HNSCC tumors are resistant to current immunotherapy drugs, this knowledge will be important to improving how we treat our patients.

With these three aims, we will learn important information about what genetic alterations in HNSCC mean for tumors and patients. Our patients need new therapies, but the current number of possible treatments being investigated in clinical trials is daunting. This study's analyses and experiments will allow us to find new genetic markers of tumor behavior - to help clinicians choose better therapies - and to find the most appropriate tumors and patients in which to investigate new therapies. Ultimately, this improved knowledge of HNSCC will help us to target our field's collective effort on the most promising new treatment strategies, to improve outcomes and reduce toxicity for patients with this cancer.

Proposal Title:	Defining the Genetic Determinants of Head and Neck Cancer Progression and Immune Evasion Through Computational and Functional Investigation
Log Number:	CA220766P1
Current PI Name:	Robert Samstein
Award Number:	HT9425-23-1-0557
Current Contracting Organization:	Icahn School of Medicine at Mount Sinai
Current Performing Organization:	Icahn School of Medicine at Mount Sinai
Web Approval Date:	10-03-2023

Our research proposal, “Defining the Genetic Determinants of Head and Neck Cancer Progression and Immune Evasion Through Computational and Functional Investigation,” seeks to improve our understanding and treatment of head and neck squamous cell carcinoma (HNSCC). Head and neck cancer is one of the fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) Topic Areas, and has particular impact on U.S. military Service Members and Veterans, due to its markedly higher incidence in these communities, and because its high symptom burden has led to elevated risks of suicidal self-directed violence in Veterans diagnosed with HNSCC.

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To tackle this significant challenge, we have formed a collaborative multi-institutional team. Our team brings together scientific experts in HNSCC tumor genetics and animal modeling as well as immunology and immunotherapy, developers of new techniques to study the functions of genes in cancer models at scale, developers of new artificial intelligence-based analyses of large cancer genetics datasets, and clinical oncology experts in HNSCC in special populations such as Veterans.

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In Aim 2, we will study the functions of these genes, and a set of genes suggested by prior research, by modeling what happens when they are altered in models of HNSCC that we have developed. These new models represent the human disease better than many existing models. We will determine how these genes affect tumor growth and metastasis, and whether these alterations we see in tumors confer resistance to chemotherapy or radiation therapy. Such treatment resistance poses major limitations in our current ability to treat patients.

In Aim 3, we will examine a new dataset of over 300 HNSCC patients treated with immunotherapy, to identify genetic alterations that allow tumors to be resistant to these treatments. We will then perform experiments in mouse models of cancer to examine how these genes, and a list of genes we have selected from prior research studies, affect the ability of tumors to evade the immune system or to develop resistance to immunotherapies. Because most HNSCC tumors are resistant to current immunotherapy drugs, this knowledge will be important to improving how we treat our patients.

With these three aims, we will learn important information about what genetic alterations in HNSCC mean for tumors and patients. Our patients need new therapies, but the current number of possible treatments being investigated in clinical trials is daunting. This study's analyses and experiments will allow us to find new genetic markers of tumor behavior - to help clinicians choose better therapies - and to find the most appropriate tumors and patients in which to investigate new therapies. Ultimately, this improved knowledge of HNSCC will help us to target our field's collective effort on the most promising new treatment strategies, to improve outcomes and reduce toxicity for patients with this cancer.

Proposal Title:	Targeting Regulatory B Cells to Augment Anti-Bladder Cancer Immunity
Log Number:	CA220769
Current PI Name:	Burles Johnson
Award Number:	HT9425-23-1-0317
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	06-09-2023

Objective and Rationale: My overall objective for the proposed research is to identify novel methods to target a bladder cancer (BC) patient's immune system in order to improve survival in BC patients. Despite recent advances in treatment for patients with metastatic BC, only a subset of patients experience significant tumor shrinkage with these therapies. Thus, most BC tumors develop resistance to approved therapies, and overall survival in patients who have had metastatic BC for 5 years is ~6%. Bladder cancer tumors hijack the immune system to promote tumor growth. Thus, there is an urgent need to better understand how this happens in order to develop therapies that can reprogram the immune system to promote antitumor immune attack. My ultimate goal is to develop novel immune-based therapies in order to improve outcomes for patients with advanced BC.

My preliminary data suggest that an immune cell called a B cell facilitates tumor growth in a clinically relevant mouse model of BC. I hypothesize this is because a subset of B cells inhibits the ability of other immune cells to kill tumors. B cells are a heterogeneous group of immune cells that can stimulate the immune system to promote tumor clearance, or suppress immunity to promote tumor growth. We have found that in patients with advanced BC who subsequently received approved immunotherapy, a subset of patients with increased tumor expression of a B cell gene signature (suggesting more B cells are present) have worse response to immunotherapy. This implicates a subset of B cells drive resistance to immunotherapy. The rationale for these experiments is that defining how immune suppressive B cells block antitumor immune activation is likely to reveal targets for therapeutic intervention in these patients. After identifying the proteins expressed by immune suppressive B cells in patients with advanced BC, I plan to target these proteins in clinical trials, which may improve survival in these patients.

Career Goals: My long-term career goal is to be an internationally recognized laboratory based BC physician-scientist whose work transforms survival for BC patients by determining how B cells block immune mediated BC eradication. By completing the proposed experiments, I expect to identify novel B cell based mechanisms that facilitate BC growth, and can be targeted in patients with advanced BC to improve their survival (PRCRP Overarching Challenge: Transform cancer treatment through the identification of new targets). My career development plan utilizes a multidisciplinary research committee committed to aggressive mentoring in basic BC biology, bioinformatics, statistics, the analysis of B cells in humans and mice, and the development of an independent research program. I will benefit from access to unique BC samples and numerous core facilities, a rich collaborative environment of clinicians and scientists, and educational seminars and coursework. By completing the proposed experiments and career development activities, I expect to identify B cell targets to transition to BC clinical trials.

Applicability and Timeline: I expect this research to improve survival of patients with BC by developing therapies to target immune-based mechanisms (discovered as a part of the proposed research) that facilitate BC growth. To the best of my knowledge, this will be the first comprehensive examination of the B cell landscape in patients with BC. As little research has been performed to define the role of B cells in BC, I estimate that it will take 2-3 years to identify the most promising B cell specific targets to pursue in clinical

trials. This will be followed by early clinical studies, which typically take 2-4 years to demonstrate whether the tested treatment results in significant improvement in clinical outcome.

Impact of Research on the Military Population: The proposed experiments will improve outcomes for current and retired military personnel and their families who have BC, by identifying B cell targets for therapeutic intervention in patients with this disease. Agent Orange is a known environmental exposure associated with military service, smoking prevalence is enriched in active and retired military members when compared with non-military, and both are known significant risk factors for development of BC. Thus, identifying novel treatments that improve outcomes in patients with BC will enrich the lives of current and retired military, along with their families.

Proposal Title:	Vulnerabilities in the Transcriptional Program of Fusion-Positive Rhabdomyosarcoma
Log Number:	CA220774
Current PI Name:	Michael Deel
Award Number:	HT9425-23-1-0228
Current Contracting Organization:	Duke University
Current Performing Organization:	Duke University
Web Approval Date:	05-04-2023

Project Overview: Many sarcomas are driven by chromosomal translocations, where a segment from one chromosome is swapped with a segment of a different chromosome, resulting in new genetic code called a 'fusion oncogene'. Some of the fusion oncogenes encode transcription factors, which are especially challenging to target as they are intrinsically disordered and lack drug-binding sites. These difficulties are exemplified by fusion- positive rhabdomyosarcoma, which is driven by the fusion oncogene PAX3-FOXO1 and remains among the most difficult to treat cancers in children and young adults. PAX3-FOXO1 disrupts normal DNA organization and regulation and also amplifies the transcription of pro-tumorigenic genes. In addition, PAX3-FOXO1 may contribute to therapy resistance by dysregulating normal cell cycle checkpoints. Radiation and cytotoxic chemotherapy causes DNA damage. Ordinarily, DNA-damaged cells would not be permitted to continue to transit the cell cycle. However, gene dysregulation caused by PAX3-FOXO1 allows the cell to progress through these checkpoints despite DNA damage. Unfortunately, attempts to directly target PAX3-FOXO1 have proven futile as it lacks good drug-binding pockets.

Although PAX3-FOXO1 is currently unable to be targeted directly, our work seeks to identify proteins that collaborate with PAX3-FOXO1 – "co-activators" – that could be exploited for therapy. We discovered co-activators that are able to regulate the activity of PAX3-FOXO1 and also are key regulators of the cell cycle in fusion-positive rhabdomyosarcoma. This project investigates how these co-activators, which can be targeted therapeutically, may be tractable vulnerabilities to PAX3-FOXO1 transcriptional programs. The goals of this proposal are to investigate mechanisms by which PAX3-FOXO1 activity is dependent on these co-activators and to use a small molecule inhibitor to exploit this dependency in our preclinical models. Using a functional genomics screen, we will also discover and investigate new druggable co-activators that are dependencies for the expression and activity of PAX3-FOXO1. Ultimately, we hope our work will inform the development of new therapeutic approaches for treating patients with fusion-positive rhabdomyosarcoma.

PI Career Goals: My career goal is to become an independent scientist leading a laboratory focused on identifying new and better ways to target the transcriptional programs orchestrated by fusion oncogenes responsible for many sarcomas. Despite numerous advances in the treatment of pediatric and adult malignancies, oncoprotein-driven sarcomas remain among the most difficult to treat tumors. These challenges are exemplified in fusion-positive rhabdomyosarcoma, which is the most common soft tissue sarcoma in adolescents and young adults. In fact, the first-line chemotherapy regimen for these tumors has not changed over the past 4 decades. Inspired by many adolescent patients who bravely battled metastatic sarcomas, I have committed my career to investigating new treatment strategies.

This research addresses two of the Fiscal Year 2022 (FY22) European Paediatric Soft Tissue Sarcoma Study Group (PRCRP) Topic Areas: (1) sarcoma and (2) pediatric, adolescent, and young adult cancers; and it is focused on advancing two of the Therapeutics-related FY22 PRCRP overarching challenges: (1) identifying new targets for a cancer frequently associated with advanced/metastatic disease, and (2) identifying and elucidating mechanisms behind cancer epigenetics to improve treatment methods. Although PAX3-FOXO1

has been unable to be targeted directly, my proposal seeks to identify and exploit its reliance on co-activators and for its transcriptional programming. Executing the Aims of this proposal and my career development plan will provide me with deep expertise in molecular oncology, chromatin biology, and sarcoma preclinical modeling. The data generated will also provide a foundation for numerous future directions in studies of rhabdomyosarcoma as well as other fusion oncogene driven sarcomas.

Research Applicability: New therapies targeting transcriptional programs of fusion oncogene-driven sarcomas are needed. To address this problem in fusion-positive rhabdomyosarcoma, I am seeking to identify tractable vulnerabilities within PAX3-FOXO1's transcriptional program. My preliminary studies suggest two therapeutically targetable co-activators cooperate with PAX3-FOXO1 and are required for its transcriptional function. I am thus seeking to exploit this reliance on co-activators as a novel therapeutic approach. This proposal includes important mechanistic studies and also tests a new class of small molecule inhibitors that interfere with the interaction between the co-activators and PAX3-FOXO1 to abrogate its transcriptional program. We propose to test this small molecule in our preclinical mouse models, which is necessary step before clinical trials in patients. If these studies confirm our promising preliminary studies, we expect this compound could be tried in clinical trials within 3-4 years.

Military Relevance: Fusion-positive rhabdomyosarcoma predominately occurs in adolescents and young adults. Because fusion-positive rhabdomyosarcoma is frequently metastatic and/or recurrent, it is associated with a dismal survival (5-yr overall survival

Proposal Title:	Understanding and Overcoming Treatment Resistance in MDM2-Amplified Esophageal Cancers
Log Number:	CA220781
Current PI Name:	Smita Sihag
Award Number:	HT9425-23-1-0250
Current Contracting Organization:	Sloan Kettering Institute for Cancer Research
Current Performing Organization:	Sloan Kettering Institute for Cancer Research
Web Approval Date:	06-02-2023

Background on Esophageal Adenocarcinoma

Esophageal adenocarcinoma (EAC) is an aggressive cancer – fewer than 20% of patients are still alive 5 years after their diagnosis. Moreover, the number of people diagnosed with the disease is rapidly rising in the United States. Most patients are diagnosed when the cancer has already spread beyond the esophagus. For these patients, the standard treatment is chemotherapy combined with radiation therapy (termed chemoradiotherapy), follow by surgery if feasible. However, around 70% of patients have little response to this chemoradiotherapy, and their subsequent risk of death is markedly higher than that of patients who have robust responses.

Therefore, we believe the greatest opportunity to improve outcomes for patients with EAC lies in predicting which patients will respond poorly to therapy, understanding the reasons for the treatment resistance, and ultimately converting such patients to responders.

Objectives and Rationale

We have discovered several genes that, when mutated or otherwise altered in an EAC, predict that the cancer is likely to be resistant to chemoradiotherapy. Two of these genes, MDM2 and TP53, stood out because they are key players in a biological process, termed the p53 pathway, which helps maintain the integrity of DNA and chromosomes. Thus, the alterations in MDM2 or TP53 in EAC could lead to chromosome damage, which in turn could account for the poor responses to chemoradiotherapy.

Our objectives are (1) to understand how MDM2 gene alterations in EAC contribute to treatment resistance and (2) to identify new approaches to therapy for these patients, for whom no effective options currently exist. For this second objective, we will assess an inhibitor of MDM2, testing its effect on models of EAC when administered alone, combined with conventional chemotherapy, and combined with immunotherapy. This work will lay the foundation for clinical trials for MDM2 inhibitors in advanced EAC.

How the Research Will Help Patients

We expect the proposed research to lead to new precision medicine strategies to greatly enhance patients' chances of surgical cure and long-term survival. This will benefit patients with EAC with MDM2 alterations (approximately 10% of patients). Because MDM2 and TP53 alterations affect the same biological pathway, we expect that our research could also benefit many of the patients with TP53-mutant EAC (approximately 80% of patients).

In addition, these findings may have relevance to other cancers that also have alterations in MDM2 or TP53, which include lung cancers and soft tissue sarcomas. In EAC, we have found that altered MDM2 and TP53 promote treatment resistance to a wide range of chemotherapies. Therefore, the knowledge we gain is likely

to be broadly applicable to other cancers with MDM2 or TP53 alterations, even if the specific treatments differ from those used for EAC.

Principal Investigator's Career Goals

Dr. Sihag is a surgeon specializing in esophageal and other thoracic cancers. Her time is evenly divided between clinical care of patients and translational research. Her central career goal is to build a productive research program in investigating the biology of esophageal cancers and devising new treatment strategies, with the ultimate goal of improving survival and quality of life for individuals affected by these diseases. She already has research expertise in two areas relevant to the proposed research: genomics and computational biology. The Career Development Award will provide training opportunities for her to acquire the additional skills and experience needed to fulfill her research goals. The award will also provide the time and resources that will enable her to launch her own independent research program.

Relevance to Overarching Challenges and Military Health

This proposal addresses two overarching challenges: "transform cancer treatment through the identification of new targets" and "advance immunotherapy across the different PRCRP Topic Areas." In 2022, esophageal cancer will affect nearly 21,000 Americans, including active-duty service members, military families, and, in particular, Veterans. Because the research is likely to lead to new advances in the treatment of EAC, we believe it will significantly improve the lives and longevity of patients affected by esophageal cancer.

Proposal Title:	Mapping Elements of DNA Architecture Responsible for Malignant Transformation in Craniospinal Sarcoma
Log Number:	CA220809
Current PI Name:	Andrew Venteicher
Award Number:	HT9425-23-1-0432
Current Contracting Organization:	Minnesota, University of, Twin Cities
Current Performing Organization:	Minnesota, University of, Twin Cities
Web Approval Date:	06-09-2023

Objective and Rationale: Chordomas are a type of sarcoma that arise from the head and spine. Because chordomas grow into the brain and/or around the spinal nerves, chordomas threaten neurologic function in patients. We currently do not have a chemotherapy that can effectively treat chordoma, so patients are treated with aggressive surgery, and if surgery is deemed too high risk, radiation. Most patients with chordomas will progress or develop metastases, and chordoma patients have an average survival of 5-10 years. This has created an urgency to develop new understanding and better options to treat patients with chordoma.

Great effort has been placed to define markers that can be used to predict patient outcome, which will help identify which patients may benefit from adjuvant therapy and to identify new therapeutic targets in subsets of chordoma patients. A great barrier to identifying such markers has been the fact that chordoma is not caused by gene mutation, the most common cause of cancer. Our lab focuses on developing new ways of studying how DNA organization contributes to sarcoma formation using patient samples collected at surgery. Our objective is to understand how DNA organization contributes to chordoma formation and how we can take advantage of this to create new tests to predict tumor behavior and to develop new therapies.

Career Goals of Principal Investigator: Dr. Andrew Venteicher is a physician-scientist at the University of Minnesota in the Department of Neurosurgery. He specializes in research and surgical treatment of patients with sarcomas of the head, neck, and spine. His career goals are to (1) use his unique position as a surgeon-scientist to advance the standard of care for patients with sarcoma, (2) develop a rigorous laboratory environment dedicated toward understanding non-traditional mechanisms of cancer formation to prevent and better treat sarcoma, (3) translate research findings into the clinic for patient care, and (4) help train the next generation of cancer researchers. In the fiscal year 2022 Peer Reviewed Cancer Research Program (PRCRP) Topic Area of Sarcoma, the FY22 PRCRP Overarching Challenge areas focused on in this proposal are to (1) elucidate the mechanisms behind cancer epigenetics/genetics and cancer development to improve prevention methods, and (2) evaluation from longitudinal collection of deep multidimensional characterization of clinically annotated research biospecimens during disease progression and treatment.

This Career Development Award creates a rich environment to interact and learn from peers who are at the start of their careers in cancer research, the ability to network with senior members of the cancer research community, and provide for research time protected from clinical responsibilities. Interacting in this environment and formal mentorship by the Career Guide, Dr. Largaespada, an international leader in the study of molecular alterations in sarcomas and brain tumors, will accelerate the ability to transition to independence and become a future leader in sarcoma research. Specifically, this award will allow Dr. Venteicher to establish experimental and computational pipelines necessary to study epigenetic and nontraditional genetic mechanisms of sarcoma formation for future studies.

Applicability of Research: Chordomas affect patients of all ages, with a propensity for aggressive chordomas to affect pediatric and young adult patients. Chordomas affect patients in all gender, racial, and ethnic

groups. The proposed project will have direct benefit to patients with chordoma by (1) finding prognostic markers that can predict which patients may benefit from adjuvant treatments and (2) identify new vulnerabilities in chordoma based on studying DNA organization. In addition, this proposal is designed so that lessons learned in chordoma will teach us more broadly about mechanisms of tumor formation applicable to other sarcomas/cancers that similarly lack a causal gene mutation. These aims will provide a crucial framework toward a more complete understanding of how cancers can form in the absence of recurrent gene mutation.

Benefit to Military Personnel: Direct and indirect benefits to active-duty military personnel and Veterans will be gained through this proposal. Direct benefit will be gained through (1) the development of new markers of more aggressive tumor behavior, (2) identifying which patients may benefit from more aggressive surgery and adjuvant therapy, and (3) preserving patient function because chordomas, by virtue of their location along the head and spine, threaten neurologic dysfunction and death. Therefore, this proposal will enhance combat readiness and reduce costs to the Military Health System. Indirectly, additional benefit will accrue through (1) identifying new vulnerabilities that may serve as therapeutic targets and (2) by creating a framework toward a deeper understanding of new ways in which cancers form in the absence of traditional gene mutation.

Proposal Title:	Optimizing Individualized Colorectal Cancer Treatment and Prognostic Prediction via Causal Machine Learning
Log Number:	CA220816
Current PI Name:	Kun-Hsing Yu
Award Number:	HT9425-23-1-0523
Current Contracting Organization:	Harvard University, Boston
Current Performing Organization:	Harvard University, Boston
Web Approval Date:	07-11-2023

Colorectal cancer affects 5.25 million people worldwide. Pathology evaluation (i.e., visual assessment of the cancer samples under the microscope) is the key to diagnosing this deadly disease. However, this standard microscopic evaluation cannot predict which patients will respond to treatments (such as immunotherapy, a type of treatment that reactivates the immune system against cancer cells) or develop undesirable side effects. The scientific objective of this proposal is to develop new artificial intelligence (AI) methods to analyze the microscopic images of colorectal cancer samples and predict treatment responses and side effects. The rationale is that AI methods can automatically identify hidden signals from a large amount of microscopic imaging data and discover previously unknown relationships from the data. We will combine AI methods with causal modeling approaches to enable reliable clinical prediction and optimize treatments for each patient.

Dr. Yu is the Principal Investigator (PI) of this proposal. His career goal is to develop reliable AI methods for analyzing high-resolution microscopic images of colorectal cancer samples. This award will significantly advance Dr. Yu's career in colorectal cancer research (a fiscal year 2022 [FY22] Peer Reviewed Cancer Research Program [PRCRP] Topic Area) by providing him with the time and resources to execute the proposed research project. The proposed study will enable accurate treatment response prediction and facilitate treatment optimization for colorectal cancer patients, which will address two of the FY22 PRCRP Overarching Challenges ("Diagnostics/Prognostics: Identify strategies to predict treatment resistance, recurrence, and the development of advanced disease" and "Therapeutics: Evaluation from longitudinal collection of deep multidimensional characterization of clinically annotated research biospecimens during disease progression and/or treatment"). The proposed research and career development plan (joint lab meetings with well-established colorectal cancer researchers, networking with leaders of the Virtual Cancer Center, and additional training in grant writing) will provide Dr. Yu with additional exposure to cutting-edge research ideas and support the PI in becoming a leader in colorectal cancer pathology research.

The ultimate applicability of the research is to establish reliable methods for predicting the treatment response and side effects of immunotherapy among colorectal cancer patients.

Results from this study will assist colorectal cancer patients in choosing the right treatments according to their individual differences. Potential clinical applications include informing each patient and their clinicians about the most likely treatment responses and side effects before initiating any treatments, which will allow patients and clinicians to make an informed joint decision on the treatment strategies. These results will benefit patients by selecting the optimal treatments and avoiding ineffective medications for their cancers. The risk associated with uncertainty in the prediction will be mitigated through informed consent and educating clinicians and patients regarding the inherent uncertainty in medical practice. We expect to complete the development and evaluation of our prediction models in 4 years using three large patient populations. Our study will advance the field of cancer pathology research by establishing reliable methods for analyzing high-resolution digital pathology images, and it will advance patient care by providing colorectal cancer patients with accurate clinical predictions.

The proposed research will benefit active-duty Service Members, Veterans, and other military beneficiaries by enabling fast and accurate clinical prediction using pathology samples. Because the patient population of colorectal cancer is getting younger in recent decades (12.1% of colorectal cancer patients in the U.S. are under 50 years old in 2020), our research has increasing relevance to Service Members and their family members.

Proposal Title:	Promoting Self-Management in Head and Neck Cancer Survivors with Lymphedema and Fibrosis (PROMISE Trial)
Log Number:	CA220818
Current PI Name:	JIE DENG
Award Number:	HT9425-23-1-0705
Current Contracting Organization:	Pennsylvania, University of
Current Performing Organization:	Pennsylvania, University of
Web Approval Date:	10-03-2023

The proposed study directly addresses the fiscal year 2022 Peer Reviewed Cancer Research Program Topic Area Head and Neck Cancer. More than half a million head and neck cancer survivors are alive in the United States. Three-quarters of them are expected to experience lymphedema (chronic swelling) and fibrosis (fibrotic tissues) in the head and neck region after their cancer therapy. Once head and neck cancer survivors develop lymphedema and fibrosis, they need to conduct life-long self management of these chronic conditions to slow their progression and reduce associated symptom burden (e.g., neck tightness) and functional issues (e.g., difficulty swallowing and speaking).

Despite the importance of self management, we identified a number of barriers which hinder head and neck cancer survivors' efforts to conduct adequate self management for lymphedema and fibrosis. To address the barriers and improve head and neck cancer survivors' quality of life, we developed an in-person self-management program. Our program centers on increasing awareness of the importance of lymphedema and fibrosis self management and provides instructions for self-management skills through guided practice. It also includes motivational assessment and strategic planning. Subsequently, we tested this program in a pilot study. The results demonstrated that the program was both feasible to implement and acceptable to participants. More importantly, survivors noted a reduction in the severity of lymphedema and fibrosis with associated decreased symptom burden. During the COVID-19 pandemic, we delivered the program via telehealth. The results showed that survivors who participated in the telehealth program had comparable outcomes to those participated in the in-person program.

Given the promising findings from our pilot study, we plan to conduct a multi-center study to further test the self-management program. This study, if successful, will have a major impact on both clinical practice and patient outcomes, specifically: (1) It addresses a significant gap regarding the lack of evidence-based interventions available to assist and direct clinicians and head and neck cancer survivors with self management for their chronic lymphedema and fibrosis. (2) It is the first Lymphedema and Fibrosis Self-Management Program developed and pilot tested to address the unique needs of the head and neck cancer population. (3) The program embraced principles of the Information-Motivation-Behavior Skills Model of health behavioral change with specific attention to critical barriers (e.g., limited lymphedema knowledge and skills, lack of motivation). (4) The program is easily followed with understandable, step-by-step instructions to ensure survivors can replicate techniques in their home. (5) The program can be delivered in person or via telehealth. We believe that telehealth is a promising approach for delivering self-management strategies for head and neck cancer survivors, which will allow improved outcomes in populations where access to in-person self-care programs (e.g., living in rural areas) is a major issue.

If successful, the program will shift supportive care programming from health care facilities to the home environment, empowering patients to engage in needed life-long self-care which is crucial to minimize long-term symptom burden and function loss and to maintain quality of life. In an era calling for self-management for chronic health conditions (e.g., cancer survivorship), our proposed intervention merits investigation, as it may serve as a model for other musculoskeletal impairments resulting from accident injury or trauma.

Proposal Title:	Transforming Radiation Therapy to Preserve Neurocognition in Children with Brain Tumors
Log Number:	CA220820
Current PI Name:	Sahaja Acharya
Award Number:	HT9425-23-1-0577
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	08-17-2023

Fiscal Year 2022 (FY22) Peer Reviewed Cancer Research Program (PRCRP) Topic Areas: “Pediatric brain tumors” and “Pediatric, adolescent, and young adult cancers”

Objective: Although advances in diagnosis and treatment have dramatically increased the survival rates for children and adolescents with brain tumors, side effects from treatment remain a major problem. The ability to remember new information (memory) and complete mental tasks in a timely fashion (processing speed) are particularly affected in children and adolescents who are treated with radiation therapy (RT). We will directly address this important problem by: (1) investigating new ways to selectively reduce RT dose to parts of the brain (i.e., substructures) that are important for memory and processing speed while treating the tumor, and (2) studying the timeline of injury to white matter pathways in the brain that we think are particularly vulnerable to cancer treatment using diffusion-weighted magnetic resonance imaging.

Rationale: We have previously shown that reducing RT dose to parts of the brain responsible for memory and processing speed is associated with improved performance on tests of memory and processing speed in children. We and others have also shown that there is white matter injury prior to RT in children who have undergone surgery, highlighting the importance of studying white matter pathways that connect the area of injury to the area of tumor.

Relevance to Behavioral Health Science Award: The intent of the Behavioral Health Science Award is to “advance behavioral health cancer science and fill gaps in the understanding of survivorship.” A key gap in knowledge for childhood survivors of brain tumors is how to reduce neurocognitive deficits in memory and processing speed, which have a significant impact on social-emotional well-being, academic performance, and the ability to live independently as an adult. Our proposal directly addresses this gap by testing novel RT strategies to reduce these deficits and by investigating the relationships between early and late white matter injury, RT dose, and neurocognitive outcomes. Furthermore, our innovative and high-reward study will shift the paradigm of RT planning from one that is substructure naïve to one that is substructure informed. Historically, the brain has been treated as a single homogeneous unit in RT planning, and substructures have not been used to optimize plans. Substructure-informed RT planning will limit RT injury to substructures that are highly susceptible to RT damage and critical for neurocognitive function, potentially reducing both the acute and long-term neurocognitive deficits.

FY22 PPRC Overarching Challenge and How it Will Make an Impact: This proposal addresses the following FY22 PRCRP Overarching Challenge: “Develop strategies to reduce short- and long-term treatment effects, including neurocognitive deficits.” Our first objective described above will reduce short-term neurocognitive deficits in memory and processing speed. Our second objective will investigate the relationship between early /late white matter injury and acute/long-term neurocognitive deficits. By deepening our understanding of white matter injury and selectively limiting dose to parts of the brain that are important for memory and processing new information, we believe we can preserve the cognitive ability of children undergoing RT therapy, which in turn will lead to better performance in school, better relationships with Family and friends,

and an overall improved quality of life. This will have an impact on a large proportion of children with cancer, because brain tumors represent the most common solid malignancy in children, and RT therapy is often required for cure.

Proposal Title:	Metastatic Progression in Medullary Thyroid Cancer
Log Number:	CA220838
Current PI Name:	Priya Dedhia
Award Number:	HT9425-23-1-0503
Current Contracting Organization:	Ohio State University, The
Current Performing Organization:	Ohio State University, The
Web Approval Date:	09-15-2023

Medullary thyroid cancer (MTC) is an aggressive thyroid cancer (a Peer Reviewed Cancer Research Program [PRCRP] Topic Area) of thyroid neuroendocrine cells. Patients with advanced MTC have a poor 10-year survival rate of 21%. Thyroid nodules are associated with increased risk for MTC. Thus, military Service Members exposed to ionizing radiation from nuclear accidents or military test sites or other toxic agents, which have been shown to cause thyroid nodules, are at increased risk for MTC. Because of the disproportionate thyroid cancer-related mortality caused by MTC and the unique exposures for military personnel, improving MTC-related outcomes for Soldiers, Veterans, and their Family Members is the focus of this proposal. Effective treatment for this appalling cancer does not exist because of an extremely limited number of research models, which do not accurately represent the complexity of the original tumor. Cell lines made from patient-derived MTC tissue only have one type of cancer cell, while the original cancer has many types of cancer cells. Furthermore, although mouse models have many types of cancer cells, the mouse cancer is too different from the human version. As a result, treatments developed using existing research models do not improve survival.

Organoids or "mini-organs," are 3-dimensional research models that incorporate features of the represented organ. For example, organoids derived from thyroid tissue will maintain features of the thyroid gland. Because organoids contain many cell types and can be made from human tissues, these models better represent the original human tissue compared to cell culture or mouse models. Organoids have been successfully used to study cancers that were previously difficult to study and treat, such as esophageal cancer, pancreatic cancer, and colon cancer. We developed the first MTC organoids, which proliferated and produced hormones similar to the original tumor.

The overall goal of this application is to support my training and development as a leader in thyroid cancer research. The career development plan includes structured mentorship and development of expertise in MTC biology, advanced stage cancer, and cancer genomics through completion of the aims. In this proposal, we use patient MTC tissues and our novel MTC organoids to understand the features of cells that lead to advanced disease. In our first aim, we implement new techniques to understand the genetics of cells that lead to advanced MTC. In our second aim, we employ a 3-dimensional organ-on-a-chip platform, which represents the human body, to study cells that escape from the MTC organoids and invade other normal tissue organoids. Using these strategies, we intend to identify the features of cells that cause advanced disease and ultimately reveal vulnerabilities that can be targeted clinically. We expect that our new techniques can be used by scientists studying other cancers, so that patients with other cancers can also benefit from this research.

As a result of this work and aligning with the PRCRP Overarching Challenges of Diagnostics/Prognostics and Therapeutics, we will be able to identify patients who are risk for advanced disease and target vulnerabilities in the most dangerous cancer cells, which cause advanced disease. After validating our findings, we are poised to develop a clinical trial to target these pathways in the next 10 years. Thus, this proposal has the potential not only to advance my scientific career, but also treatment of MTC and other cancers.

Proposal Title:	Targeting Unusual Nutrient Acquisition Routes of Nutrient-Deprived Cancers
Log Number:	CA220856
Current PI Name:	Kacper Rogala
Award Number:	HT9425-23-1-0723
Current Contracting Organization:	Leland Stanford Junior University, The
Current Performing Organization:	Stanford University
Web Approval Date:	10-03-2023

Nutrients - carbohydrates, proteins, and fats - are the currency of cellular growth. This is particularly true for cancer cells, which do what they can to acquire as much of these nutrients as possible to sustain their uncontrolled growth. The opposite of growth is tumor shrinkage and eventual death of cancer cells, which is precisely what chemotherapy and radiation treatments aim to achieve. Yet, somehow, there are cancer cells that survive these harsh conditions and eventually begin growing again once the treatments are discontinued. What is so special about these remaining cancer cells that allows them to survive malnutrition and a barrage of toxins?

It appears that many of these cancer cells have a mutation in the infamous gene called RAS, which rewires the way these cancers acquire nutrients. Trapped inside tumor cores or other malnutritioned tissues following chemotherapy, these cancer cells no longer wait for blood vessels to deliver nutrients. Instead, they start scavenging food from their surroundings, and they also cannibalize dead cells. Every cell needs to digest this scavenged food inside cellular stomachs called “lysosomes” before it can be recycled back to the cell. These recycled nutrients are then converted into energy, and used as building blocks for the growth of the cell.

To fight those cancers, many researchers are currently trying to find ways to stop cancer cells from scavenging food, by either blocking the scavenging directly or by attacking mutant RAS. However, what if, instead of preventing cancer cells from eating, we simply trapped all the food inside the cellular stomach?

Most recently, a number of molecular machines, called nutrient transporters, were found to act as gates for releasing digested nutrients from the cellular stomach, and our plan is to immobilize that gate with drugs. Imagine a situation where the cancer cell consumes all of the food that it can scavenge, but after digestion, the food becomes trapped inside of its stomach compartment. The release no longer works because of the drug that blocked the food gate. After having their main food supply cut off, cancer cells that are addicted to scavenging will stop growing. At the same time, normal cells would ultimately not care, because their food and energy does not come from scavenging.

This Career Development Award from the U.S. Department of Defense will allow Dr. Kacper Rogala to build an entire therapeutic program in his laboratory at Stanford University by focusing on drugging survivor cancer cells that scavenge food from their surroundings. Dr. Rogala wants to specifically focus this project on two fiscal year 2022 (FY22) Peer Reviewed Cancer Research Program Topic Areas: Bladder Cancer and Colorectal Cancer.

Approximately half of these cancers carry mutations in RAS and a few other genes that transform them into food scavengers. This project will develop new prototype anti-cancer therapeutics addressing one of the FY22 PRCRP Overarching Challenges: Therapeutics - Transform cancer treatment through the identification of new targets, especially for advanced disease and metastasis. Importantly, this Award will also allow Dr. Rogala to grow as a cancer researcher by learning new research skills and expertise. And most importantly, because Dr. Rogala was trained as a laboratory scientist, with limited experience working with oncologists

or patients, this proposal will fill that gap. Through participation in the Virtual Cancer Center program, and interactions with the medical staff and patients at the Stanford Cancer Institute, Dr. Rogala will learn about the most pressing therapeutic needs and how his laboratory discoveries can be translated to the clinic for the benefit of patients.

Military personnel are at an elevated risk of developing bladder and colorectal cancers, mainly due to repeated exposure to toxic substances in the field and in military bases. This project will specifically focus on researching how these cancers can be stopped from growing, even in situations where standard therapies are no longer effective. We will start the development of specific drugs that trap nutrients inside cellular stomachs, and we will use powerful electron microscopes to take pictures of the process of how the stomach food gate works. These detailed microscopic images will help us and other researchers in designing drugs that are more potent and more tolerable by the patient's body. This project will allow us to begin advancing the total arsenal of drugs that doctors have at their disposal to attack food-scavenging cancers. And importantly, because these drugs will function via a novel mechanism, they will be suitable for use in combination with RAS-targeting drugs, effectively minimizing the risk of developing drug resistance by patients.

Proposal Title:	Development of a Stable and Effective Off-the-Shelf Wound Care Treatment for Combat Burn Injuries
Log Number:	DM220030
Current PI Name:	Young Min Ju
Award Number:	HT9425-23-1-0823
Current Contracting Organization:	Wake Forest University Health Sciences
Current Performing Organization:	Wake Forest University Health Sciences
Web Approval Date:	09-26-2023

FY22 DMRDP BWMIR Focus Area: Understanding combat traumatic wound physiology and wound progression through preclinical and clinical studies to inform clinical practice guidelines and standard of care efficacy and gaps.

Extensive burns and full-thickness wounds present a major clinical challenge for patients, even after treatment. Despite technological advancement in treatments, healing rates remain below 50% success. Patients who suffer from either of these types of injuries benefit from rapid treatments that result in complete closure and protection of the wounds. In particular, burn patients who receive delayed treatments are often subject to extensive scarring that can result in negative long- term physiological effects.

Currently, the use of spilt-thickness autografts is currently the gold standard of treatment, but this option is often limited by the availability of a suitable area of healthy donor skin to harvest. Cellular and non-cellular biological skin-equivalents are commonly used as an alternative treatment option for these patients, however, these treatments usually involve multiple surgical procedures and associated high costs for production and repeated wound treatment.

To overcome these limitations, cellular therapy is a promising alternative to biological skin-equivalents because a successful cell-based technique could rapidly cover wounds and accelerate healing using living components. Among the available cell sources, human stem cells are an attractive cell source for applications in regenerative medicine because of their high proliferation capacity, multipotency, immunomodulatory activity, and lack of significant immunogenicity. Many studies have shown that stem cells promote healing in acute full-thickness wound care; regenerative effects are believed to be primarily due to the paracrine effects of the growth factors and cytokines released by the stem cells, defined as the secretome.

Recent research has shown that stem cell-derived secretomes promote angiogenesis and accelerate tissue regeneration. Secretomes have been used as a conditioned medium since high levels of various growth factors and tissue-repairing agents from therapeutic cells are released into the culture medium. However, the translation of conditioned media into a treatment for human applications is not possible due to numerous undefined factors in the conditioned medium, thus creating safety concerns from the regulatory perspective.

To overcome the regulatory challenges, our approach was to identify and select the stem cell- derived proteins involved in accelerating wound healing to develop an effective cell-free therapeutic platform consisting of highly potent recombinant proteins. In this proposed study, we will fabricate the alginate-gelatin foam with stem cell-derived recombinant proteins and evaluate the clinical feasibility for wound healing in a preclinical porcine full-thickness skin injury model.

We believe that our prototype product will an alternative option for the rapid recovery of normal skin tissue and function in military Service Members and civilians who suffered from a full-thickness deep skin wound.

Proposal Title:	Antimicrobial Shape Memory Polymer Foams for Rapid Hemorrhage Control and Infection Prevention in Traumatic Wounds
Log Number:	DM220031
Current PI Name:	Mary Beth Monroe
Award Number:	HT9425-23-1-0756
Current Contracting Organization:	Syracuse University
Current Performing Organization:	Syracuse University
Web Approval Date:	09-26-2023

Objective/Rationale: HemostatiX is the newest member of the family of shape memory polymer (SMP) foam-based medical devices for embolic applications. Shape Memory Medical, Inc., (SMM) has successfully translated TrelliX and IMPEDE foam devices for clinical use in aneurysm control and peripheral vascular occlusion based on their excellent biocompatibility and rapid blood clotting. In this proposal, a research team comprised of academic, clinical, and industrial experts from Syracuse University, SUNY Upstate Medical University, and SMM will characterize HemostatiX in animal models of noncompressible hemorrhage control and infected wounds. HemostatiX is a fully-synthetic material with natural, non-drug-based antimicrobial phenolic acids. In the long-term, HemostatiX could be stored in first-aid kits at home and on the battlefield in an easy-to-use applicator in their compressed state. They could be applied to deep, irregularly-shaped bleeds, upon which they safely expand to shape-fill the bleed site without inducing high, damaging pressures on surrounding tissues and organs. They can induce blood clotting to stabilize patients during transport to a fixed care facility. Their antimicrobial properties enable initial wound disinfection and long-term infection and biofilm prevention to improve healing outcomes. Here, we will characterize the safety and efficacy of these antimicrobial dressings in a noncompressible porcine liver injury in comparison to non-antimicrobial control SMP foams and a clinically- available hemostatic dressing. We will also evaluate infection control and would healing in a small animal model in comparison with clinical controls.

FY22 DMRDP BWMIR Focus Area: This research will optimize prolonged care management of penetrating torso injury by developing solutions for prevention/management of deep space infections (e.g., bacterial or fungal) and delays in care of penetrating abdominal injury.

Research and Clinical Applications, Benefits, and Risks: Uncontrolled hemorrhage is responsible for ~1.5 million deaths each year, 30-50% of which occur before getting the patient to a hospital. Current hemorrhage control strategies that employ pressure dressings and tourniquets are insufficient for noncompressible wounds (e.g., to the torso), which comprise up to 80% of bleeds. Of patients who survive the initial injury, almost half develop infections in their wounds in the first week after injury. Wound infection treatment is often complicated by biofilm formation and/or drug-resistant bacteria. An improved antimicrobial hemostatic dressing could stabilize patients en route to a fixed care facility while protecting wounds from bacteria without the need for drug- based antibiotics to which microorganisms readily develop resistance. Existing hemostatic dressings are limited by poor clotting, difficulty in application or removal, potential for internal damage from high pressures, and/or a lack of antimicrobial activity. A safe and effective antimicrobial hemostatic dressing that is easy-to-use could overcome these limitations to improve hemorrhage treatment.

Projected Timeline: In year 3 of this project, we will leverage data obtained in the porcine studies to submit the control SMP foam to the FDA, leveraging prior safety and efficacy data obtained by SMM. Within 1 year of the completion of this 3-year project and upon obtaining regulatory clearance for the control foam

formulation, the antimicrobial HemostatiX dressing will be submitted for regulatory clearance. We anticipate that both the control foam and the HenostatiX formulation can be made available on the market within 4 and 5 years of this proposal submission, respectively (by fall 2026 and 2027).

Military and Public Benefit: This hemostatic dressing could be employed with current tourniquets to more quickly stop bleeding in extremity wounds and/or in noncompressible bleeds where tourniquet use is not appropriate. There is a significant gap in current hemorrhage control for military and civilian trauma cases, particularly in noncompressible bleeds and after safe tourniquet use time during patient transport from remote locations. Current hemostatic dressing concerns including difficulty of use and/or ineffective clotting. HemostatiX foam expansion and rapid clotting properties directly address these issues. Furthermore, the current standard of care in trauma is to administer antibiotics, which contributes to the rise of drug-resistance and lacks efficacy at preventing polymicrobial infections that form in half of traumatic wounds. By providing a localized non-drug-based antimicrobial at the time of injury, HemostatiX could reduce long-term infection risks. HemostatiX could provide a safe, easy-to-use, and effective antimicrobial hemostatic dressing option to reduce potentially survivable military and civilian deaths from the huge number of uncontrolled hemorrhages that occur each year and improve healing outcomes by reducing infection.

Proposal Title:	A Novel Transdermal Delivery Technology to Expedite Wound Healing and Attenuate Carbapenem-Resistant Acinetobacter baumannii Bacterial Infections
Log Number:	DM220078
Current PI Name:	Hongmin Sun
Award Number:	HT9425-23-1-0841
Current Contracting Organization:	Missouri, University of, The Curators of the
Current Performing Organization:	Missouri, University of, The Curators of the
Web Approval Date:	09-26-2023

Objectives and Rationale: Wound infections are a common cause of morbidity and mortality in combat casualty care as well as civilian trauma. Traumatic injuries break the protective barrier of the skin allowing bacteria to initially colonize and ultimately infect wounds. Combat sustained wounds are particularly unique due to the destructive force applied during wounding and the dirty environment of battle. A complicating factor is that many of the existing antibiotics are no longer effective against bacteria due to drug resistance and the emergence of so called “superbugs.” This has created a growing public health crisis for the combat wounded, Veterans and civilians alike. For the military, the emergence of antimicrobial resistance among bacteria affecting combat-related injuries is a serious problem with major regional and global implications. The initial wound management and surgical care of combat-injured military personnel and the chronic wound care of Veterans would benefit from novel treatment paradigm to both help preventing and treating infections of wounds caused by antibiotic resistant bacteria.

Focus Area: Droplette micromist technology device (DMTD) is a small portable device developed and patented by Droplette, Inc., and provided to the CoI (Pulakat) via a Material Transfer Agreement between Tufts Medical Center and Droplette, Inc., (both institutions at Boston). Based on our published and preliminary data, we propose that we can deliver high molecular weight, broad spectrum antibiotics that are used to treat antibiotic resistant bacteria to treat infections by one of the most notorious biofilm forming multi-drug resistant pathogen, Acinetobacter baumannii. Thus our study will fit into the focus area: “Understanding appropriate wound prophylaxis/empiric treatment strategies throughout continuum of care, regardless of injury status, through preclinical and clinical studies to inform clinical practice guidelines for expanding the understanding of antibiotic use in tissue injury (e.g., systemic versus topical), especially in the context of hemorrhage/resuscitation, blast, and/or delayed evacuation times.”

Potential Research and Clinical Applications, Benefits, and Risks: The antibiotics used to treat “superbug” infections, though powerful against the drug-resistant bacteria, can cause organ toxicity when administrated intravenously. These drugs also have high molecular weight, thus are unable to penetrate skin to reach the bacteria causing the infections deep in the wounds. As a result, they can only be administrated intravenously. We are proposing to test localized transdermal delivery of high molecular weight, broad spectrum antibiotic to treat infections by multi-drug-resistant Acinetobacter baumannii, thus minimizing the toxicity to the patient.

Projected Timeline: We will optimize the dosing regimen of drug administration in the first year, then test the safety and efficacy of the treatment regimen in both a small animal (rat) and a large animal (pig) infection model in year 2 and 3 to validate the utility of this novel device in wound care. These animal studies are necessary steps before human clinical trials. We expect to demonstrate and validate the usefulness of DMTD in delivering these life-saving powerful drugs to fight antibiotic resistant pathogens and

reducing their toxicity at the same time before the end of the project, and prepare proposals to support future clinical studies to bring this treatment ready for military adoption. It is estimated that it will take about 2-3 years to finish the clinical trials and 18 months to get the FDA approval for this technology to be applied to patient care.

Benefit Service Members, and/or the American Public: This technology could reduce the morbidity and mortality not only of active-duty military suffering from combat associated wounds, but Veterans and the general public who may suffer from chronic wounds such as diabetic or pressure ulcers. The device is light and portable and can be utilized with limited training to deliver antibiotics deep into wounds and skin tissues with little side toxicity to the patient. As a result, the device can be used in the austere nature of the environment of battlefield hospitals and incorporated directly into the wound care at all levels of combat casualty care.

Proposal Title:	Achieving Homeostasis in Traumatic Injuries with a Nitric Oxide-Driven Antimicrobial Dressing Combined with a Rapid Blood Clotting Agent
Log Number:	DM220089
Current PI Name:	Ronald Shebuski
Award Number:	HT9425-23-1-0955
Current Contracting Organization:	Nytricx Inc.
Current Performing Organization:	Nytricx Inc.
Web Approval Date:	09-26-2023

FY22 DMRDP BWMIR Focus Area for Project: Nytricx, Inc., is responding to the FY22 DMRDP, Battlefield Wound Management and Infection Research (BWMIR), specifically to understanding appropriate wound prophylaxis/empiric treatment strategies throughout continuum of care, regardless of injury status, through preclinical studies to inform clinical practice guidelines. The technology described in this application is designed to manage hemorrhagic shock, super-massive transfusion, traumatic limb ischemia (secondary to vascular disruption or tourniquet use), complex soft tissue injury/blast injury, open fracture, and/or frost bite, including evaluation of antimicrobial dosing and tissue penetration studies. This project encompasses a new, innovative wound dressing that can be applied in the field in the “golden hour” following injury, and when conventional medical treatment may be delayed for days due to combat circumstances and logistics.

Problem: Most dressings developed to-date for treating acute wounds are used as a cover to possibly prevent on- set of infection, and act as a secondary dressing. Combat wounded often experience bleeding and potential exposure to austere environment making wounds vulnerable to infection. There remains an unmet need to provide a therapy that will both prevent infection and rapidly stop bleeding.

Solution: The proposed wound dressing combines tranexamic acid (TXA), Propolis (a natural product), and the nitric oxide (NO) donor, S-nitroso-N-acetylpenicillamine (SNAP) termed TPS. Propolis is an antibacterial resin that provides a sticky matrix that is embedded with the antifibrinolytic agent TXA and also acts as the boundary layer between SNAP and TXA. This boundary layer promotes SNAP’s antibacterial effect while limiting putative NO interference with clot formation. TXA is then able to reinforce and enhance blood clot stability.

Objective: Our overall goal is to develop a wound dressing with dual capacity of preventing infection and stop hemorrhage post injury by promoting formation of a rapid blood clot. We will achieve this by fabricating and optimizing the TPS wound dressing and evaluate in vivo using porcine wound healing models.

Applicability and Impact: Nytricx wound dressing design focuses on rapid application and ease of use in conflict environments, and delivers a “shelf-stable” product focused on rapid application and will be available in a variety of sizes (Including very large), which potentially can be applied even by untrained personnel. The technology will bring a life-saving field treatment modality to the point of injury by managing threats of bacterial infection and promoting healing for several days until acute medical care is available. These practical attributes, combined with the product’s wound healing abilities and infection control, will increase medical readiness.

Benefit to Service Members: Combat-injured Service Members often lack a wound dressing that has addressed both infection and hemorrhage problem in tandem. Lack of an effective intervention immediately

after an injury may delay timely treatments to address key issues like infection control, worsening of burn wound and consequently will impact outcomes. We envision deploying the TPS wound dressing as close to geographical location of Service Members and making it available for prolonged field application. In particular, the dressing will benefit Service Members; those who sustain manageable size combat wounds can get appropriate treatment close to area of operation, which ultimately helps to preserve combat power. In addition, the dressing can also be employed outside of war zones such as subterranean and dense urban environments (DUE) and in different chemical, biological, radiological and nuclear events (CBRN) scenarios.

Potential Clinical Applications, Benefits, and Risks: The wound dressing described will be appropriate for wounds other than burns and acute injuries, including surgical incisions, plastic surgery reconstructions and chronic wounds. The device will provide tremendous clinical benefits to civilian populations and also to military veterans, many of whom are older and have co-morbidities such as cardiovascular disease and diabetes, which can result in poorly healing wounds. A dressing that can remain anti-infective and stabilize wound bed for 7+ days will provide additional protection and healing to persons who cannot receive immediate medical treatment. The TPS wound dressing provides (a) an improved injury outcome, (b) less time for infection to develop, (c) better mobility, (d) faster recovery, (e) fewer medical treatments, (f) faster reentry to service or civilian life and (g) lower logistical and economic burden on the healthcare system, patients, families and caretakers during and following the treatment period.

Proposal Title:	Prevention of Post-Traumatic Epilepsy by Inhibiting the Initiation of Innate Immune Reaction
Log Number:	EP220010
Current PI Name:	Xiaoming Jin
Award Number:	HT9425-23-1-0213
Current Contracting Organization:	Indiana University
Current Performing Organization:	Indiana University
Web Approval Date:	03-23-2023

Posttraumatic epilepsy (PTE) is a disabling neurological disorder that develops in some patients after a traumatic brain injury (TBI). It is poorly controlled by the currently available antiepileptic drugs and constitutes one of the major conditions that compromise functional outcome and quality of life in TBI patients. Because the mechanism is not well understood, this disease is difficult to control and cannot be prevented at present. There is an urgent need to develop novel treatments for PTE prevention. Inflammation has been found to play an important role in epilepsy development and seizure induction, but our knowledge on this is limited.

Recently, we made a novel finding that a type of immune cell called natural killer T cells (NKT) may be critical for PTE development. These cells are important in inflammatory responses, but we found that these cells may play an important role in inducing PTE. For example, if we genetically delete these cells in mice, the development of PTE is also prevented. More importantly, our early study showed that statins, a type of widely used lipid lowering medicines, can block the signal that activates these NKT cells. Therefore, we will further study how these immune cells cause epilepsy development and how statins may prevent PTE. The goal of this project is to study where and how NKT cells cause PTE development and how to target these cells for PTE prevention. In our research team, Dr. Xiaoming Jin is an experienced neuroscientist with expertise in studying the mechanism and prevention of PTE; Dr. Randy Brutkiewicz is an immunologist with expertise in innate immune function, particularly the signaling and activation of NKT cells. This highly interdisciplinary collaboration brings innovation and supports the success of the project.

The current project will make several contributions to the PTE research field. It will reveal a new mechanism about whether and how NKT cells cause the development of PTE after a TBI, so that we can obtain new knowledge and develop new approaches to prevent or treat it. Because inflammation is such a universal response after brain injuries, our study will be generally applicable to epilepsy development after different types of TBI. The study on statins will not only discover how this drug may control inflammation, but also provide a new method using statins for the prevention of PTE. Particularly, because statins are a U.S. Food and Drug Administration (FDA)-approved medication and are quite tolerable with few adverse effects, our studies may be quickly translated into the clinic, so that Soldiers and patients with a TBI may use statins for preventing PTE. Therefore, this project will have a strong potential for translational applications to the military, Veteran, and civilian communities.

Proposal Title:	Mitochondrial Pathways in Epileptogenesis Following Traumatic Brain Injury
Log Number:	EP220018
Current PI Name:	Chad Frasier
Award Number:	HT9425-23-1-0334
Current Contracting Organization:	East Tennessee State University
Current Performing Organization:	East Tennessee State University
Web Approval Date:	04-04-2023

Annually approximately three million Americans experience traumatic brain injury (TBI), putting them at an increased risk of developing epilepsy. TBI represents a major cause of death and permanent disability in the population. In addition, patients with TBI are at an increased risk of developing post-traumatic epilepsy (PTE). It's been estimated that PTE cases account for approximately 20% of total epilepsy cases. Despite significant work to uncover mechanisms of that underly seizures in models of epilepsy, the use of these therapeutic avenues in treating epileptogenesis has been underwhelming. Unfortunately, treatments targeted at the processes to decrease the incidence of PTE have been largely unsuccessful. This proposal will investigate both regional and temporal differences in mitochondrial function that underly the progression of PTE following TBI.

The mitochondria lay at the intersection of several pathways in energy production, cellular excitability, cell signaling, and cell death. Recently, there has been an increase in the interest in the role of bioenergetics in epilepsy as well as other neuronal disorders. Mitochondria play a vital role in the overall function of individual cells and the brain as a system, and a deeper understanding of how mitochondria are at the intersection of epileptogenesis may be critical for advancing current therapies for treating epilepsy. Our central hypothesis is that altered mitochondrial energetics, redox balance, and cell death pathways are compromised during the epileptogenesis process leading to PTE.

This proposal uses novel approaches to investigate how key mitochondrial pathways are altered in an animal model of TBI. Our first aim will look at cellular respiration, reactive oxygen species, calcium flux, and mitochondrial membrane potential following TBI. Our second aim will investigate the role of the mitochondrial signaling and excitability in mice that develop PTE. Finally, we will investigate if the ketogenic diet represents a therapeutic target for preventing PTE. Our experiments are carefully designed to provide valuable insight into the regional and temporal changes that effect the brain following TBI. All three aims in this proposal have yet to be investigated in TBI, and our ability to provide both spatial and temporal resolution in these processes will likely lead to the discovery of potential therapeutic targets for further study. In addition, we have chosen dietary therapy as it has high potential to be useful in the field. The inclusion of the ketogenic diet as a potential therapeutic avenue in the third aim has the potential for quick translation should our results be positive. We've chosen to test different delivery timepoints to test effectiveness to further increase clinical translation. In addition to the ketogenic diet, our proposal has the potential to impact clinical outcomes, as novel therapeutic agents targeting the mitochondria are under active development and understanding the pathways involved in PTE can lead to a more targeted pharmacologic approach.

The Principal Investigator's participation in the Virtual P-TERC is also well suited for his career goals. Despite success in cardiovascular research, he has always maintained his interest in neuroscience and has a strong desire to pursue an active research career in PTE. His previous research experience in a lab with a

focus on epilepsy has given him a strong foundation to build upon, and the team assembled in place will ensure his success in this endeavor. His participation in the P-TERC can only serve to further enhance his career goals, training, and chances of sustaining a well-funded PTE research program.

Proposal Title:	Post-Traumatic Epileptogenesis: Role of Neocortical-Hippocampal Interactions
Log Number:	EP220027
Current PI Name:	Paul Koch
Award Number:	HT9425-23-1-0363
Current Contracting Organization:	Virginia Commonwealth University
Current Performing Organization:	Virginia Commonwealth University
Web Approval Date:	04-12-2023

After severe traumatic brain injury (TBI), up to 20%-50% of patients may develop delayed seizures, or post-traumatic epilepsy (PTE), several months to several years after injury in a process called epileptogenesis. We have no effective therapies to prevent it. There is evidence that injury to a part of the brain called the temporal lobe increases one's risk of PTE, yet most seizures that occur in PTE do not seem to originate from the temporal lobe, which is a common location for non-traumatic epileptic seizures to originate. Given the apparent discrepancy, this research proposal seeks to understand whether temporal lobe injury after TBI plays a role in the development of PTE even if few temporal lobe seizures result. The lateral fluid percussion injury (FPI) rat model of TBI parallels this seeming paradox in humans: FPI causes characteristic damage to a structure in the temporal lobe called the hippocampus, but leads to seizures coming predominantly from a different area of the brain, the overlying neocortex. Damage after FPI makes the hippocampus more excitable, exhibiting exaggerated responses to inputs, but whether this contributes to PTE is unknown. The hippocampus receives information from nearly all of the neocortex and is responsible for forming memories from that information. The hippocampus, in turn, communicates encoded memories back to the neocortex for long-term storage. The mechanisms by which this bidirectional communication occurs may be corrupted by the epileptogenic process. This study seeks to understand how the circuitry within the hippocampus may change over time after its initial injury following FPI and whether those changes can contribute to abnormal activity in the neocortex that ultimately leads to PTE. To do this, we will use the FPI rat model of TBI. In the first set of experiments, rats will undergo FPI and then will be implanted with electrodes in the hippocampus and the neocortex. We will monitor the rats for up to 24 weeks after FPI, looking for the evolution of abnormal signals recorded from the hippocampus and neocortex that suggest progressive hippocampal hyperexcitability and abnormal communication between these structures, as well as whether these abnormalities predict the development of PTE. In the second set of experiments, we will attempt to reverse the hyperexcitability of the hippocampus after injury by activating a particular set of cells called interneurons within the hippocampus (some of which are damaged after FPI) via delivery of light using a technique called optogenetics. We will test whether reducing the injury-induced hyperexcitability in the hippocampus can prevent, or attenuate, the development of PTE.

The military population bears a disproportionate burden of PTE, occurring in up to 53% of cases of severe TBI, compared to 10%-20% in civilians. Delayed onset of PTE and associated cognitive and memory impairment after TBI leads to increased risk of death as well as further loss of functional independence, reduced quality of life, and increased economic burden. Effective preventative therapies (of which there are currently none) are urgently needed to improve function and quality of life after TBI, particularly in the military population. The immediate goal of this proposed research is to identify key changes in the circuitry of the hippocampus that contribute to the development of PTE. As an intermediate step for subsequent preclinical studies, strategies for identifying and reversing these key changes would then be developed and tested for effectiveness in reducing PTE. The long-term goal is two-fold: to define key hippocampus circuitry changes that both (1) predict development of PTE and thus help define the population of TBI patients that should receive preventative therapy, and (2) can be intervened upon as an effective therapy to prevent, or attenuate, the development of PTE. Modifying the function of brain circuits to treat disease using

implantable stimulating electrodes has had great success in diseases such as Parkinson's disease and essential tremor. Thus, applying such existing technology to new brain targets makes the development of a novel "anti-PTE" therapy achievable within 10 years. Use of more experimental therapeutic technologies, such as the targeted delivery of gene therapy products to parts of the brain, would likely increase that time.

Achievement of this project's goals will directly enable the Principal Investigator's (PI's) career goal in PTE research, which is to understand how brain circuits become progressively dysfunctional after TBI leading to PTE, identifying novel circuit targets for intervention to reverse that dysfunction and prevent the development of PTE. The PI's participation in the Virtual P-TERC is an ideal environment to find PTE-specific mentorship that will complement his Virginia Commonwealth University (VCU) team of collaborators in neural data analysis and optogenetics, to engage national collaborators with complementary approaches to the same, or similar problems that strengthen the scientific foundation and therapeutic potential of his strategy, and gain feedback from the national PTE scientific community. Similarly, the PI's Career Development and Sustainment Plan includes specific research and writing skills to acquire, as well as milestones for the local, regional, and national dissemination of findings and establishment of collaborative projects, culminating in the application for independent funding and publication of novel findings, which will support the PI's career goal by giving him the skills to maintain a sustainable and relevant research program.

Proposal Title:	The Neurophysiology of Post-Traumatic Epilepsy: Unraveling Epileptogenesis Signals (NEPTUNE)
Log Number:	EP220036
Current PI Name:	Edilberto Amorim De Cerqueira Filho
Award Number:	HT9425-23-1-0242
Current Contracting Organization:	California, University of, San Francisco
Current Performing Organization:	California, University of, San Francisco
Web Approval Date:	03-29-2023

Focus Area:

(1) Markers and Mechanisms and (2) Innovative Research.

Objectives and Rationale:

Post-traumatic epilepsy (PTE) affects 20% of civilians and near half of military Service Members with severe traumatic brain injury (TBI). Seizures and other brain activity following acute injury are likely contributing to PTE in humans; however, the specific early changes in brain wave characteristics that emerge before epilepsy starts are unknown. In this Idea Development Award proposal, Dr. Amorim will test whether quantitative analysis of brain waves can help predict seizures within 7 days and PTE diagnosis 6 months after TBI.

The central hypothesis is that evolution in shapes and features of brain waveforms soon after TBI reflect the biology of epilepsy. Dr. Amorim already collected data from 85 patients with TBI who had electrodes placed on the brain's surface to record seizures during life-saving TBI surgery, and he plans to recruit 60 additional patients. Effects of anti-seizure medications (ASM) given in the hospital will be connected to the brain wave data to determine which ASM better prevent early seizures and their association with PTE risk. Dr. Geoffrey Manley, Professor of Neurosurgery at the University of California, San Francisco (UCSF), and Dr. Lowenstein, Professor of Neurology at UCSF, are world-renowned leaders in the field of TBI and epilepsy who are committed to Dr. Amorim's project and his career development. This project is expected to discover new knowledge about brain activity after TBI that may: (1) help future studies select patients most likely to benefit from interventions to prevent seizures and PTE and (2) help select the right medication for the right patient to prevent seizure recurrence and prevent side effects. This research opportunity will ideally position Dr. Amorim to lead studies to prevent PTE and advance treatments.

Applications and Benefits:

A successful project will expand our knowledge about PTE biology and guide the care of patients with TBI to (1) improve survival without disability from seizures or PTE and (2) improve selection and dosing of ASM.

- What populations will it help, and how will it help them?

This work will have immediate impact to patients and military Service Members with TBI who are at highest risk for PTE: patients with moderate to severe TBI or penetrating injuries. The overarching goal is to create patient-specific treatment strategies to avoid PTE from developing early on after TBI.

- What are the potential applications, benefits, and risks?

This study will support point-of-care applications for acute seizure diagnosis after TBI, early predictors of PTE, and individualized ASM use. Importantly, this information will support future studies testing interventions to prevent epilepsy in patients at highest risk. Participants in this study may benefit from epilepsy evaluation with experts in PTE. This study has limited risks as there are no interventions; however, it may involve a small risk of loss of privacy as patient information will be collected.

- What are the likely contributions of the proposed research project to advancing PTE research, patient care, and/or quality of life?

Understanding the specific biological changes in brain activity that predict epilepsy after TBI will help clinicians educate patients and families about epilepsy risk and select interventions for studies to stop acute seizures and PTE. Side effects from ASM are common in PTE; therefore, ASM selection to maximize effectiveness and prevent side effects could have a big impact in a patient's quality of life.

Timeline:

We plan to complete this project in 24 months. We will enroll patients and pursue brain wave analysis for 18 months and complete the needed PTE follow-up by 24 months. In the last 6 months of the project, we plan to submit a proposal for a study in PTE prevention that uses brain wave responses to ASM within days from TBI to guide medication selection. If this new proposal is awarded, patients would be invited to participate in the study within 6-12 months after the completion of the Idea Development Award.

Proposal Title:	Spreading Depolarizations as a Predictive Biomarker of Post-Traumatic Epilepsy
Log Number:	EP220039
Current PI Name:	Laura Ngwenya
Award Number:	HT9425-23-1-0483
Current Contracting Organization:	University of Cincinnati
Current Performing Organization:	University of Cincinnati
Web Approval Date:	05-18-2023

Traumatic brain injury (TBI) results from mechanical forces, direct cranial impact or pressure wave, causing injury to the brain. TBI happens to tens of thousands of military personnel and hundreds of thousands of civilians every year and is a significant public health concern. The effects of TBI can be life changing and permanent. One of the common consequences of TBI is the development of new-onset seizures, termed post-traumatic epilepsy (PTE). PTE develops weeks, months, or years after TBI and can have significant negative impact on quality of life. We currently have a poor understanding of the processes underlying the development of PTE and very little ability to predict who may be at risk. After TBI, a large number of secondary cellular and molecular processes expand the injury. One of the more dramatic processes occurring after TBI is cortical spreading depolarizations (CSDs), also known as “brain tsunamis.” CSDs are periods of complete silencing of electrical activity that move in a slow wave across the brain surface. We have shown in our clinical studies, that when CSDs occur after TBI, they can be associated with seizures within the first week of hospitalization.

However, it is not known whether CSDs contribute to the development of seizures in weeks to months after TBI, and whether CSDs cause pathology that predisposes one to PTE. One of the consequences of CSDs is increased neurogenesis, the generation of adult-born neurons in a specialized area of the brain. Improper regulation of neurogenesis, a process that includes proliferation, maturation, and integration of new neurons into neural circuits, is associated with development of epilepsy. We also know that neuronal cell death in this region of the brain can be associated with seizures. We hypothesize that CSDs serve as a “second hit” after injury, causing excess neuronal cell death and accumulation of abnormal adult-born neurons, thereby facilitating later development of PTE. We will test whether the combination of TBI+CSDs increases the incidence of seizures 3-6 months after injury. We will evaluate for excess neuronal cell death as a consequence of CSDs in injured brain. Lastly, we will determine if abnormal adult-born neurons contribute to the development of seizures in TBI+CSDs by blocking creation of new neurons for 2 weeks after injury and observing for differences in seizure frequency at 3-6 months. Our team has expertise in both basic science and clinical management of TBI, epilepsy, and CSDs. We have recently demonstrated that in TBI patients we can detect and treat CSDs at bedside. Therefore, success of this project will define CSDs as a pathology that predicts later development of seizures, and our team is poised to translate our findings to the bedside to help prevent development of PTE.

Proposal Title:	Temporal Dynamics of Astrocytic Activation and Function in Post-Traumatic Epilepsy (PTE) Genesis and Progression
Log Number:	EP220047
Current PI Name:	Lauren Harte-Hargrove
Award Number:	HT9425-23-1-0184
Current Contracting Organization:	CURE (Citizens United for Research in Epilepsy)
Current Performing Organization:	CURE (Citizens United for Research in Epilepsy)
Web Approval Date:	04-26-2023

Objectives and Rationale

Post-traumatic epilepsy (PTE) is when someone experiences recurring and unprovoked seizures after sustaining a traumatic brain injury (TBI). PTE is a common and serious complication of TBI. There are no U. S. Food and Drug Administration (FDA)-approved medicines for prevention or treatment of PTE beyond current antiepileptic drugs, and there are no blood tests based on biomarkers for PTE. The disease course from TBI to PTE is not well understood, which further hampers the development of novel biomarkers and therapeutic targets. This project aims to identify specific changes in astrocytes, which are special star-shaped cells in the central nervous system, and how they contribute to the transition from TBI to PTE. Based on evidence we gathered in our previously funded Department of Defense grant, which revealed molecules associated with astrocytes are activated in mice with PTE, compared to mice without PTE, we believe that astrocyte signatures have a key role in the progression from TBI to PTE. We think that by clarifying how these signatures impact TBI/PTE, we can identify novel PTE-associated molecules for future blood tests and therapeutics for PTE. We will do this by integrating various neuroimaging and large-scale molecular analyses to characterize the temporal dynamics of astrocytic signatures both before and after PTE starts, in a mouse model and in patient samples.

Applicability and Impact

This project will ultimately lead to the identification of TBI patients who are at risk of developing PTE. It will provide markers for early detection and monitoring of PTE, and therapeutic targets to help prevent the progression or improve symptoms once they develop. The target population includes combat Soldiers and Veterans who may experience TBI. The outcomes of this project could lead to the prevention or improvement of the disease course from TBI to PTE, and therefore could help military and civilian victims of TBI/PTE alike. Identifying combat casualties with novel blood tests for PTE-associated biomarkers, followed by treatment with novel therapeutics for PTE, will improve care, management, and disposition for PTE, including return-to-duty decisions.

To progress to clinical research, the following outcomes will be achieved in this project: (1) various neuroimaging and large-scale molecular measures that underly astrocytic signatures associated with the presence or absence of PTE in different brain regions and (2) a defined time course for astrocytic signatures that are predictive of PTE in blood samples from TBI patients.

This project will lead to a greater understanding of the key astrocytic signatures underlying TBI progression towards PTE, which will advance PTE research, as currently little is known of the mechanisms and markers of TBI-induced PTE. The biomarkers and therapeutic targets identified in this project can be used to improve patient care after someone has experienced TBI by identifying their risk of developing PTE, monitoring their

progression from TBI to PTE, and treating them before they develop PTE, as well as treating them to after they develop PTE to reduce the severity of PTE. In conclusion, this project will help to reduce the burden of PTE on military TBI casualties – improving the quality of life for PTE patients and their families.

Proposal Title:	Individualized Prediction of Post-Traumatic Epilepsy Risk and Associated Cognitive Deficits Using Connectome Analysis and Machine Learning
Log Number:	EP220064
Current PI Name:	Anand Joshi
Award Number:	HT9425-23-1-0149
Current Contracting Organization:	University of Southern California
Current Performing Organization:	University of Southern California
Web Approval Date:	02-22-2023

Traumatic brain injury (TBI) is a significant health hazard for Service Members and Veterans during times of both peace and war. TBI is one of the major causes of epilepsy, yet the link between TBI and post-traumatic epilepsy (PTE) is not well understood. Prediction and, if possible, prevention of the development of PTE is a major unmet challenge. The goal of the proposed project is to use magnetic resonance imaging (MRI) data collected immediately after the injury to predict the onset of PTE on an individual basis. This critical insight will allow early intervention and targeted therapies for individuals identified as being at high risk of developing PTE.

This 3-year project on individualized prediction of PTE leverages and extends the team's previous work, funded by the 2018-2022 Epilepsy Research Program (ERP) award on finding imaging-based biomarkers for post-traumatic epilepsy using MRI data. The project also directly addresses one of the ERP focus areas: "Markers and Mechanisms," and subarea "Predictive biomarkers of epileptogenesis (acute and chronic)."

A large pre-collected longitudinal dataset of TBI patients available in the FITBIR database, with a subpopulation of PTE, offers a unique opportunity for identifying brain regions and networks involved in epileptogenesis as well as for designing and training algorithms to predict PTE. The overall aim and focus of this project are to identify imaging and connectome features that are associated with PTE, and then to use these features to predict PTE onset and cognitive deficits over time, on an individual subject basis. The preliminary results obtained by our team on the MagNeTS dataset, presented in the proposal and our publications in this area, suggest that high predictivity is achievable for PTE onset and cognitive trajectory.

To achieve our goals, we will analyze data from three large studies involving military as well as non-military personnel with TBI. The TBI data on military personnel is collected at the National Intrepid Center of Excellence at the Walter Reed National Military Medical Center. The non-military personnel data will be from the TRACK-TBI consortium and Maryland MagNeTS study. All the data is available to us from the FITBIR database. The use of multiple datasets will ensure that the results of the study are not dataset specific.

We will apply novel methods for lesion mapping, anomaly detection, and mapping brain connectivity changes for quantifying local structural and network changes in the brain based on MRI data. Through classical statistical analysis, we will then identify the subset of these imaging markers, and their location in the brain, that are most strongly correlated with the onset of PTE. We will also use the markers as the basis for developing and training a deep neural-net for individualized prediction of PTE. Additionally, we will establish bounds on the uncertainty in our predictions.

The connectome analysis conducted as part of this work may help in identifying the future origins of epileptic seizures in the brain, and potentially even the severity/recurrence rate for seizures as they develop.

This information in turn may help to select appropriate therapies to reduce the effects of disrupted brain networks. Leveraging the team's expertise in machine learning and connectomics, the project is aimed at modeling the complex interactions and relationships between risk factors, as reflected in imaging data collected shortly after injury, and epilepsy. The multivariate statistical analysis we will perform to determine these relationships may also provide insights into the pathogenesis of epilepsy. In summary, this project, if successful, would allow improved targeting of preventive care for TBI-affected Service Members and Veterans.

Proposal Title:	Calcium Channel Modulation to Prevent Post-Traumatic Epilepsy
Log Number:	EP220067
Current PI Name:	Terence O'Brien
Award Number:	HT9425-23-1-0354
Current Contracting Organization:	Monash University
Current Performing Organization:	Monash University
Web Approval Date:	04-30-2023

Epilepsy, with uncontrolled, unprovoked, and recurrent seizures, is a common, disabling, and all too often fatal, long-term consequence of a traumatic brain injury (TBI), in both the general population as well as in military personnel and Veterans. For those that develop epilepsy, this condition adds a considerable additional disability burden to their lives and risk of mortality. In addition to the seizures, people with epilepsy after TBI also commonly develop significant psychiatric (anxiety, depression) and memory problems that compound the disability burden on their life. To control the seizures, patients often are required to take more than one drug. The cumulative effect of taking multiple anti-seizure medications, in addition to the epilepsy and psychiatric comorbidities, create a massive toll on the quality of life of people with epilepsy. To date, no intervention can prevent the development of epilepsy and its associated comorbidities (psychiatric and memory problems). The available treatments cannot control the seizures in more than 30% of the people suffering from post-traumatic epilepsy. In addition, the current available therapies do not improve the anxiety, depression, mobility and memory problems that haunt patients with epilepsy and TBI. Therefore, there is a huge unmet need to prevent the development of epilepsy after TBI. In epilepsy, the current medicines or surgical interventions focus on treating the illness or injury rather than keeping it from happening. However, the ideal in medicine is to prevent a person from developing epilepsy.

In this proposal, we hypothesize that a novel treatment could help prevent the development of the symptoms of epilepsy, which means less seizures, and no psychiatric, memory or mobility problems, which will result in a significant improvement in the quality of life of people that suffer epilepsy after TBI. We have preliminary data that shows encouraging evidence that our intervention would be the “holy grail” for patients that suffer from TBI. The results of this study will prove if this novel approach can become a clinical intervention that can prevent the development of epilepsy after TBI. Similarly, our novel treatment could be the first-ever described to prevent or reverse the psychiatric, memory or mobility problems in epilepsy after TBI, which until now were thought to be irreversible.

Aim 1 of this proposal will test if our novel treatment is able to prevent epilepsy after TBI using a well-established rat model of the disease. Aim 2 using the same model, we will evaluate if our novel treatment is able to reverse the psychiatric (anxiety), memory and mobility problems associated with epilepsy after TBI. Unlike ever before, we will investigate the severity of psychiatric, cognitive, and neurobehavioral comorbidities in males and females and collect information whether the response to the treatment will show sex dependence.

This proposal is particularly unique and innovative as it offers a new approach to help people that suffer this devastating disorder, addressing aspects of the PTE beyond just that of seizures, including cognitive and mental health aspects of the condition. We integrate experts across different disciplines: neuroscience, neuropharmacology, clinical neurology, neuroimaging, and molecular biology. Together, these studies will represent a transformational advancement in understanding the mechanisms that underlie the development of epilepsy after TBI. Findings are anticipated to validate our novel treatment as a therapy to prevent epilepsy and its associated mental health disorders, with critical implications to improve the quality of life of military, Veterans, and civilian populations.

Proposal Title:	Phenotypes of Epilepsy Etiology and Drug Resistance (PEER)
Log Number:	EP220089
Current PI Name:	Eamonn Kennedy
Award Number:	HT9425-23-1-0221
Current Contracting Organization:	Utah, University of
Current Performing Organization:	Utah, University of
Web Approval Date:	04-02-2023

Post-traumatic epilepsy (PTE) refers to epilepsy that emerges and persists for more than 7 days after a traumatic brain injury (TBI). The likelihood of PTE depends on the severity of the brain injury, and there is normally a significant duration of time, often years, between a brain injury and the onset of recurring seizures. The delay between injury and epilepsy may offer a clinical window of opportunity since it represents a period of time prior to the emergence of epilepsy where we can affect change for those at higher risk for epilepsy following TBI. For example, if we compiled the right information together in the right ways, like the severity of brain injury, relevant medical history, and medications, it may be possible to infer who will develop epilepsy after brain injury. Very high accuracy is not required for this to be useful. For example, if we estimated the top one thousand Service Members we think are at risk of epilepsy after TBI, and only one hundred of them go on to develop epilepsy, then we will still have given some advanced warning to a hundred people that they are going to have a serious life-changing disease in the future. This means they can be allocated resources, targeted for interventions or trials before epilepsy emerges, and they can begin to prepare for different eventualities.

However, a simple model that looks at the medical history of Veterans and tries to guess who will be diagnosed with epilepsy in the future may not work for a few reasons. First, reporting rates of head injury are very low, and injury information from before military service or elsewhere may not be available. We propose to solve this problem directly by using the responses of thousands of Veteran volunteers with epilepsy and TBI who were asked about the dates and details of their epilepsy and history of lifetime head trauma in a study conducted by project co-investigator Dr. Mary Jo Pugh. We have agreements to reuse this data, which also documents measures of quality of life, medication use, combat exposure, and many other factors. Combined with decade-long medical histories, we can use this data offered by Veterans to greatly improve our model timing and event date accuracies.

Aside from event timing, there is another challenge. There is no guarantee that prior medical conditions and demographics contain enough information to make good future predictions. The strongest warning signs that come before an epilepsy diagnosis are often subjective, like unexplained fatigue, absent mindedness, spacing out, or changes in behavior. To capture these valuable pre-diagnostic warning signs, we propose to examine “multimodal” data. We will capture this information by proxy by analyzing big data repositories that detail how people use health services over time, dates, and dosages of medications that are administered, what health problems emerge, and what services are used and how frequently. A core feature of this study is that we believe the order of all these things combined might add a lot of predictive power. Whether a medication is prescribed in the presence or absence, or before or after certain medical diagnosis is informative.

Little work has explored whether specific health comorbidities interact with injury characteristics to increase risk for PTE, and military health questions of interest to the Department of Defense remain unanswered. We propose to enact the solutions to these hurdles we have described and specifically focus on medication differences and outcomes in our first aim. In our second aim, we will develop risk scores for PTE and drug-resistant epilepsy (DRE) following TBI. We want to try and exploit the delayed window of time between TBI and epilepsy diagnosis for detection and to buy time to prepare. The right preceding information could provide a summary measure of epilepsy risk following TBI, which could offer new opportunities for intervention and health care planning. The causal nature of epilepsy acquisition after TBI requires specific

methods like network models that can map the directed links between TBI, epilepsy, comorbidities, treatments, and key like seizure freedom, and quality of life. Aim 2 will focus on ways to fine tune these data and models to maximize predictive performance.

After developing these risk scores, we will test how well they perform in new datasets in our third aim. We will trial the risk scores in two ongoing studies that are currently screening for epilepsy. If we can predict epilepsy before it happens in ongoing studies, that is strong evidence that the risk scores are working well and could even be rolled out to the whole population. It is not easy to guess how well these risk scores will perform up front, but there are encouraging correlates, and our group has the clinical and technical expertise to achieve success. Our proposal offers new perspectives on how to enhance treatment and innovations in data collection and analysis. We will use multimodal data to build and deploy new tools that seek to flag the early warnings for epilepsy risk with prospective validation building on our extensive clinical and technical expertise.

Proposal Title:	Late-Stage Preclinical Development of Teixobactin to Treat Drug-Resistant Infections
Log Number:	JW220010
Current PI Name:	Dallas Hughes
Award Number:	HT9425-23-1-0376
Current Contracting Organization:	NovoBiotic Pharmaceuticals
Current Performing Organization:	NovoBiotic Pharmaceuticals
Web Approval Date:	04-27-2023

This project describes the late preclinical development of teixobactin, a new class of antibiotic to treat skin and lung infections and other serious infections in both military personnel and the general population. Teixobactin is very effective in animal models of infection, has not shown drug resistance, and has great promise for treating drug-resistant infections, as it kills the infecting bacteria through different mechanisms than current antibiotics.

We have completed most of the safety and toxicology studies that are required before a drug can be tested in humans. As the next steps in drug development, we are required to develop methods to manufacture the compound under current Good Manufacturing Practice (cGMP) compliance and to determine safe maximum starting doses in human. The studies in this proposal will help to determine what dosages, dose frequency (e. g., once or twice daily, etc.), route of drug administration (e.g., injection, intravenous infusion, etc.), and how to manufacture and package the drug for delivery in a first-in-human clinical trial. Developing the manufacturing of the clinic-ready drug product is a main goal of this proposal. In addition, we propose an animal model study aimed at confirming the effective dose for humans. The Fiscal Year 2022 (FY22) Joint Warfighter Medical Research Program (JWMRP) Focus Area to be addressed by the proposed effort is the Endemic and Emerging Disease Threats: Therapeutic measures for infectious diseases, including combat wound care in austere and prolonged field care environments.

Introducing a novel antibiotic into the clinic would be a great advance, considering the very few novel antibiotics approved since 2000, and the rise of antimicrobial resistance. Teixobactin’s attractive mode of action, lack of resistance, and activity against drug-resistant strains suggests that it may be able to address the clinical need of combating several serious infections faced by active-duty Service personnel, Veterans, and their families. Drug-resistant infections pose a particular threat for the military population due to environmental and occupational exposures unique to military service. These exposures may facilitate transmission of infections and thereby compromise Force health and operational readiness. This population remains at significant risk for infections caused by methicillin-resistant Staphylococcus aureus (MRSA) due to environmental and occupational exposures, such as combat associated deployments. Because infections can also interrupt training cycles and compromise operational readiness, effective treatment strategies for military populations are critically needed. For the general population, 2.8 million antibiotic-resistant infections occur in the U.S. every year, resulting in 35,000 fatalities.

Teixobactin is a very potent antibiotic with a broad range of clinical applications including skin infections, bacterial pneumonia, tuberculosis, and anthrax. Since it is currently evaluated as an intravenously administered drug, its application will be for patients in the hospital setting. Teixobactin’s potential benefits include activity against serious bacterial infections, lack of resistance, and shorter treatment times with lower doses than required for current antibiotics. Although unlikely, the main risk is unacceptable side effect(s), which could prevent the administration of an effective dose. However, our animal studies to date suggest an

effective dose of the compound can be safely delivered. The potential side effects (risks), if any, will be closely monitored in the clinic. The projected dosing schedule will be 7 to 10 days treatment with a daily intravenous infusion of 15 to 30 minutes duration.

At the end of this project, we will be able to submit an Investigational New Drug application to the U.S. Food and Drug Administration (FDA) to obtain approval to test teixobactin in human clinical trials. The contributions of this proposal are essential, as they will provide the drug product and dosing schedule necessary to conduct first-in-human trials.

Proposal Title:	Delineate Tumor-Immune Contexture That Shapes ccRCC Metastatic Progression and Response to Immunotherapy
Log Number:	KC220003
Current PI Name:	Srinivas Malladi
Award Number:	HT9425-23-1-0867
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	09-01-2023

Objective and Rationale: Renal adenocarcinomas are molecularly and clinically distinct, with clear cell and papillary type accounting for 90% of the cases. Despite increased early detection of primary tumor, approximately 25% of patients are presented with metastatic disease and more than one-third of the patients who undergo nephrectomy with curative intent experience local or distal relapse. Around 78% of metastases occur within 5 years and 11% occur after more than 10 years of initial diagnosis. Despite early detection and increased efforts to treat metastatic disease, we have limited understanding of why some invasive ccRCC tumors metastasize and some do not -- and why immunotherapies are effective in some metastatic patients and not all.

Innovation: By implanting PDXs in humanized mice models we have developed a novel approach to define the immune contexture and activity status in tumors that metastasize versus that don’t and in tumors that respond to immunotherapy versus that do not.

Applicability of Research: If successful, in the short term the insights gained from these studies will identify patients likely to develop metastatic disease and predict response to immunotherapy. Moreover, the gene expression data obtained from tumors will be the basis for testing several novel hypothesis such as the features associated with metastatic tumors and immunotherapy response. Insights gained from non-metastatic and therapy-resistant ccRCC tumors is invaluable to identify mediators that resist metastatic progression and immunotherapy. Strategies designed to modulate activity of these mediators will result in long-term cures.

The application aligns well with the FY22 KCRP focus area on conducting basic biology research to better understand etiology and cancer progression, metastatic disease, refractory disease and therapeutic resistance, genetic and environmental risk factors, and the prevention of kidney cancer. The findings from this project has broad implications on how we approach clinical management of not only metastatic ccRCC patients but also other cancers in Service Members, their Families, Veterans and the American public.

Proposal Title:	Targeting Environmental Ferroptosis Protection as a Novel Therapeutic Strategy for Metastatic Renal Cell Carcinoma
Log Number:	KC220008
Current PI Name:	Jen Tsan Chi
Award Number:	HT9425-23-1-0850
Current Contracting Organization:	Duke University
Current Performing Organization:	Duke University
Web Approval Date:	09-01-2023

Scientific Objective and Rationale for the Proposed Project: Despite all the new development in diagnosis and treatment for kidney cancer, the prognosis and outcomes are still unacceptable for patients with advanced and metastatic kidney cancer. One special characteristic of kidney cancer is its association with blood. Many kidney cancers will grow into blood, a process called "vascular invasion." In addition, most of the metastasis of kidney cancer occurs through the bloodstream instead of the lymphatic system. This preferential association with blood has been a mystery and solving such a mystery may unlock new insights to reduce the metastasis of kidney cancers. The proposed study is based on an exciting insight we have gained into a potential explanation. We and other scientists have found that most kidney cancer cells are very vulnerable to ferroptosis, a special kind of iron-dependent cell death we are just learning about. In addition, many changes during metastasis would make them even more sensitive to ferroptosis. One study on melanoma cells has confirmed our prediction that traveling tumor cells in the bloodstream undergo ferroptosis. If this is the case, how can traveling kidney cancer cells survive ferroptosis and establish metastasis? We may have solved the mystery. We have found that while the metastatic RCC are highly sensitive to ferroptosis, they can be protected from ferroptosis by the blood and serum. Therefore, the traveling RCC tumor cells in the bloodstream are continuously protected and chaperoned by the blood during their journey to metastasis which can allow survival and establish metastasis.

FY22 KCRP Focus Area(s) to be addressed: (1) Conduct basic biology research better to understand etiology and cancer progression, metastatic disease. (2) Develop novel therapeutic strategies for the treatment of kidney cancer.

Describe the ultimate applicability of the research: There are multiple ways the proposed research can be applied to help patients. First, it is possible that bezafibrate and other lipid-lower drugs can be safely used to prevent metastasis by disabling the ferroptosis protection by blood. In addition, these methods can be used to treat metastatic kidney cancers by combining with the drugs that kill RCC by ferroptosis, especially when the RCC has become unresponsive to other treatments. Finally, ferroptosis has been shown to enhance immunotherapy and may provide combination therapeutics to immunotherapy for patients with kidney cancer.

What types of patients will it help, and how will it help them? Most patients diagnosed with kidney cancer are at risk for metastasis and clear-cell types are particularly sensitive to ferroptosis. Therefore, our efforts to reduce or potentially reduce or eliminate metastatic kidney cancer will benefit most patients at risk for metastasis and for whom there are currently no effective treatments.

What are the potential clinical applications, benefits, and risks? We will find out whether interfering with blood protection can be used to eliminate metastatic RCC by triggering ferroptosis. If successful, this will lead to an entirely new regimen of treatment that targets metastatic tumor cells' survival in the blood. A potential benefit is the ability to reduce metastatic RCC. Risks include undesirable side effects, as is true with any drug.

What is the projected time it may take to achieve a clinically relevant outcome? Our research has pointed to a potential for FDA-approved bezafibrate used by billions of people. Several ferroptosis-inducing agents are in early clinical development. Therefore, depending on the progress of drug development and regulatory procedures, they could be available in 5 to 10 years.

What are the likely contributions of this study to advancing the field of cancer research and/or patient care? Find out the novel role of serum and blood as determinants of metastasis by ferroptosis protection.

Short-term and long-term impact for Service Members, their Families, Veterans, and the American public: Military Service Members are vulnerable to kidney cancer, potentially increasing the risk of exposure to ionizing radiation and toxic chemicals during their service. The novel therapies will potentially benefit the health and welfare of these service members and their families by reducing and hopefully even preventing metastasis.

Innovative aspects of the proposed research project: The project's innovation is the concept that blood and its lipids provide unexpected protection against ferroptosis in the circulating kidney cancer cells. Our findings explain why kidney cancer tends to spread in the bloodstream and how we can interfere with this blood protection to prevent or treat metastasis

Proposal Title:	Multifunctional Immunotherapy Nanoparticle to Enable Innate Immunotherapy for Kidney Cancer
Log Number:	KC220012
Current PI Name:	Andrew Wang
Award Number:	HT9425-23-1-0516
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	09-01-2023

Scientific Objective and Rationale: Immunotherapy options for metastatic kidney cancer are rapidly expanding, including five first-line treatment strategies for metastatic clear cell kidney cancer patients. These include immunotherapy activating the adaptive immune system (T-cells), targeted therapy directed against blood vessel formation, and the combination of both. However, complete responses are rare, and durable responses are even rarer.

In order to enhance responses to immune checkpoint inhibitors, other immunotherapies to activate the innate immune system are currently under development. Natural killer (NK) cells are a significant part of the innate immune system. Recent publications show that NK cells play a complimentary role to T-cells in tumor cell killing, and therefore are a good target for developmental therapies. We have preliminary data that kidney tumors lacking in NK cell gene expression do not respond to immune checkpoint inhibitors as well as kidney tumors that do have NK cell gene expression. We have developed an innovative approach using nanoparticles to activate NK cells in a tumor-specific fashion. We propose to use the multifunctional immunotherapy nanoparticle (MINP) technology to activate NK cells targeted to metastatic renal cell carcinoma (RCC). We propose to develop MINPs that are targeted against RCC and evaluate these using mouse models and patient-derived tumor graft models of RCC. Our proposal thus addresses key KCRP area of emphasis of therapeutic development.

Innovation: Our approach is innovative in several aspects. We are proposing to activate NK cells of the innate immune system to improve upon immunotherapy options for metastatic RCC. The MINPs tailored specifically to RCC tumors are innovative in using concepts important in bioengineering, tumor immunology, and clinical oncology. The proposed MINPs can target ccRCC, papillary RCC and potentially other histologies. Importantly, the MINPs will activate NK cells to specifically kill RCC tumors. Finally, the tumor-based and peripheral NK cell population may also provide a biomarker to track during treatment of these tumors.

Clinical Applicability: The successful completion of this research will help many patients with metastatic RCC in developing a new class of immunotherapy therapeutic agents. This proposal will generate the preclinical data necessary to translate these new therapies to the clinical setting. There are pending patents for NK-cell activating MINP technology, and the patents has been licensed by Archimmune Therapeutics, a startup biotechnology company. The successful completion of these proposed studies may enable rapid clinical translation of MINP technology for renal cell carcinoma, within several years for clinical development. The MINP technology can also be tailored for different cancer types and could become a platform to develop NK-activating therapies for different tumor types of kidney cancers. Ultimately, we hope to improve immunotherapies for all kidney cancer patients including Veterans as well as the American public.

Proposal Title:	Uncovering the Tumor-Immune Microenvironmental Determinants of Immunotherapy Response in Renal Cell Carcinoma Through Ex Vivo Patient-Derived Models
Log Number:	KC220016
Current PI Name:	David Braun
Award Number:	HT9425-23-1-0735
Current Contracting Organization:	Yale University
Current Performing Organization:	Yale University
Web Approval Date:	09-01-2023

Despite tremendous advances in treatment through the development of medications that stimulate the immune system, advanced kidney cancer is still responsible for approximately 15,000 deaths each year in the United States. Kidney cancer is particularly common among Veterans, and so there is a fundamental need to better understand why kidney cancer tumors do or do not respond to current medicines, and to develop new types of treatments to help more patients.

A kidney tumor is often composed of hundreds of millions or even billions of individual cells, but not all of those cells are part of the cancer itself. The tumor is actually a diverse ecosystem composed of dozens of different types of cells, including immune cells that may either be trying to attack and eliminate the cancer cells, or conversely, may actually be aiding the growth of the cancer. Altogether, all of the cells and the way they interact with each other is called the "microenvironment" of the tumor. Prior research from our laboratory and others have studied this tumor microenvironment to try to identify exactly what types of cells are present, how they might interact or "talk to each other," and how this might change when kidney cancer grows from a small tumor to a larger one or spreads to other parts of the body. We found that when kidney cancer grows and spreads, the immune cells within the tumor actually become part of the problem. The T cells, which are normally responsible for attacking cancer cells and are the main target of current standardly available immune therapies (called immune checkpoint inhibitors), lose their normal function through a process called "exhaustion". However, these T cells do not act alone -- there is another immune cell type in the tumor, the macrophage, that also change in a way that prevents a normal immune response against kidney cancer and promotes the growth of cancer cells. Importantly, these two types of cells appear to be talking to each other (i.e., interacting), with the macrophages instructing the T cells to stop any anti-cancer attack, and the T cells also instructing the macrophages to continue to act in this unproductive fashion. We believe that this crosstalk between these critical immune cell types within the tumor prevents the body's immune system from successfully attacking and controlling kidney cancer. We identified numerous ways that these cell types use to communicate (or "interact"), and so the next question is clear -- which of these specific interactions can we target with a new drug to restore the normal functioning of the immune system and help it successful eliminate kidney cancer cells? Therefore, our Kidney Cancer Research Program Focus Area is centered on developing novel therapeutic strategies for the treatment of kidney cancer.

We believe that the best method for studying this is to use actual kidney cancer tumors from patients to understand these immune cells in a true human context. Our lab has developed and optimized multiple techniques for studying kidney cancer tumor directly from patients -- one system where we isolate just two types of cells and study them in isolation, and another where we actually preserve the full three-dimensional structure of a tumor and all of the dozens of components. These innovative new systems will allow us to ask fundamental questions about kidney cancer: Which interactions can we target with new drugs in order to restore the immune system’s ability to successfully control or eliminate kidney cancer? Once we have

identified new targets to bolster the immune system to attack kidney cancer, we next ask which patients are most likely to benefit from a new therapy? To do this, we re-analyze a large set of data that we previously published to ask which of these new targets or interactions are more commonly found in tumors that are resistant to current kidney cancer therapies, as we believe patients with these therapy-resistant tumors would be most likely to benefit from a novel drug.

We hope and believe that this proposed study will be directly applicable to patients with advanced kidney cancer, and particularly those with the most common subtype (called "clear cell" kidney cancer). This study will aim to identify new immune targets that will re-invigorate T cells and other immune cell types to effectively attack kidney cancer cells, and will seek to identify which patients are most likely to benefit from new drugs that affect these targets. We would aim to successfully identify new immune targets over the course of this grant (i.e., within the 3-year award period), and then to rapidly translate these findings into new early phase clinical trials for patients with advanced kidney cancer (as I have previously done with an early phase I study of a cancer vaccine in kidney cancer as a co-Principal Investigator). Our goal is that this research provides both short-term benefit in terms of improving our knowledge of how kidney cancer grows and operates, and longer-term benefit with respect to developing new therapies that can help treat more patients with kidney cancer, including Service Members, their Families, Veterans, and the general American public.

Proposal Title:	DROSHA Regulates Mesenchymal Expression and Chemosensitivity in Wilms Tumor
Log Number:	KC220019
Current PI Name:	Kenneth Chen
Award Number:	HT9425-23-1-0727
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	09-02-2023

Scientific Objective and Rationale: Wilms tumor is the most common kidney cancer in children. It usually arises in toddlers, and most patients can be cured with a combination of chemotherapy, surgery, and radiation. Although this therapy is often successful, many survivors are left with long-term health conditions caused by their therapy. In addition, some types of Wilms tumors remain harder difficult to cure. Recently, a newer chemotherapy named irinotecan showed promise in Wilms tumor, and irinotecan is now being tested in other types of Wilms tumor. However, we do not know which Wilms tumor patients will benefit the most from irinotecan. More effective treatments are needed, and the treatments that are given need to be better at targeting the patients who will most benefit. However, our ability to adapt treatments to Wilms tumor subgroups is constrained by our limited understanding of how Wilms tumor mutations cause cancer.

One harder-to-treat group of Wilms tumors is known as "blastemal-predominant" based on how their cells look under a microscope. Wilms tumors generally look like undeveloped kidney cells, but "blastemal-predominant" Wilms tumors look like the earliest type of undeveloped kidney cells. We found that many of these tumors are caused by tumor-specific mutations in a pathway that makes regulatory molecules called microRNAs. Normally, microRNAs turn off other genes, so cells without microRNAs cannot turn off specific genes. However, many questions remain. We do not know how loss of microRNAs causes cancer. We do not know how this locks cells into the earliest state of kidney development. We do not know how this should affect therapy decisions.

To study the effects of losing microRNA production, we studied Wilms tumor cells in the lab; Wilms tumor samples from patients; and mice with the most common mutation seen in Wilms tumor. From these studies, we found that a particular gene named HMGA2 is normally turned off by microRNAs in the first step of kidney development. However, Wilms tumors lacking microRNAs are unable to turn off this gene and are unable to undergo this first step. HMGA2 helps a cell turn other genes on and off, and we believe that high levels of HMGA2 lock a Wilms tumor cell into a primitive state. Studies in colon cancer suggest that cells with HMGA2 respond best to irinotecan. However, little is known about how HMGA2 functions in Wilms tumor.

FY22 KCRP Focus Area: “Conduct basic biology research to better understand etiology and … therapeutic resistance … of kidney cancer.” Wilms tumor is one of the most common cancers in children. Although cure rates are high, the regimen involves about one year of frequent hospitalizations, multiple surgeries, and multiple chemotherapy drugs. These therapies carry a high risk of side-effects and complications; some of these side-effects are temporary, but some may continue for the rest of a survivor’s life. In addition to taking time away from other activities, therapy becomes a significant financial burden and emotional burden for Service Members and their Families.

Ultimate Applicability of this Research: This project seeks to improve our understanding of how Wilms tumors form and how they respond to therapy. Immediately, this will help us prioritize which patients should receive irinotecan therapy and which may avoid it. In the longer term, our project will provide fundamental

details about how Wilms tumors form. Although we only study one subtype of Wilms tumor here, this is an important subtype to study because it is harder to treat. In addition, because this subtype looks like the earliest kidney cells, improving therapy for this subtype will improve therapy for other types of Wilms tumors that arise from these early cells. Lastly, this project will contribute to our understanding of how kidneys form. Based on the development of promising technologies, we may one day be able to grow a kidney from scratch. This will require a precise balance between growth and development. Getting that balance wrong may lead to Wilms tumor, but little is known about how to optimize that balance. Our project studies the fundamental biology of Wilms tumor and may provide insights into how to control the balance between growth and development.

Innovative Aspects: This project will benefit from several innovative aspects. First, we use a new mouse model of the most common mutation in Wilms tumor. This led to a paradigm shift where we are now studying a fundamental cellular process at the beginning of kidney development. Our focus on how microRNAs regulate this step is unexplored. Second, we use new technologies to manipulate gene expression using CRISPR. This technology a more precise way to understand the effects of a specific gene than traditional technologies. Lastly, irinotecan is emerging as a promising therapy in Wilms tumor and other pediatric cancers, and no studies have looked at the biology behind why specific tumors respond.

Proposal Title:	Epigenetic Changes as a Biomarker in African Americans with Papillary Renal Cell Carcinoma and a Link to the Tumor Microenvironment
Log Number:	KC220020
Current PI Name:	Brandon Manley
Award Number:	HT9425-23-1-0882
Current Contracting Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Current Performing Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Web Approval Date:	09-02-2023

Our project seeks to evaluate features of epigenetics in papillary renal cell carcinoma (pRCC) and if they can be used as a blood-based biomarker for disease recurrence and if African American patients harbor unique changes relative to European ancestry patients. Epigenetics literally means "above" or "on top of" genetics. It denotes alterations to DNA that turn genes "on" or "off." These modifications do not change the DNA sequence, but instead, they affect how cells "read" genes. One of the most common way cells make this modification is through methylation. While these changes are a normal biological phenomenon in how cells grow and divide, they can become pathologically altered in cancer states. In fact, these changes can be so characteristic of a tumor type that these "marks" be used as a method to detect "tumor DNA" from "normal DNA". One of the main objectives of this study is to evaluate the detection of these "tumor DNA" epigenetic changes as a possible blood-based biomarker that could improve detection and sensitivity of recurrence above current standards that require frequent and less specific CT/MRI imaging.

Though surgery can be curative for some of these patients, there is a high risk of disease recurrence for those with pRCC tumors. In the first part of this proposal, we will test the performance of a molecular assay to detect candidate methylation markers levels in the plasma (a component of blood) of patients with pRCC. This test is also known as a liquid biopsy. Our initial testing has demonstrated the ability of our assay to identify these candidate methylation markers in the plasma of pRCC patients. We will evaluate detection levels of these markers in a prospective group of patients before and after surgery or tumor ablation, both of which will be performed as curative treatment. By detecting whether any, or all, of the markers are still present in the patient's plasma after treatment, we believe we will be able to identify patients with early disease recurrence (before radiographic imaging can detect recurrence). Such patients could be ideally suited for altered surveillance protocols (i.e., more frequent) or possible adjuvant treatment in the context of a clinical trial. This method is like how persistent prostate-specific antigen levels are used as a sign of disease recurrence after prostate cancer treatment. We will also examine if the presence of these markers in patient blood can serve as a surrogate for changes and features in the tumor itself (i.e. the tumor microenvironment). An important feature of our study will also focus on African American patients as these patients have increased risk of pRCC and worse clinical outcomes even when controlling for things like pathological stage. The biological rationale for why these patients are at more risk and have worse outcomes is not understood and our study will examine if epigenetic changes could be a contributing factor. In line with the Congressional Metastatic Cancer Task Force, our study seeks to fill a gap in the care of these patients by developing practical liquid biopsy test for patients with pRCC.

With respect to our project's ability to impact current kidney cancer patients, we have both short-term (1-2 years) and long-term (3-6 years) objectives. In the short term, we hope to validate and incorporate the use of these methylation markers to identify patients with early disease recurrence after presumed curative treatment. We also hope to have a liquid biopsy test that can inform clinicians and researchers about a patient's tumor features without the need for invasive tissue biopsies. The results of this study will focus on the difference between epigenetic markers between African American patients and their tumors compared to those from European ancestry. In the long term, these markers could also be evaluated as a screening test for

patient without a diagnosis of pRCC, especially among groups at higher risk of development, such as those with hereditary renal cell cancer syndromes in which pRCC is a frequent histology. We also believe that by identifying unique epigenetic features present in African American tumors we can provide scientific insight on how to address the observed health disparity seen among pRCC patients.

Proposal Title:	Epigenetic Changes as a Biomarker in African Americans with Papillary Renal Cell Carcinoma and a Link to the Tumor Microenvironment
Log Number:	KC220020P1
Current PI Name:	Liang Wang
Award Number:	HT9425-23-1-0883
Current Contracting Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Current Performing Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Web Approval Date:	09-02-2023

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Though surgery can be curative for some of these patients, there is a high risk of disease recurrence for those with pRCC tumors. In the first part of this proposal, we will test the performance of a molecular assay to detect candidate methylation markers levels in the plasma (a component of blood) of patients with pRCC. This test is also known as a liquid biopsy. Our initial testing has demonstrated the ability of our assay to identify these candidate methylation markers in the plasma of pRCC patients. We will evaluate detection levels of these markers in a prospective group of patients before and after surgery or tumor ablation, both of which will be performed as curative treatment. By detecting whether any, or all, of the markers are still present in the patient's plasma after treatment, we believe we will be able to identify patients with early disease recurrence (before radiographic imaging can detect recurrence). Such patients could be ideally suited for altered surveillance protocols (i.e., more frequent) or possible adjuvant treatment in the context of a clinical trial. This method is like how persistent prostate-specific antigen levels are used as a sign of disease recurrence after prostate cancer treatment. We will also examine if the presence of these markers in patient blood can serve as a surrogate for changes and features in the tumor itself (i.e. the tumor microenvironment). An important feature of our study will also focus on African American patients as these patients have increased risk of pRCC and worse clinical outcomes even when controlling for things like pathological stage. The biological rationale for why these patients are at more risk and have worse outcomes is not understood and our study will examine if epigenetic changes could be a contributing factor. In line with the Congressional Metastatic Cancer Task Force, our study seeks to fill a gap in the care of these patients by developing practical liquid biopsy test for patients with pRCC.

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patient without a diagnosis of pRCC, especially among groups at higher risk of development, such as those with hereditary renal cell cancer syndromes in which pRCC is a frequent histology. We also believe that by identifying unique epigenetic features present in African American tumors we can provide scientific insight on how to address the observed health disparity seen among pRCC patients.

Proposal Title:	Characterization of Epigenetic and Metabolic Vulnerability in VHL-Deficient ccRCC and Its Therapeutic Potential
Log Number:	KC220035
Current PI Name:	Weibo Luo
Award Number:	HT9425-23-1-0863
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	09-02-2023

The current proposal focuses on clear cell renal cell carcinoma (ccRCC) and addresses one of Fiscal Year 2022 Kidney Cancer Research Program Focus Areas, that is, develop novel therapeutic strategies for the treatment of kidney cancer, such as novel drug targets, therapeutic modalities and agents, treatment combinations and drug delivery systems.

ccRCC accounts for more than 75% of all renal cancer cases but is currently incurable. Loss of the tumor suppressor gene VHL is a key feature in ccRCC, which results in activation of hypoxia-inducible factor (HIF) to drive tumor development. Targeting HIF signaling has been recently approved by the FDA as a first-line drug to treat ccRCC. However, a large population of ccRCC patients are resistant to this targeted therapy. Therefore, identification of alternative therapeutic approaches for ccRCC treatment is an unmet clinical need. Our recent studies identified a next-generation DNA methyltransferase inhibitor that could selectively kill VHL-deficient ccRCC cells. However, the underlying mechanism remains obscure. In the current proposal, we will perform preclinical studies to evaluate this selective killing approaches in clinically relevant VHL-deficient ccRCC models and investigate the underlying mechanism. These proposed studies are highly innovative because they are expected to define a specific therapeutic approach for the treatment of VHL-deficient ccRCC.

The short-term outcomes of this project are to determine the therapeutic potential of DNA methyltransferase inhibitor and understand the mechanism behind this targeted therapy. The goal will be achieved based on our expertise and through close collaborations with Dr. James Brugarolas (an internationally recognized leader in kidney cancer and a director of the NCI kidney cancer SPORE), Dr. Ralph DeBerardinis (an expert in cancer metabolism), and Dr. Chao Xing (an expert in bioinformatics) at UT Southwestern. In the long term, successful completion of this project will yield a rational therapeutic option for killing the most common and currently incurable subset of kidney cancer in patients including Service Members, their Families, Veterans, and the American public in near future.

Proposal Title:	Tumor Extravasation in Zebrafish as a Prognostic Marker and a Therapeutic Target for Metastasis of Kidney Cancer
Log Number:	KC220043
Current PI Name:	Kiyoshi Ariizumi
Award Number:	HT9425-23-1-0596
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	09-02-2023

Objective and Rationale: In the U.S., kidney cancer is the 7th most common cancer in men, and the 9th most common in women, with renal cell carcinoma (RCC) accounting for over 90% of cases. Recurrence after surgical removal of RCC is very high (~40%) and survival in the presence of metastasis is poor (approximately 10% in 5 years). Thus predicting and treating metastasis are crucial aspects for patients with RCC. Our proposal addresses these problems and offers novel solutions.

It is widely assumed that cancer cells are released into circulation even in patients with no detectable metastasis. While metastasis involves several phases, it stands to reason that the juncture at which tumor cells exit from blood is a crucial determinant of metastatic risk. To determine whether this component can be used to predict metastasis, we will use zebrafish larvae (in which human tumor cells are allowed to survive) as a vehicle for measuring tumor-cell traffic in and out of circulation (TExZ score). An existing platform for RCC at our institution will allow us to correlate this measure with metastatic risk based on the clinical profile (including treatment outcomes).

Having discovered DC-HIL and its ligand rHS (unique carbohydrates) as novel immune checkpoint molecules that inhibit T-cell defense against cancer, promote tumor angiogenesis, and regulate T-cell extravasation, we will use our monoclonal antibody (mAb) against rHS in zebrafish and mouse models to develop prognostic markers and treatments for metastatic RCC.

FY22 KCRP Focus Area(s): “Develop new strategies for accurate prognosis prediction and for novel therapeutic modalities of kidney cancers”.

What types of patients will it help, and how will it help them? We focus on patients with RCC to validate markers for predicting and treating metastasis. Our results can be applied to managing other solid cancers.

What are the potential clinical applications, benefits, and risks? Zebrafish is a relatively low-cost (vs. mice and humans) and rapid mode of predicting metastasis. Targeting rHS as treatment (for which we have already created the mAb) is a new modality for RCC and potentially other cancers.

What is the projected time it may take to achieve a clinically relevant outcome? 3-4 years of research.

What are the likely contributions of this study to advancing the field of kidney cancer research? Our studies will expand the utility of the existing Kidney Cancer Program at UT Southwestern. It will be a game changer in predicting risk for metastasis and in preventing metastasis. Finally we should get useful information regarding how RCC behaves and how cancer cells leave blood circulation to reach secondary organs.

The impact that the proposed research project results might have on the field of kidney cancer research and /or patient care in the short term and/or long term for Service Members, their Families, Veterans, and the American public. RCC is a growing cause of death and sickness among Americans, Veterans, and Military

Service members and Families. Reliable predictive markers are lacking and the cancer is very unresponsiveness to currently available treatments.

The innovative aspects of the proposed research project.

(1) Discoveries of DC-HIL and its ligand (rHS) as regulators of immune defense vs. cancer progression.

(2) Exiting of cancer cells from blood circulation is a key process of metastasis, so measuring is important.

(3) Kidney Cancer Program at UT Southwestern provides a reservoir and registry of renal cell cancers.

(4) Zebrafish model is a relatively economical and rapid way of studying metastasis.

(5) Develop our monoclonal antibody against rHS to treat and prevent metastasis.

Proposal Title:	Developing Clinically Relevant Genetic Mouse Models for Upper Track Urothelial Cancer
Log Number:	KC220053
Current PI Name:	Guocan Wang
Award Number:	HT9425-23-1-0305
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	02-07-2023

Background and scientific objective: Upper tract urothelial carcinoma (UTUC), a rare cancer type that occurs in the urinary system, including kidney, accounts for approximately 10% of renal tumors and 5% of all urothelial carcinomas. The treatment options for UTUC are still limited to chemotherapy and surgery and patients with advanced UTUC remain incurable. There is an urgent need to develop better therapeutic strategies. However, our understanding of UTUC development and progression remains limited. Recent studies of human UTUC have led to the identification of changes in patients' genomic DNA sequences. Although patient-derived xenografts (PDXs) and cell lines generated from human UTUCs serve as valuable research tools, they cannot be easily used to study the impact of the immune system on UTUC progression and on the responses of UTUC to various cancer therapies, including immunotherapy. Currently, there are no genetic mouse models for UTUC, which hampers our efforts to fully harness the power of immunotherapy to achieve durable control or cure of UTUC. Thus, there is an unmet need to develop UTUC genetically engineered mouse models. The objective of this study is to develop clinically relevant UTUC GEMMs, which will facilitate basic and translational research.

Innovation: Our proposed study will lead to the development of a novel UTUC genetic mouse model and cell lines harboring clinically relevant genetic aberrations. Also, our study will provide novel insights into the molecular and cellular process driving UTUC progression.

Impact: In the short term, our proposed research will lead to a better understanding of how UTUC develops and progress to advanced stages. Also, our study will lead to the generation of new research tools for preclinical research, which will allow researchers to study the functions of UTUC-related genes and test novel therapeutics. Ultimately, our study will improve the clinical outcome of UTUC in the long term.

Proposal Title:	Targeting P2X7 Receptor in Renal Cell Carcinoma
Log Number:	KC220055
Current PI Name:	Vladimir Kolenko
Award Number:	HT9425-23-1-0833
Current Contracting Organization:	Institute for Cancer Research
Current Performing Organization:	Institute for Cancer Research
Web Approval Date:	09-02-2023

Renal cell carcinoma (RCC) is a lethal disease whose incidence is on the rise. Clear cell RCC (ccRCC) is the most frequent (75%to 80%) subtype of RCC. Traditional chemo- and radiation therapies are largely ineffective in the treatment of all RCC subtypes. The development of multi-targeted tyrosine kinase inhibitors (TKIs) and immunotherapeutic agents notably changed the treatment paradigm of advanced kidney cancer. However, despite the therapeutic progress, complete and durable responses have been noted in only a few cases. Thus, ccRCC continues to be a treatment-resistant malignancy. Adenosine 5'-triphosphate (ATP) is the principal molecule for storing and transferring energy in cells. In the process of tumorigenesis, a large amount of intracellular ATP is released into the tumor microenvironment (TME). Extracellular ATP levels are more than 1,000-fold higher in the TME than in normal tissues and can reach high micromolar concentrations. Besides energy transduction, ATP plays a critical role in extracellular signaling activating ATP-gated purinergic receptors. Among all ATP-gated receptors, P2X7 receptor (P2X7R) has unique characteristics including a very high activation threshold. Therefore, P2X7R function is expected to be highly relevant for TME with extremely high abundance of extracellular ATP. Indeed, P2X7R contributes to tumor progression and therapeutic resistance in various cancer types. Importantly, the expression of P2X7R is increased in ccRCC at both mRNA and protein levels.

Our preliminary studies demonstrate that stimulation of P2X7R results in the activation of pro-tumorigenic signaling and the augmented expression of several key immunosuppressive and pro-angiogenic factors in ccRCC cells. P2X7R has two active isoforms, full-length P2X7A and truncated P2X7B, that impart different functional properties. P2X7B might be of special relevance in cancer as P2X7B retains P2X7A growth-promoting activity while it lacks P2X7A-associated cytotoxicity. Therefore, P2X7A may act as a tumor suppressor, whereas P2X7B may function as a tumor promoter. Given that high extracellular ATP levels in solid tumors are capable of inducing P2X7A-mediated pore formation and thereby promote cell death, it is unlikely that cells expressing P2X7A could survive in the TME. Therefore, it is anticipated that malignant cells express P2X7R predominantly in a form where pore activity is attenuated, such as P2X7B isoform. Thus, tumor cells can gain a selective advantage by silencing the negative (death-inducing) response linked to P2X7A activation and keeping only the positive (pro-tumorigenic) P2X7B-associated properties. Based on our preliminary findings and published data, we hypothesize that high levels of extracellular ATP activate pro-survival and pro-tumorigenic signaling in ccRCC by stimulating P2X7B. Therefore, antagonism of P2X7B could have significant negative effect on survival and tumorigenic potential of ccRCC cells.

To test our hypotheses and to elucidate the role of P2X7R as a potential prognostic biomarker and a novel therapeutic target in ccRCC, we propose the following Specific Aims: (1) Evaluate P2X7R as a potential therapeutic target in ccRCC. First, we will examine the role of P2X7R in supporting pro-tumorigenic properties of ccRCC cells. Next, we will delineate the role of P2X7A and P2X7B isoforms in regulating ccRCC tumorigenesis in vivo. In addition, we will examine the therapeutic potential of P2X7R inhibition in cell and animal models of human ccRCC. (2) Establish the association of P2X7R with ccRCC clinical outcomes. Response to TKIs remains unpredictable in individual ccRCC patients. There are currently no validated markers to allow selection of patients destined to gain maximal clinical benefits. The studies proposed in this aim will address this unmet medical need by establishing the relationship between the expression levels of P2X7R isoforms and clinical outcomes providing prognostic and potentially predictive biomarkers to optimize treatment management of ccRCC patients.

The current application addresses the following FY22 KCRP Focus Areas:

*Identify and develop new strategies for screening, early-stage detection, and accurate diagnosis and prognosis prediction of kidney cancers, with examples including biomarkers and imaging.

*Develop novel therapeutic strategies for the treatment of kidney cancer, such as novel drug targets, therapeutic modalities and agents, treatment combinations and drug delivery systems.

Proposal Title:	PET Imaging of HIF-2a in Renal Cancer
Log Number:	KC220060
Current PI Name:	Xiankai Sun
Award Number:	HT9425-23-1-0903
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	09-02-2023

Despite extensive new therapies, most patients with metastatic renal cell carcinoma (RCC) do not reach the 5- year benchmark. Hypoxia inducible factor 2 alpha (HIF2alpha) is arguably the most important driver of clear cell RCC (ccRCC), but it was previously thought to be undruggable. This project is built upon an extraordinary journey from gene discovery to the recent approval by the U.S. Food and Drug Administration (FDA) of an HIF2alpha inhibitor, belzutifan, for patients with von Hippel-Lindau disease who require therapy for associated RCC, central nervous system hemangioblastomas, or pancreatic neuroendocrine tumors. Notably, this is a unique University of Texas Southwestern Medical Center (UT Southwestern) journey that started with the discovery of the HIF2alpha gene (EPAS1), which was followed by structural studies of the protein that recognized a vulnerability and then a chemical screen that identified small molecule inhibitors.

While HIF2alpha represents a core dependency, only about 50% of ccRCC patients benefit from HIF2alpha inhibitors and biomarkers to identify these patients are lacking. While circulating erythropoietin is controlled by HIF2alpha and has been used as a pharmacodynamic marker, erythropoietin downregulation by HIF2alpha inhibitors simply indicates that appropriate circulating levels of the drug have been achieved, and it does not speak to the tumor dependency on HIF2alpha. Importantly, our previous studies show that HIF2alpha-dependent tumors have higher levels of HIF2alpha expression than those that are resistant. Thus, we hypothesize that HIF2alpha levels may serve as a predictive biomarker of HIF2alpha inhibitor activity. However, measuring HIF2alpha levels in tissues is challenging. Indeed, RCC is well known for its heterogeneity. Furthermore, it has been shown that multiple biopsies are necessary to comprehensively sample a tumor. This is, however, not feasibly clinically and alternatives to evaluate potentially predictive biomarkers, such as HIF2alpha, are urgently needed. A viable approach may involve molecular imaging. Imaging approaches would also have the advantage of offering noninvasive, quantitative, and real-time measurements of HIF2alpha levels across sites of disease.

We have been successful in the generation of an [18F]PT2385 radiotracer that is identical to PT2385, a first-generation HIF2alpha inhibitor, and obtained an investigational new drug approval (IND156933) from the FDA and opened a clinical trial (NCT04989959). To date, we have completed seven positron emission tomography (PET) scans with [18F]PT2385 in human subjects, which showed the high net influx of the radiotracer in 2/3 of HIF2alpha positive tumors demonstrating the promising potential of deriving HIF2alpha inhibitors to develop radiotracers for HIF2alpha imaging. However, the tumor uptake was limited likely due to rapid metabolism of the radiotracer (PT2385 glucuronidation occurs in humans but not in mice). While [18F]PT2385 may find applications to evaluate the biliary tree, which represents an unmet medical need, in this project we propose to develop an optimized radiotracer to image HIF2alpha using a second-generation HIF2alpha inhibitor, PT2977, which was developed, precisely, to escape glucuronidation in humans. In this project, we have proposed a practical synthetic route to [18F]PT2977 based on our established experience in developing [18F]PT2385. To evaluate the proposed imaging of HIF2alpha, we will perform a set of experiments with [18F]PT2977 in mouse models using RCC tumorgraft lines expressing high and low HIF2alpha levels to validate the anticipated correlation between PET signal readout and expression of HIF2alpha. Radiation dosimetry will be assessed by using bio-distribution along with the clearance data. Later, we will follow the same procedures as we did with [18F]PT2385 to validate the production reliability and reproducibility for an FDA IND application towards the translational studies of [18F]PT2977.

If successful, this project will yield a noninvasive, quantitative, and real-time imaging method to query HIF2alpha expression in ccRCC tumor for both efficacious HIF2alpha-targeted therapy and clinical evaluation of tumor resistance to therapies with HIF2alpha inhibitors. Notably, this method can be also used to identify other tumor types that may similarly be HIF2-dependent. We believe the method developed in this project may become transformative not only in oncology, but also in other diseases such as cardiac ischemia or strokes. Given the fact that both oncologists and cancer patients have become more and more acceptive to theranostic methods (therapeutic + diagnostic) thanks to the great success of prostate-specific membrane antigen (PSMA) targeted radiopharmaceuticals in prostate cancer, we believe it will not take more than 5 years for our proposed theranostic HIF2alpha method to achieve a clinically relevant outcome after we obtain FDA's IND approval of clinical trials with [18F]PT2977.

FY22 KCRP Focus Area: Identify and develop new strategies for screening, early-stage detection, and accurate diagnosis and prognosis prediction of kidney cancers, with examples including biomarkers and imaging.

Proposal Title:	PET Imaging of HIF-2a in Renal Cancer
Log Number:	KC220060P1
Current PI Name:	James Brugarolas
Award Number:	HT9425-23-1-0904
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	09-02-2023

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FY22 KCRP Focus Area: Identify and develop new strategies for screening, early-stage detection, and accurate diagnosis and prognosis prediction of kidney cancers, with examples including biomarkers and imaging.

Proposal Title:	Peripheral Systemic Response Assessment in RCC
Log Number:	KC220067
Current PI Name:	Kathryn Beckermann
Award Number:	HT9425-23-1-0921
Current Contracting Organization:	Vanderbilt University Medical Center
Current Performing Organization:	Vanderbilt University Medical Center
Web Approval Date:	09-02-2023

There are no biologic methods currently available to predict or monitor response to standard systemic treatment for patients with kidney cancer. Our prior research showed that the nutrition and energy metabolism of T cells are necessary for patients to respond to immune based therapies and control cancer cells. We found that T cells from patient’s kidney tumors were exhausted and lacked activity but that these T cells could renew their function and activity to enable tumor cell killing after energy pathways such as glucose and mitochondrial metabolism were increased. During these studies we found T cells in the blood of the same patients that showed similar exhausted characteristics and altered metabolism. Because blood T cells are readily accessible and may provide a marker for antitumor immunity, we next examined blood of kidney cancer patients receiving standard of care immune therapy treatments for changes to T cell populations. Preliminary data suggests T cells subsets defined by metabolic and exhausted characteristics changed during treatment and may correlate to a patient's response on therapy.

This proposal seeks to understand if measuring the metabolic characteristics of T cells in the peripheral blood of patients can reflect tumor experienced T cells and may serve as noninvasive method to correlate with how a patient may respond to immune therapy. The work proposed in this grant will touch upon multiple KCRP Focus areas including (1) conducting basic biologic research to further our understanding of kidney cancer, (2) to identify and develop novel methods to understand and predict response to treatment with biomarkers.

The proposed work to understand peripheral blood immune metabolism as it relates to the tumor immune microenvironment and response to immune therapy may lead to a noninvasive liquid biopsy that would serve as a reflection of a patient's tumor and correlate to response seen in immune based therapy. While the focus of this work is on patients with clear cell type kidney cancer, it is possible that understanding immune cell changes in the peripheral blood will be more broadly applicable across different histologic types of cancer. We believe these experiments could be directly translatable into clinical trials for prospective validation of findings with a goal of informing patient care in the short term. This grant application has several innovative approaches including use of immune metabolism as a reflection of the tumor immune microenvironment and use of liquid biopsy capturing response to systemic immune therapy.

Proposal Title:	Mechanisms and Predictors of Adoptive Cytokine-Induced Memorylike NK Cell Therapy in Renal Cell Carcinoma
Log Number:	KC220072
Current PI Name:	Toni Choueiri
Award Number:	HT9425-23-1-0477
Current Contracting Organization:	Dana-Farber Cancer Institute
Current Performing Organization:	Dana-Farber Cancer Institute
Web Approval Date:	09-02-2023

Despite recent advances in treatment, metastatic clear cell kidney cancer (ccRCC) remains fatal for most patients. Natural killer (NK) cells are an important part of the body's natural immune defenses against cancer. Prior experiments have shown that among patients with clear cell kidney cancer, decreasing NK cell function is linked with kidney cancer progression and metastasis. Restoring the function of NK cells might be a promising treatment strategy. In prior work, we have shown that NK cells taken from patients can be treated in the lab with cytokine proteins, which causes them to change into cytokine induced memory-like NK (CIML-NK) cells.

These CIML-NK cells have improved survival, improved ability to grow and persist, and improved ability to kill cancer cells. We have previously shown that CIML-NK cells can lead to tumor regression in patients with myeloid leukemia and head and neck cancer. We are conducting a clinical trial to treat patients with ccRCC with CIML-NK cells. Using samples from these patients, the goal of this project is to understand how CIML-NK cell treatment works in patients. This will then help us improve the treatment for future patients.

In our first aim, we will perform a deep analysis and comparison of NK cells collected prior to treatment, after cytokine treatment (to generate CIML-NK cells), and then collected from the patient after treatment. We will assess whether the treatment caused the NK cells to survive longer and whether there are changes in the NK cells' protein and gene expression. We will test these NK cells, collected at different time points, in the lab to see whether our treatment strategy improves the NK cells' ability to kill cancer cells. We will also analyze biopsies taken from patients to understand what effects the treatment is having on tumor cells and the tumor environment in the actual patient. Overall, this first aim will help us ensure that the CIML-NK treatment protocol is having the intended effects on patients and give us a better understanding of how these cells work in patients with ccRCC.

In our second aim, we will look for signals that can indicate successful treatment, and examine potential reasons why treatment may or may not work for individual patients. This will be done by comparing various measurements (amount of NK cells collected and infused, changes in cytokine protein levels in the blood, changes in levels of other immune cells in the blood and tumor environment, changes in circulating tumor genetic material) and seeing if these are associated with the response to treatment. In patients who do not have a good response to treatment, we will check if the tumor is causing changes that cause the CIML-NK cells to be evaded or inactivated. Overall, this second aim will help us to personalize treatment to individual patients and potentially anticipate and overcome cancer resistance to treatment.

The goal of the project as a whole is to help create a new treatment option for patients with clear cell kidney cancer. If successful, this will help patients to live longer and have fewer symptoms from their cancer. Since this is a new type of treatment, we seek to understand in a detailed way how the body responds to treatment, what effect the treatment has on tumors, and what factors might be associated with patients whose cancers respond versus do not respond.

This research could directly benefit patients depending on the results of our clinical trial. If many responses to treatment are seen, we could move forward into larger clinical trials in the next 1 to 2 years. If few responses are seen, this project would allow us to make adjustments to future protocols that can better choose which patients are most likely to benefit from this treatment. We would also better understand how to make changes to the protocol to overcome resistance to treatment.

While the focus of our study will be on CIML-NK cell treatment, this will also improve our understanding of NK cell biology in kidney cancer in general. In the future, the scientific data generated from this study could also prove helpful in the development of other NK cell treatments including for example chimeric antigen receptor (CAR) NK cells.

Proposal Title:	Mechanisms and Predictors of Adoptive Cytokine-Induced Memorylike NK Cell Therapy in Renal Cell Carcinoma
Log Number:	KC220072P1
Current PI Name:	Rizwan Romee
Award Number:	HT9425-23-1-0478
Current Contracting Organization:	Dana-Farber Cancer Institute
Current Performing Organization:	Dana-Farber Cancer Institute
Web Approval Date:	09-02-2023

Despite recent advances in treatment, metastatic clear cell kidney cancer (ccRCC) remains fatal for most patients. Natural killer (NK) cells are an important part of the body's natural immune defenses against cancer. Prior experiments have shown that among patients with clear cell kidney cancer, decreasing NK cell function is linked with kidney cancer progression and metastasis. Restoring the function of NK cells might be a promising treatment strategy. In prior work, we have shown that NK cells taken from patients can be treated in the lab with cytokine proteins, which causes them to change into cytokine induced memory-like NK (CIML-NK) cells.

These CIML-NK cells have improved survival, improved ability to grow and persist, and improved ability to kill cancer cells. We have previously shown that CIML-NK cells can lead to tumor regression in patients with myeloid leukemia and head and neck cancer. We are conducting a clinical trial to treat patients with ccRCC with CIML-NK cells. Using samples from these patients, the goal of this project is to understand how CIML-NK cell treatment works in patients. This will then help us improve the treatment for future patients.

In our first aim, we will perform a deep analysis and comparison of NK cells collected prior to treatment, after cytokine treatment (to generate CIML-NK cells), and then collected from the patient after treatment. We will assess whether the treatment caused the NK cells to survive longer and whether there are changes in the NK cells' protein and gene expression. We will test these NK cells, collected at different time points, in the lab to see whether our treatment strategy improves the NK cells' ability to kill cancer cells. We will also analyze biopsies taken from patients to understand what effects the treatment is having on tumor cells and the tumor environment in the actual patient. Overall, this first aim will help us ensure that the CIML-NK treatment protocol is having the intended effects on patients and give us a better understanding of how these cells work in patients with ccRCC.

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The goal of the project as a whole is to help create a new treatment option for patients with clear cell kidney cancer. If successful, this will help patients to live longer and have fewer symptoms from their cancer. Since this is a new type of treatment, we seek to understand in a detailed way how the body responds to treatment, what effect the treatment has on tumors, and what factors might be associated with patients whose cancers respond versus do not respond.

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While the focus of our study will be on CIML-NK cell treatment, this will also improve our understanding of NK cell biology in kidney cancer in general. In the future, the scientific data generated from this study could also prove helpful in the development of other NK cell treatments including for example chimeric antigen receptor (CAR) NK cells.

Proposal Title:	Leveraging SCARB1 Overexpression for the Treatment of ccRCC with Low-Density Lipoprotein Nanocarriers
Log Number:	KC220076
Current PI Name:	Ian Corbin
Award Number:	HT9425-23-1-0866
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	09-02-2023

Despite current advances in new molecular and immunotherapy, the 5-year survival rate for advanced kidney cancer remains around 12%. This finding clearly highlights an urgent need for novel treatments. The most common form of kidney cancer exhibits a unique metabolic derangement encompassing an addiction to cholesterol where copious amounts of this lipid is needed for the cancer cell's survival. To date this is an understudied area in kidney cancer biology. N o other research teams have tried to leverage this deviant metabolic activity for therapeutic purposes against kidney cancer.

The focus of the current Kidney Cancer Research Program Idea Development Award is to exploit the overactive cholesterol uptake pathway in kidney cancer cells using an engineered cholesterol carrier (low-density lipoprotein (LDL)) that is reformulated with the natural omega-3 fatty acid called docosahexaenoic acid (DHA). The LDL-DHA formulation has previously been shown to kill tumors cells without injury to normal cells. For the current grant application we anticipate the LDL-DHA formulation will be cytotoxic to kidney tumors, while being non-injurious to the normal kidneys. As such, this grant proposal directly addresses the FY22 KCRP Focus Areas to "Develop novel therapeutic strategies for the treatment of kidney cancer, such as novel drug targets, therapeutic modalities and agents, treatment combinations, and drug delivery systems."

The proposed grant will explore the following specific aims: First we will investigate the binding, uptake, and cytotoxicity of the LDL- DHA formulation against a panel of normal and malignant kidney cells.

Secondly, we will develop a rat model of kidney cancer to assess LDL-DHA drug delivery kinetics, safety and short term anticancer effects.

Lastly, we will compare effectiveness of LDL-DHA vs current front-line treatment, sunitinib, to provide sustained long term antitumor control in the rat model of kidney cancer.

This work is highly innovative in that the LDL-DHA treatment consists of completely natural components at are well tolerated by the body, thus minimizing the likelihood for adverse effects. At the completion of these studies we will have demonstrated that targeting the abnormal cholesterol uptake pathway in kidney tumors is a viable strategy for treating this cancer; LDL-DHA is an effective new treatment against kidney cancer that is safe and actually provides health benefits to the normal kidneys. Novel therapies like LDL-DHA that demonstrate selective tumor toxicity and kidney protective properties are critical for the management of high-risk kidney cancer patients who have underlying conditions like kidney disease. LDL-DHA can be a viable treatment option for difficult to treat patients who have few therapeutic options. Ultimately, it is our goal to bring this technology to the kidney cancer patients where it is anticipated to provide a safe and efficacious approach to managing this aggressive cancer.

Proposal Title:	Multiplexed Quantification of Urinary Biomarkers for Noninvasive Classification of Imaged Kidney Tumors
Log Number:	KC220085
Current PI Name:	Srikanth Singamaneni
Award Number:	HT9425-23-1-0996
Current Contracting Organization:	Washington University in St Louis
Current Performing Organization:	Washington University in St Louis
Web Approval Date:	09-02-2023

According to the National Cancer Institute, more than 79,000 new cases of kidney cancer are diagnosed annually, and almost 14,000 patients die. Nationally, kidney cancer is more common than leukemia and pancreatic cancer in men, and ovarian and pancreatic cancer in women, and it now accounts for almost 4% of adult cancers. Unfortunately, kidney cancer is 5-6 times greater in the military than the civilian population. There is currently no approved method for widespread assessment for kidney cancer. Providing proper care is predicated on the detection of kidney tumors either incidentally through abdominal imaging or by means of screening at-risk groups. Radiologic screening is impractical and expensive and, even if a kidney mass is detected, CT imaging cannot reliably differentiate malignant from benign tumors.

The objective of this project is to develop a simple urine test to: (1) rule in the presence of malignant kidney cancer in unsuspecting patients; (2) rule out malignant kidney cancer through pre-surgical differential diagnosis of benign from malignant CT-imaged kidney masses, all to guide and inform physicians for appropriate treatment.

Our laboratory has been instrumental in identifying the first-ever urine biomarkers for the non-invasive diagnosis of metastatic kidney cancer and in developing highly sensitive and specific nanotechnology-based assays for various biodiagnostic applications. In addition to the urine protein biomarkers that we have identified in the past, our collaborators have recently identified novel biomarkers of metastatic and benign tumors of the kidney. Over the next 3 years, we propose to combine kidney cancer biomarker assays with ultrasensitive fluorescence-based biosensors introduced by our lab to fully develop a rapid informative urine test to identify metastatic and benign kidney tumors. We will develop technology to simultaneously measure the concentration of five proteins in urine using an ultrabright nanostructure developed in our lab. We will measure protein biomarkers in the urine identified as diagnostic of metastatic or benign kidney tumors of patients with CT-imaged kidney masses, and compare results to the urine of demographically matched control individuals. Based on the concentrations of five proteins in the urine, we will develop a formula to accurately classify the tumors as benign or malignant. Our study will help fulfill the unmet need of a sensitive and specific diagnostic assay to non-invasively differentially diagnose imaged kidney masses or to identify individuals in at-risk groups who harbor a silent kidney tumor. Our study will rule in or rule out metastatic kidney cancer and guide pre-surgical decisions to best tailor treatment.

This technology would enable non-invasive diagnosis of kidney cancer and avoid a costly and risky invasive needle biopsy of tumors that were incidentally discovered by imaging of patient abdomens. While developing a test to evaluate kidney tumors discovered by CT-imaging, our study will set the stage for development of a non-invasive diagnostic screening test to identify individuals in at risk populations harboring asymptomatic kidney tumors. If successful, this project will establish a novel diagnostic assay to help identify people with a devastating disease that severely impacts individuals and families both financially and emotionally within the military and civilian populations. Funding for this project would put the Department of Defense in position to make this test available to help people both in the military and civilian sectors, and to reduce medical costs and risks associated with kidney tumors.

By using a non-invasive urinary biomarker test as a first-line diagnostic tool to molecularly diagnose an imaged kidney mass, we may be able to avoid both surgical overtreatment of small kidney tumors and overextending health care budgets. Avoiding surgery of benign small tumors of the kidney could significantly decrease patient morbidity, loss of productivity, and risk of developing or exacerbating chronic kidney disease from functional kidney loss; all of benefit to the military and civilian populations. Unfortunately, the default clinical approach of removing all kidneys with a tumor, in order not to miss cancer, tragically removes a large percentage of otherwise normal kidneys because at least 20% of tumors are benign. It is becoming increasingly important to preserve as much functioning kidney as possible to avoid a future decline in overall kidney performance. Progressive loss of kidney function inexorably leads to non-malignant chronic kidney disease only mitigated by expensive dialysis or kidney transplant with an attendant reduction in the quality of life. The availability of a reliable test for kidney cancer would help reassure patients with a kidney tumor that they are not losing a portion of or even a whole good kidney.

Proposal Title:	Multimodal AI-Based Renal Cancer Patient Care
Log Number:	KC220086
Current PI Name:	Christopher Weight
Award Number:	HT9425-23-1-0918
Current Contracting Organization:	Cleveland Clinic Foundation
Current Performing Organization:	Cleveland Clinic Foundation
Web Approval Date:	09-02-2023

Kidney cancer is considered one of the most common cancers in the United States (approximately 80,000 new cases per year) and worldwide (450,000 new cases per year) and one of the most rapidly increasing cancers in American adults between 25 and 50 years old. Most kidney cancers, if caught early, can be treated and cured with surgery, however in about 30%-40% of patients, the cancer will metastasize. When kidney cancer spreads to other organs, about 85% of those patients will die within 5 years. Fortunately, most of the recent increase in kidney tumors have been diagnosed as small renal masses (tumor size less than 4 cm), which often turn out to be either benign (30%) or indolent (50%). However, with existing tools in clinical practice, it is difficult to sort out which kidney tumors are dangerous and which tumors can be left alone. This has led to overdiagnosis and over-treatment in recent years with little reduction in the number of people dying from kidney cancer. It is estimated that tens of thousands of patients each year may undergo surgery for a concerning kidney tumor that turns out to be benign or indolent, leading to an estimated national cost of $153 million ($55,573/individual) on kidney cancer treatments that may not help a patient live longer or better.

Unfortunately, doctors often cannot differentiate between a benign kidney mass and kidney cancer on the patient's preoperative imaging. Biopsy for diagnosis of the kidney mass is not routinely used by most physicians, as it is an invasive procedure with a risk of complications and is sometimes inaccurate or non-conclusive. Thus, we aim to develop reliable, accurate, non-invasive tools that use artificial intelligence (AI) to evaluate patient information, including medical images, blood tests and other details like age, gender, etc., to accurately differentiate the benign or indolent masses from the potentially aggressive tumors in the preoperative setting. Our proposed tools will fill in this missing knowledge gap to assist the doctor and patient in choosing personalized treatment, and we will compare them in terms of time, efficiency, and accuracy to existing clinical tools.

Current treatments for kidney cancer can be as significant a threat to patient outcome as the risk of a metastatic tumor. In the case of early kidney cancer, the main non-cancer related risk is the loss of kidney function during partial or total kidney removal surgery to take out the tumor. It is important to be able to predict what the kidney function will be after the surgery to help plan whether to remove all or part of the kidney when removing the tumor. We aim to create an automated tool using AI and preexisting information that all kidney tumor patients have collected during routine care, a patient's baseline kidney function plus preoperative computed tomography (CT) scans to accurately estimate kidney function. If we are successful, patients and doctors will have more accurate ways to predict kidney function so they can balance cancer and non-cancer risks and personalize the decision to remove all or just part of the kidney when operating on kidney tumors.

To help advance the field rapidly, we will make some of our models and data publicly available so researchers around the world can evaluate our work, generate their own models from our data, and build on it and implement it in practice. When we complete our project, some patients can experience clinical benefit immediately, as the tool will be validated and ready to implement in the clinic. If successful, it will serve to increase confidence in detecting benign or indolent kidney masses preoperatively, potentially sparing thousands of patients from unnecessary surgery and treatment. Instead, patients with benign/indolent masses will be safely watched, potentially saving more than $150 million dollars and sparing patients the

complications of unnecessary interventions. Furthermore, patients with malignant kidney tumors will have more accurate information about anticipated cancer risk and kidney function outcomes, which will allow a more personalized approach to treatment of their kidney tumor.

Proposal Title:	Multimodal AI-Based Renal Cancer Patient Care
Log Number:	KC220086P1
Current PI Name:	Michal Rosen-Zvi
Award Number:	HT9425-23-1-0919
Current Contracting Organization:	IBM Thomas J Watson Research Center
Current Performing Organization:	IBM Thomas J Watson Research Center
Web Approval Date:	09-02-2023

Kidney cancer is considered one of the most common cancers in the United States (approximately 80,000 new cases per year) and worldwide (450,000 new cases per year) and one of the most rapidly increasing cancers in American adults between 25 and 50 years old. Most kidney cancers, if caught early, can be treated and cured with surgery, however in about 30%-40% of patients, the cancer will metastasize. When kidney cancer spreads to other organs, about 85% of those patients will die within 5 years. Fortunately, most of the recent increase in kidney tumors have been diagnosed as small renal masses (tumor size less than 4 cm), which often turn out to be either benign (30%) or indolent (50%). However, with existing tools in clinical practice, it is difficult to sort out which kidney tumors are dangerous and which tumors can be left alone. This has led to overdiagnosis and over-treatment in recent years with little reduction in the number of people dying from kidney cancer. It is estimated that tens of thousands of patients each year may undergo surgery for a concerning kidney tumor that turns out to be benign or indolent, leading to an estimated national cost of $153 million ($55,573/individual) on kidney cancer treatments that may not help a patient live longer or better.

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complications of unnecessary interventions. Furthermore, patients with malignant kidney tumors will have more accurate information about anticipated cancer risk and kidney function outcomes, which will allow a more personalized approach to treatment of their kidney tumor.

Proposal Title:	Interrogating the Therapeutic Relevance of Targeting the Antiapoptotic BCL-XL Protein in Kidney Cancer
Log Number:	KC220095
Current PI Name:	Abhishek Chakraborty
Award Number:	HT9425-23-1-0771
Current Contracting Organization:	Cleveland Clinic Foundation
Current Performing Organization:	Cleveland Clinic Foundation
Web Approval Date:	09-02-2023

Significance: Kidney cancer is among the top 10 most common forms of human cancer. This disease not only affects the general public, but also has a negative impact on military personnel and their families. The kidney is one of the major detoxifying organs in our body and exposure to environmental toxins, either during conflict or due to contaminated water sources around military bases, leads to direct harm to the kidney tissue. Lifestyle risk factors, including obesity and smoking, also negatively impact defense personnel. Together, these environmental and lifestyle factors predispose military personnel to an increased risk of kidney cancer. Despite major breakthroughs in the clinical management of kidney cancer in recent years, this disease remains ultimately incurable. There is, therefore, a clear clinical need to identify novel drugs to treat kidney cancer.

Kidney cancer is stubbornly non-responsive to traditional chemotherapy, virtually eliminating the possibility of treatment with a large class of molecules that work against other cancers. This is particularly surprising because the cellular machinery that triggers cell death in response to chemotherapy is normally intact in kidney cancer cells. Why then do these tumors not die? And could we reprogram these cells to begin dying?

Tumor cells constantly make "life" or "death" decisions in response to environmental cues. And, typically, even when faced with adversity that would prompt a normal cell to mount suicidal "cell death" responses, tumor cells choose "life". Simply put, tumor cells are like revved-up cars facing the edge of a cliff, but protected by the action of some "brake" proteins. Making these "brakes" fail could be a therapeutic strategy.

To identify these "brake" proteins, we exploited recent large-scale studies that have measured the impact of eliminating virtually every known protein on the growth of cancer cells. Our analysis identified BCL-XL, as a new "brake" protein in kidney cancer. Indeed, we found that eliminating functional BCL-XL triggers significant cell death in a number of kidney cancer cells. We argue, in this proposal, that turning BCL-XL off eliminates the cell's protective shield, causing them to die. This could be a novel usable therapeutic strategy in kidney cancer.

Focus Area: Develop novel therapeutic strategies for treatment.

Hypothesis and Objective: We hypothesize in this proposal that kidney cells are protected from cell death by the BCL-XL "brake" and propose the use of BCL-XL blockers as new drugs in kidney cancer therapy.

Specific Aims: We have distilled this project into three aims. First, we propose to study a large collection of (age- and sex-matched) human kidney tumors to catalog their relative sensitivity to BCL-XL blockers. Based on this, we propose to identify "biomarkers" -- genes whose presence/absence can faithfully predict if a given tumor will respond to anti-BCL-XL therapy. [A "biomarker" is any easily measurable feature that can faithfully predict a clinical pathology or response. For example, the well-known A1C test is a "biomarker" for blood glucose monitoring in diabetic patients.]

Second, we propose to combine BCL-XL blockade with existing therapies in kidney cancer. We reason that removing the protective BCL-XL "brake" would send kidney cancer cells hurtling toward (and maybe even over the) edge of the cliff. At least, we hope that BCL-XL blockers will make kidney tumors vulnerable to other therapies, such as rapalogues and immunotherapy. Finally, we propose to use cutting-edge CRISPR /Cas9 tools to find ways to make kidney tumors more responsive to anti-BCL-XL therapy. Altogether, our proposal not only addresses the biological role of the BCL-XL "brake" protein in kidney cancer, but also interrogates approaches that can accelerate the use of anti-BCL-XL therapy in the clinic.

Innovation and Impact: Kidney cancer accounts for approximately 79,000 new diagnoses and approximately 14,000 deaths annually and the therapeutic outlook for patients with advanced kidney cancer remains poor. Our proposal is novel because it demonstrates for the first time that BCL-XL blockade could promote the killing of kidney cancer cells. Yet, this alone is not sufficient to make our approach clinically usable. In this proposal, we line up a set of rigorous studies, which include the development of diagnostic tests and the use of therapeutic combos with BCL-XL blockade, to establish the long term clinical utility of our approach. Notably, BCL-XL blockers are already being clinically tested in other cancers, and can be rapidly repurposed for use against kidney cancer. Therefore, if we succeed, a clinical transition by repurposing existing BCL-XL blockers can be achieved rapidly.

To ensure success in our goals, we have put together a well-balanced team of scientists and clinicians who are committed to improving outcomes in kidney cancer patients, and see through the development of this idea. In summary, we anticipate that this proposal will lay the foundations for the future clinical analysis of BCL-XL's role in human kidney tumors and support our long-term vision to establish the use of BCL-XL blockers as a new therapeutic strategy in kidney cancer. Altogether, this could have a profound impact on the clinical outcomes of the general public and the military personnel burdened by kidney cancer.

Proposal Title:	A Safe, Small-Molecule Approach to Prevent Kidney Cancer Metastasis
Log Number:	KC220097
Current PI Name:	Lily Wu
Award Number:	HT9425-23-1-0858
Current Contracting Organization:	California, University of, Los Angeles
Current Performing Organization:	California, University of, Los Angeles
Web Approval Date:	09-02-2023

Background/Rationale: Kidney cancer is the seventh leading cancer in the United States. Each year, more than 70,000 Americans are diagnosed with kidney cancer. Clear cell renal cell carcinoma (ccRCC) is the most common subtype, accounting for 80% of all cancers of the kidney. Patients with ccRCC tumor confined to the kidney have favorable outcome with 5-year survival of 75%-80% after surgical removal. However, 40% of ccRCC patients are at risk of developing disseminated disease (metastasis) that most frequently spreading to the lung. Patients who develop lung metastasis will have a very poor outcome with median survival of only 7 months. There are several likely reasons for poor outcome of metastatic ccRCC. First, the current treatments for metastatic ccRCC are unable to achieve a complete and durable response. Numerous new promising treatments, in the form of a combination of multiple drugs, are currently under investigation in multiple clinical trials. The efficacy, the appropriate utilization, and the potential treatment related side effects of the multitude of combination treatments will be challenging to determine that will require extensive time, efforts and cost. A factor that contributes to the inadequate treatment of metastatic ccRCC is the lack of in-depth understanding of the biology of metastasis as it is a disease that evolves over time that is difficult to study in patients. There is also a lack of clinically relevant mouse models of metastatic ccRCC.

Research Approaches: We discovered that ccRCC tumors from patients commonly contain two different cell populations (i.e., VHL(-) and VHL(+) cells). Mouse models using a mixture of these two types of tumor cells revealed that the cross- communication between the two populations dictates the metastatic process, and the VHL(-) cells are the metastatic drivers. Based on this novel metastatic model, we initiated an unbiased drug screening approach to select drugs that would selectively inhibit VHL(-) cells. We identified 9 hits from a 2,500 library of U.S. Food and Drug Administration (FDA)-approved drugs. Remarkably, four out of the nine hits all belongs to a family of commonly used drugs used to prevent cardiovascular diseases. We further verified that these hits are able to inhibit tumor metastasis in animal models. The key goal of this KCRP project is to further develop these promising small molecule drugs to create them as novel potent metastasis blockers. The three research approaches to achieve this goal are threefold. First, we will determine the cellular target of our lead compounds by genetic and proteomic experiments. Second, we will take on a medicinal chemistry approach to improve the potency of our leads by modifying the chemical structure of the hits. Third, we will validate the activity of the optimized lead drugs and test their therapeutic efficacy in animal models of mRCC. We will also verify the safety of these lead drugs in mice. The proposed research will address two of the fiscal year 2022 Kidney Cancer Research Program Focus Areas, namely (i) conducting basic biology research to understand cancer progression and metastasis, and (ii) to develop a new therapeutic strategy to treat metastatic disease.

Innovation: This project is unique and innovative in many aspects. First, this line of investigation is based on several preclinical metastatic models of RCC we developed that informed on an entirely new mechanism of metastasis. Furthermore, the hit compounds identified thus far belong to a class of drugs that has not been used to treat RCC or metastatic ccRCC in the past. Given the four hits identified belong to a class FDA-approved drug taken orally for a long time to prevent heart disease, we anticipate new derivatives of the hits

developed from this project might likewise be safe for long term use. With the novel concept and a great number of valuable resources at hand, we are well-situated to advance the development of a new class of safe, small molecule drugs to prevent the lethal metastatic stage of kidney cancer.

The long-term benefit is to offer a safe and effective treatment to prevent and treat metastatic ccRCC. Currently, the treatment for metastatic disease is inadequate, resulting in the very poor outcome of metastatic ccRCC. Hence, this project holds a great promise in addressing a clear unmet need in the field and improve the outlook for patients with metastatic ccRCC.

Proposal Title:	Targeting Branched Chain Amino Acids in Kidney Cancer
Log Number:	KC220099
Current PI Name:	Marie Simon
Award Number:	HT9425-23-1-0859
Current Contracting Organization:	Pennsylvania, University of
Current Performing Organization:	Pennsylvania, University of
Web Approval Date:	09-02-2023

Kidney cancer is one of the 10 most prevalent malignancies in the world. In 2022, approximately 79,000 new cases and 13,920 deaths due to renal cancer will occur in the United States, with >430,000 new cases diagnosed worldwide. There are multiple subtypes of kidney cancer, each characterized by distinct histological appearances, clinical courses, and therapeutic responses. Clear cell renal cell carcinoma (ccRCC) is the most common, accounting for greater than 75% of all diagnoses. Because of obesity and an aging population, the incidence of ccRCC has steadily risen over the last decade. If ccRCC is detected early and can be surgically resected, 5-year survival rates are relatively favorable compared to many cancers. However, metastatic disease has a catastrophic 5-year survival rate of 10%-12%. At that stage, treatments are sometimes ineffective due to the established resistance of ccRCC to conventional forms of multiple chemotherapies and radiation. Beyond the use of certain cytotoxic drugs, the ccRCC treatment arsenal includes targeted therapies and immunotherapies. Each of these have proven to be somewhat effective, but only in a subset of patients. This lack of consistently effective therapies, particularly in metastatic ccRCC, highlights an urgent need for developing new targets, which could be beneficial to a broader range of ccRCC patients. The overall goal of our proposal is to identify novel treatment strategies that would ultimately benefit most, if not all, ccRCC patients. We have found highly consistent metabolic changes in renal tumors compared to healthy kidney and hope to exploit these with novel approaches.

As such, this Kidney Cancer Research Program (KCRP) Idea Development Award application addresses several KCRP Overarching Strategic Goals to (1) increase understanding of kidney cancer biology; (2) develop novel therapeutic strategies for the treatment of kidney cancer; and (3) improve patient care for kidney cancer. The highly innovative aspects of this proposal are its in-depth evaluation of altered branched chain amino acid metabolism typical of ccRCC tumors detected in patients, and revelation of specific enzymes in these pathways as novel druggable targets. We hope to inhibit key metabolic steps in kidney tumors in a way that spares healthy tissue. We propose developing new compounds for ccRCC, and ultimately testing them in individuals with both localized disease and distant metastases in the lung, brain, and bones. Overall, the proposed studies endeavor to rapidly expand treatment options to patients at all stages of disease, especially those that fail other options currently in the clinic.

Proposal Title:	Engineered Neutrophils for Intratumoral Delivery and Targeting
Log Number:	KC220106
Current PI Name:	Craig Lefort
Award Number:	HT9425-23-1-0824
Current Contracting Organization:	Rhode Island Hospital
Current Performing Organization:	Rhode Island Hospital
Web Approval Date:	09-02-2023

Therapies that modulate the immune system have shown great promise for treatment of cancer in the past decade. However, there are still barriers to overcome to realize their full potential against solid tumors, including renal cell carcinoma (RCC). The major hurdle to overcome is that tumor cells are able to produce factors that thwart the immune cells that these therapies target. In our proposed studies, we investigate the potential for using a specific type of innate immune cell called a neutrophil as a vehicle to deliver factors that re-activate the cancer fighting activities of immune cells within the tumor. Our proposed research will address the following KCRP Focus Area: "Develop novel therapeutic strategies for the treatment of kidney cancer, such as novel drug targets, therapeutic modalities and agents, treatment combinations and drug delivery systems."

Neutrophils are the foot soldiers of the innate immune system and are typically present in abundance wherever inflammation occurs, including at tumor sites. Our research team has extensive expertise in studying neutrophils. We recently developed a precursor cell line that when injected into mice has the ability to take up residence in the bone marrow (where most immune cell development occurs) and produce large numbers of neutrophils. We call this new cell line a Neutrophil Progenitor (NP) and we have further shown that we can alter its activity using standard genetic engineering techniques. The discovery of NPs that can be maintained and modified in the laboratory opened up new avenues to investigate whether they can be altered so that they can treat disease. In our proposed study, we take advantage of the fact that neutrophils normally travel from the bone marrow to sites of RCC tumors. Our goal is to genetically modify NPs so that when are injected into tumor-bearing mice, they produce neutrophils that will travel to RCC tumors and release factors that directly and indirectly cause the tumor cells to die or prevent them from metastasizing.

If successful in an experimental animal model, future work will aim to translate our findings to humans and the eventual treatment of kidney cancer patients with a cell therapy based on NPs. Given the barriers that the RCC tumor presents to current therapies, this project has the potential for high impact to advance kidney cancer research.

Proposal Title:	Leveraging Biophysicochemical Motifs in T-Cell Receptor Antigen Binding Regions and Antigen Co-occurrence to Predict Response to Immune Checkpoint Inhibitors
Log Number:	KC220107
Current PI Name:	Lindsay Cowell
Award Number:	HT9425-23-1-0793
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	09-02-2023

Traditionally, patients with metastatic clear cell renal cell carcinoma have been treated with therapies that target the tumor blood vessels. More recently, immune-based therapies have emerged that have significantly changed the treatment and outcomes for our patients. Immune checkpoint inhibitors (ICIs) are drugs that use the body's immune system to fight diseases such as cancer. Today, patients with metastatic renal cell carcinoma are treated with a combination of drugs that involve ICI and/or drugs that target the tumor blood vessels. However, none of these treatments uniformly benefit all patients and many suffer from lot of side effects from these drugs. Different drugs target different molecular pathways and possibly benefit different groups of patients and therefore there is a need for markers that can tell us if the patient will respond to one group of drugs or not (ICI or those that target blood vessels). We also need to understand what induces the anti-tumor immunity, so we can develop new approaches to bring the benefits of ICI to more RCC patients.

Past studies have tried to identify patients that may respond to ICIs by correlating levels of a protein called PD-L1 on tumors to how patients respond to treatment. These studies have found only a minimal correlation between clinical outcomes and PD-L1 levels, and patients with no PD-L1 expression may also respond to the ICIs.

We and others have shown previously that tumors that have alterations in a gene called BAP1 tend to have more inflammation and respond better to ICI. This contrasts with tumors that have alterations in another frequently altered gene PBRM1. In this proposal, we will obtain sequencing data from immune cells in the tumors and integrate it with the sequencing data from the tumor cells. We have built a state-of-the-art model that we will apply to identify the unique properties of the immune cells present in tumors with BAP1 alterations and compare them with tumors with PBRM1 alterations. These results will enable us to understand the unique features of the immune cells that provide antitumor immunity and build a model to predict response to ICI.

If successful, our efforts will lead to the first integrated predictive biomarker in renal cell carcinoma. This will enable appropriate allocation of drugs to the patients who will show treatment benefit and not be given to those patients who will not benefit and thus minimize toxicity.

Proposal Title:	Leveraging Biophysicochemical Motifs in T-Cell Receptor Antigen Binding Regions and Antigen Co-occurrence to Predict Response to Immune Checkpoint Inhibitors
Log Number:	KC220107P1
Current PI Name:	Payal Kapur
Award Number:	HT9425-23-1-0794
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	09-02-2023

Traditionally, patients with metastatic clear cell renal cell carcinoma have been treated with therapies that target the tumor blood vessels. More recently, immune-based therapies have emerged that have significantly changed the treatment and outcomes for our patients. Immune checkpoint inhibitors (ICIs) are drugs that use the body's immune system to fight diseases such as cancer. Today, patients with metastatic renal cell carcinoma are treated with a combination of drugs that involve ICI and/or drugs that target the tumor blood vessels. However, none of these treatments uniformly benefit all patients and many suffer from lot of side effects from these drugs. Different drugs target different molecular pathways and possibly benefit different groups of patients and therefore there is a need for markers that can tell us if the patient will respond to one group of drugs or not (ICI or those that target blood vessels). We also need to understand what induces the anti-tumor immunity, so we can develop new approaches to bring the benefits of ICI to more RCC patients.

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If successful, our efforts will lead to the first integrated predictive biomarker in renal cell carcinoma. This will enable appropriate allocation of drugs to the patients who will show treatment benefit and not be given to those patients who will not benefit and thus minimize toxicity.

Proposal Title:	Targeting Immunosuppressive Adenosine to Enhance Vaccinia Virus Renal Cancer Oncolysis
Log Number:	KC220113
Current PI Name:	Jaime Merchan
Award Number:	HT9425-23-1-0839
Current Contracting Organization:	Miami, University of, Coral Gables
Current Performing Organization:	Miami, University of, Coral Gables
Web Approval Date:	09-02-2023

Rationale and Scientific Objective: Renal Cell Carcinoma (RCC) is expected to affect 79,000 Americans in the and cause about 14,000 deaths in 2022. While RCC can be cured in most cases by surgery when diagnosed and treated early, renal cell cancer recurs in about half of those patients. Due to its aggressive nature, spread to vital organs, and the resistant nature of the cancer, RCC is a fatal disease when advanced. While significant advances have been made in the understanding the biological basis of RCC, leading to improved treatment options (targeted therapies and immunotherapies) and survival, many patients with metastatic RCC patients eventually stop responding to treatment and succumb to their disease. Therefore, there is an urgent need to find new strategies to overcome resistance and cure renal cell cancer.

Oncolytic viruses are promising anticancer therapies, designed to preferentially target and kill tumor over normal cells, and by stimulating antitumor immune responses. Unfortunately, and as with other treatments, including immunotherapies, only a small percent of patients enjoy durable, complete responses from oncolytic virus treatments. The causes of the limited efficacy to these therapies are not fully understood but may be similar to those causing resistance to immunotherapies. Among the mechanisms responsible for limited treatment efficacy, the adenosine pathway plays a critical role. Adenosine, a by-product of ATP, is produced in tumors during hypoxia (low oxygen), tissue necrosis, cell death and inflammation, and often, because of anticancer treatments. Tumor adenosine causes treatment resistance by suppressing antitumor immune responses, limiting the action of the immune cells activated by viruses and immunotherapies. Therefore, targeting the adenosine pathway is a promising strategy to overcome resistance to immune and virus therapies.

Our long-term goals are to improve clinical outcomes and cure rates in advanced renal cell carcinoma by using oncolytic virus-based biotherapies. The objective of this application, which is a step toward achieving our long-term goals, is to investigate the role of, and target tumor adenosine to improve the efficacy of oncolytic viral therapies in preclinical models of renal cell carcinoma.

The FY21 KCRP Focus Area to be addressed is "Developing novel therapeutic strategies for the treatment of kidney cancer". To achieve our goals, we will use a novel oncolytic vaccinia virus, JX-594, which is currently being used in a clinical trial in RCC. This viral agent has shown safety in patients with RCC and early evidence of promising activity. As we will use mouse models of kidney cancer, we will use the mouse version of JX-594, to be able to mimic the effects we see in humans. Our experiments will test the hypothesis that vaccinia virus induces adenosine production in the tumors by increasing levels of CD39 and CD73, which mediate the production of adenosine, and that blocking adenosine will enhance the antitumor efficacy of the vaccinia virus based immuno-virotherapies.

Applicability of the research: Positive results from our research will advance the fields of oncolytic virotherapy and renal cell cancer, as they will validate the adenosine pathway as a target to improve oncolytic virus efficacy and clinical outcomes in RCC. Our studies can be applied to both clear cell and non-clear cell renal cell carcinoma patients because the virus has potent activity in both RCC subtypes

(demonstrated in preliminary data). They will generate the knowledge necessary for future studies investigating the effects of alternative viral vectors on the adenosine pathway and novel adenosine targeting strategies beyond those proposed by us. Moreover, our preclinical studies will provide a strong rationale for future clinical trials (in the next 2 to 3 years) in patients with metastatic RCC who have failed currently approved therapies. We envision that in the long term, the benefit of the proposed combinations will go beyond heavily pretreated RCC patients and may especially benefit patients who are treatment naive (first line) or in the second line setting. Importantly, our studies have the great potential to rapidly move from the laboratory to clinical trials, because the proposed agents to be tested in this study are clinically available, either as experimental agents (JX-594, adenosine pathway blockers) or already U.S. Food and Drug Administration-approved (anti-PD1 agents). In summary, the novelty, significance, and short-term applicability of our approach to thousands of patients with advanced RCC in the US and the world make this proposal highly impactful.

Proposal Title:	Purinergic Receptor Antagonist Therapeutics in Treating Metastatic Renal Cancer
Log Number:	KC220116
Current PI Name:	Jean Jiang
Award Number:	HT9425-23-1-0495
Current Contracting Organization:	Texas, University of, Health Science Center at San Antonio
Current Performing Organization:	Texas, University of, Health Science Center at San Antonio
Web Approval Date:	09-02-2023

Kidney cancer is the 8th most frequently diagnosed malignancy and it occurs almost twice as often in men than women. It is usually without symptoms in the early stages and is often not diagnosed until it is advanced. The biggest challenge facing renal cancer patients is recurrence and metastasis and a lack of effective therapies for advanced renal cell carcinoma (RCC). Clear cell RCC (ccRCC) is the most common subtype and advanced ccRCC has a poor prognosis, rapidly progressing to distant metastases leading to an extremely poor prognosis. ccRCC is typically non-responsive to traditional chemotherapy drugs and other treatment options are very limited. Treatment for ccRCC includes surgery, radiation therapy, immunotherapy (aldesleukin (Proleukin®)), and targeted therapy (sorafenib (Nexavar®) and temsirolilmus (Torisel®)). Newer generation immunotherapies represent a promising approach for ccRCC patients with the potential for a long duration of response, but many patients are not responsive to these treatments. Additionally, for patients who initially respond, the cancer will often eventually progress on treatment as the tumors develop resistance.

Studies by our research team and others show that the naturally occurring adenosine nucleotide has a cancer-promoting effect. This molecule binds to its receptors on the cell surface and increases cancer cell growth and migration. The data from our animal studies and other researchers shows that preventing binding of adenosine to these receptors decreases cancer growth and metastasis. Drugs that can block these receptors represent a new, novel therapy option for cancer treatment. We recently generated a group of molecules that target adenosine receptors and suppress breast cancer bone metastasis as compared to untreated animals. This treatment is highly effective even with less frequent administration. Our pilot study showed that these compounds also inhibit ccRCC cell growth and migration. The major objective of this idea development proposal is to identify a new drug candidate that is specific for this novel drug target for treating ccRCC with high efficacy and low toxicity. A specific Focus Area listed in the FY22 KCRP will be addressed: (1) Develop novel therapeutic strategies for the treatment of kidney cancer, such as novel drug targets, therapeutic modalities and agents, treatment combinations and drug delivery systems. In this study, we propose three specific aims. First, we will identify the lead molecules that reduce ccRCC cell growth, migration, and invasion in cell models via alteration of A2 purinergic pathways. Second, we will evaluate the molecules' effectiveness in animal models through the assessment of primary and metastatic ccRCC tumor growth and body immune responses. Third, we will select the compound with the best treatment profile and study its stability within the body and further validate its effectiveness using a mouse model with patient-derived ccRCC cells. These efforts will lead to a finalized drug candidate for the next stage of preclinical study and clinical trials.

The outcome from this Department of Defense Kidney Cancer Research Program drug development proposal will generate an entirely new class of therapies that target novel mechanisms with high efficacy and low toxicity. Globally, about 270,000 cases of kidney cancer are diagnosed annually, and 116,000 people die from the disease each year, including active-duty U.S. Service Members, retirees, Veterans, and their Family members. The drug developed in this proposal will offer enormous benefits to these patients with the reduction of kidney cancer-associated symptoms and improvement in survival rate.

The estimated time for the proposed project is 3 years. Within the 3 years, we will provide research data on the efficacy of our lead compounds in treating ccRCC growth and metastasis and improving antitumor immunity with various models. Moreover, we will provide important data containing pharmacological parameters of the selected drug candidate. In the latter part of year 3, we will move toward the finalization of the drug candidate for further investigation of drug properties, safety profile and toxicity, leading to the preparation of an Investigational New Drug (IND) application for regulatory approval and subsequent clinical trials.

Proposal Title:	Targeting Sema5B in RCC Immunotherapy
Log Number:	KC220120
Current PI Name:	Anirban Kundu
Award Number:	HT9425-23-1-0339
Current Contracting Organization:	Alabama, University of, at Birmingham
Current Performing Organization:	Alabama, University of, at Birmingham
Web Approval Date:	02-07-2023

The kidney removes wastes from our body and maintains a healthy balance of water, salts, and minerals. When we are exposed to toxins, our two kidneys help eliminate them from our bodies as well. Hence, the toxins, one point in time, travel through the kidneys increasing the chance of kidney cancer. Exposure to toxic chemicals while on base or during deployment can lead to kidney cancer among a great deal of Veterans. Not only Veterans but any other people can be diagnosed with kidney cancer in their lifetime for some known and unknown reasons. In the United States, kidney cancer is among the top ten malignancies in both men and women. Often the disease is diagnosed at an advanced stage when it spreads to the other organs (metastasis) leading to their death in 2-3 years. In recent years, immunotherapy seems to be a promising way to treat advanced-stage and metastatic kidney cancer patients. Immunotherapy is a type of cancer treatment that boosts the body's natural defenses (immunity) to fight cancer. Our body's immunity is maintained by many types of cells, called immune cells. The immune cells keep themselves from attacking normal cells in the body by "checkpoints". Kidney cancer cells sometimes use these checkpoints to avoid being attacked by the immune system. Immunotherapies are designed to inhibit the checkpoints so the immune cells can kill the cancer cells. Blocking checkpoints might lead the immune cells to kill our normal cells, as well, which is called autoimmunity, and this is even worse if the patients have preexisting autoimmune disorders. Moreover, kidney cancer cells develop unique mechanisms to develop resistance against these checkpoint inhibitors. In fact, only a small percentage of kidney cancer patients have benefited from immunotherapy, which urges researchers to develop highly specific and targeted immunotherapies for the disease. Semaphorins are the proteins found in many parts of our body where they serve many similar or different biological functions. There are many types of semaphorins in our system. Semaphorin 5B (Sema5B) is known to eliminate cell-to-cell communications in our nerve cells, thereby regulating wrong nerve signals. Interestingly, Sema5B level is highly elevated in kidney cancer cells compared to any other parts of our body. Immune cells must contact tumor cells to initiate their killing. Given the high Sema5B content in kidney cancer cells and its ability to prune cell-to-cell contact, the cancer cells will likely avoid contacting immune cells, eventually making them safe from being killed by the immune cells. The proposal is a proof-of-principle study to know whether Sema5B helps kidney cancer cells to escape from immune cell-mediated killing. The knowledge gleaned from this proposal will be important to develop novel immunotherapy against kidney cancer and, given that Sema5B level is highest in kidney cancer tissues compared to any other normal parts of our body, the therapy will have minimum off-target toxicity.

Proposal Title:	Using WEE1 Inhibitor to Convert "Immune Cold" into "Immune Hot" for Aggressive Clear Cell Renal Cell Carcinoma with SETD2 Deficiency
Log Number:	KC220122
Current PI Name:	Gangning Liang
Award Number:	HT9425-23-1-0160
Current Contracting Organization:	University of Southern California
Current Performing Organization:	University of Southern California
Web Approval Date:	02-07-2023

Large-scale sequencing efforts of human cancers have identified recurrent mutations and deletions in a variety of chromatin regulatory proteins that modulate DNA methylation, histone modifications, and nucleosome positioning. ,Decreased or depleted levels of histone H3K36 tri-methylation (H3K36me3) have been shown to play an important role in diverse forms of cancer due to somatic mutations in or down-regulation of SETD2 (H3K36 trimethylase), most notably in clear cell renal cell carcinoma (ccRCC). Altered chromatin modelers related to impaired H3K36me3 occupancy are linked to renal cancer aggressiveness. The relative 5-year survival rate of localized ccRCC is 91%, however, survival rates drop to 11% once the ccRCC has metastasized (advanced ccRCC). Thus, there is an urgent need to develop novel treatments to target H3K36me3-compromised cancer cells, as these alterations may represent an attractive target for diagnostic and treatment purposes.

Previous studies suggest that cancer cells displaying loss or depletion of H3K36 methylation show increased sensitivity to WEE1 inhibitor. In addition, the WEE1 inhibitor also can stimulate an immune response, meaning, the body's own immune system can be directed to kill the ccRCC by this approach and may further provide a specific synergistic therapeutic effect by a combination therapy with WEE1 inhibitor and immune checkpoint inhibitor. This proposal describes the use of the WEE1 inhibitor, alone or in combination immune checkpoint inhibitor, in preclinical models to improve treatment efficacy.

Specifically, we will examine whether the defects of impaired H3K36 methylation are potential mechanisms for WEE1 inhibitor sensitivity and identify potential therapeutic target genes using in vitro approaches in Aim 1. In Aim 2, we will evaluate the antitumor and epigenetic changes caused by combination treatments of the WEE1 inhibitor and immune checkpoint inhibitor in preclinical kidney cancer models with genetic changes similar to human disease inclusive of Setd2 loss.

We anticipate these preclinical models will allow us to evaluate and better understand the antitumor effects of the WEE1 inhibitor and immune checkpoint inhibitor on kidney cancer cells with altered chromatin modelers and subsequent impaired H3K36 methylation, as well as to challenge the current standard of care in developing personalized and precision treatments that impact survival times, especially aggressive kidney cancers. We believe that the success of this project will immediately lead to clinical trials for aggressive kidney cancers with impaired H3K36me3 levels using a precision medicine-based approach.

Proposal Title:	A Novel Trispecific Immunocytokine Approach to Enhance Antitumor Immunity in Renal Cancer
Log Number:	KC220124
Current PI Name:	Petr Makhov
Award Number:	HT9425-23-1-0536
Current Contracting Organization:	Institute for Cancer Research
Current Performing Organization:	Institute for Cancer Research
Web Approval Date:	Pending

Abstract not yet available.

Proposal Title:	Novel Therapeutic Target of Renal Medullary Carcinoma
Log Number:	KC220127
Current PI Name:	Yongdong Su
Award Number:	HT9425-23-1-0609
Current Contracting Organization:	Emory University
Current Performing Organization:	Emory University
Web Approval Date:	09-02-2023

Renal medullary carcinoma (RMC) is a rare renal malignancy that has been associated with sickle cell trait or sickle cell disease. It occurs primarily in adolescents and young adults of African ancestry. RMC is aggressive, with a median survival rate of 13 months. Despite various therapies being explored and applied clinically, limited success has been seen. It is, therefore, necessary to identify new therapeutic targets for patient with RMC.

Our prior data along with others identified the proteasome as a therapeutic target in RMC. Since monotherapy rarely leads to cure, we will interrogate previously identified targeted therapies with proteasome inhibitors to determine if there is an additive or synergistic effect in vitro and in vivo. We hypothesize that novel efficacious combination strategies using the proteasome inhibitor and another targeted therapy might benefit those military beneficiaries with children who are affected by RMC. Specifically, we will test the effect of inhibiting the cell cycle as well as inhibiting how TP53 is degraded on cell survival and tumor growth individually and in combination with proteasome inhibitors. Success of this grant will give children with RMC access to new therapeutic combinations and potentially improve outcomes for this aggressive renal tumor.

The Department of Defense Kidney Cancer Research Program Postdoctoral and Clinical Fellowship Award will help the PI (Dr. Su) to achieve his long-term research interests in the development of becoming a kidney cancer researcher. Furthermore, it will provide the training opportunity for the PI to gain the experience needed to become an independent researcher. Dr. Hong, his mentor, is a pediatric oncologist and physician-scientist with significant experience in the treatment and research of childhood kidney cancer. Altogether, this award will provide PI the necessary experience to facilitate his successful transition into an independent kidney cancer investigator.

Proposal Title:	Heme-Targeting Agent and Immunotherapy Combinations in Renal Cell Carcinoma
Log Number:	KC220131
Current PI Name:	Tianyuan Wang
Award Number:	HT9425-23-1-0220
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	02-08-2023

The number of patients suffering from kidney cancer is rising around the world. As the most common type of kidney cancer, renal cell carcinoma (RCC) emerges from highly vascularized tumors. In recent years several innovative treatments have emerged for RCC with significant improvements in outcomes. Respectively, the kinase inhibitors everolimus, sunitinib, and cabozantinib revolutionized targeted treatment and achieved significant tumor response in many patients, but ultimately tumor progression was observed. Most recently, immunotherapy with checkpoint inhibitors such as nivolumab and ipilimumab has shown remarkable success in some patients (typically 30%), even for multiply pre-treated tumors. A recent innovation is belzutifan, a HIF-2 antagonist, invented at UT Southwestern, developed by Peloton Pharmaceuticals and acquired by Merck, which just gained FDA approval for RCC and has shown benefits in patients resistant to sunitinib. However, most RCC patients who initially respond to therapy eventually develop resistance allowing tumor progression. Thus, there remains an unmet need to develop new therapies that may be effective in their own right or augment the success of existing treatments. The invasive neovasculature of tumors is immature, lacks pericyte support, and exhibits increased permeability that leads to hypoxia, providing an attractive target for therapy. Our team has recently demonstrated the therapeutic efficacy of a novel heme-targeting agent, cyclopamine tartrate (CycT) in non-small cell lung cancer (NSCLC) cells and mouse tumor models. Specifically, we found that CycT effectively inhibits angiogenesis, normalizes tumor vasculature, and alleviates tumor hypoxia. These findings have established heme targeting by CycT as an effective strategy for suppressing tumors.

Our preliminary study indicates that CycT effectively normalized vasculature and inhibited the progression of a patient-derived RCC xenograft model without apparent systemic toxicity. Recognizing the emerging success of immunotherapy, we anticipate that heme-targeting agent-induced vasculature normalization will reprogram the tumor microenvironment (TME) for enhanced immunotherapy response.

The overarching goal of the proposed research is to characterize the effects of heme-targeting agent CycT alone or in combination with immunotherapy on tumor growth, metastasis, and microenvironment in RCC RENCA tumors growing in immunocompetent animals. We place considerable emphasis on imaging, which can provide important insights into the time course of drug effect and early indication of efficacy. Multispectral optoacoustic tomography (MSOT) is a newly available method that will directly reveal the extent of vascular normalization. It should provide a prognostic imaging biomarker to evaluate kidney tumor response to CycT and combination therapy. In addition, investigating the mechanism of CycT action in RCC will be essential for understanding its activity at the molecular level and predicting potential off target effects, providing information to circumvent drug resistance, and exploring new targets along the activation pathways.

Proposal Title:	Provoking the Immunotherapy Efficacy of Renal Medullary Carcinoma
Log Number:	KC220138
Current PI Name:	Zilong Zhao
Award Number:	HT9425-23-1-0132
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	02-09-2023

Renal medullary carcinoma (RMC) is a highly lethal cancer that predominantly affects young individuals of African descent with blood disorders such as sickle cell disease (SCD). Sickle cell disease causes a structural change in red blood cells, which causes them to take on a crescent-like shape. This change in red blood cells of the kidney tissue is linked to the incidence of RMC. Less than 5% of patients with RMC survive beyond 3 years. In order to prevent, diagnose, and treat this disease there is a need to develop strategies informed by a deeper understanding of its distinct molecular changes.

Ferroptosis is a form of regulated cell death that is mediated by iron ion overload. In SCD, excess iron is released from the abnormal red blood cells and causes ferroptosis. Our findings found that immune T cells from both mice and human patients with SCD showed significant upregulation of ferroptosis markers compared to wild-type controls or healthy donors. We hypothesize that administration of hydrogen sulfide may enhance antitumor immunity by interacting with the free iron ions released by red blood cells and inhibiting ferroptosis. We therefore aim to (1) define that SCD promotes tumor growth of the RMC tumor, (2) determine whether ferroptosis induces immune T cell exhaustion in the setting of SCD, and (3) examine whether hydrogen sulfide administration of mice with SCD can sensitize RMC tumor to immunotherapy.

Overall, the project will determine the linkage between SCD and RMC tumors, which will fill in current gaps of knowledge in the field of cancer biology. Additionally, we will provide a pioneering therapeutic strategy for treating SCD-related RMC in the clinical setting.

Proposal Title:	Radio-Reprogramming of Tumor-Associated Macrophages in Renal Cell Carcinoma
Log Number:	KC220144
Current PI Name:	Jason Muhitch
Award Number:	HT9425-23-1-0666
Current Contracting Organization:	Health Research Inc., Roswell Park Division
Current Performing Organization:	Health Research Inc., Roswell Park Division
Web Approval Date:	Pending

Abstract not yet available.

Proposal Title:	Generation of Patient-Specific Humanized Mouse Models to Recapitulate Human Tumor Microenvironments for Optimal CAR-T Cell Therapy in Kidney Cancer
Log Number:	KC220154
Current PI Name:	Sophie Hanina
Award Number:	HT9425-23-1-0335
Current Contracting Organization:	Sloan Kettering Institute for Cancer Research
Current Performing Organization:	Sloan Kettering Institute for Cancer Research
Web Approval Date:	02-09-2023

The survival rate of people with advanced kidney cancer remains dismal, with the majority surviving around 13 months despite multiple treatment options. There is an urgent need to develop new and effective treatments. A state-of-the-art therapy called CAR-T cells has shown outstanding success in certain blood cancers leading to cures in patients. CAR-T cells are a new class of living drugs that are made from a patient's own immune cells. The immune cells are taken out of the patient's blood and are modified to express a receptor (analogous to a missile) that is specific for a target expressed on the surface of cancer cells. We call these modified patient immune cells CAR-T cells, and we put them back into the patient. Like a missile that seeks out its target, the CAR-T cells seek out and destroy the cancer, sparing normal healthy tissue.

Our goal is to make a CAR-T cell that destroys kidney cancer and cures patients. Unfortunately, to date, the results of using CAR-T cells for non-blood cancers like kidney cancers have not been successful. The treatment has either made the patient sick or not worked at all. The good results obtained when CAR-T cells are tested in the laboratory do not seem to translate into the good results we hope for when used in patients.

Since it would be dangerous to try to fine-tune these living drugs in humans, we use a special breed of mice instead. These special mice are useful because they were created to have no immune system, which allows us to grow a human cancer inside the animal without the cancer being attacked by the immune system. However, because these mice lack an immune system, the human cancers grown inside of them are not fully representative of a patient's cancer, which by contrast is full of many different types of immune cells supporting the spread of cancer and preventing CAR-T cells from working effectively. Therefore, testing CAR-T cells in these special mice without an immune system is like scoring a touchdown without the defense present to block you.

The overarching aim of this proposal is to make a mouse model that resembles the patient scenario as closely as possible so that we can figure out ways to make our treatments better and safer before they are used in patients. To make our "human-mouse" model, we will replace a mouse immune system with a human immune system specifically from a kidney cancer patient. We get the patient's immune cells by taking a small sample of blood from the hip bone at the time of kidney cancer surgery. We use this blood to make a patient-specific "human-mouse": All the organs and tissues are still mouse but the blood, and all the cells inside it, are human. We then implant tumor donated by the same patient into this "human-mouse". We now have a "human-mouse" with tumor and an immune system from the same patient, in which we can test our CAR-T cells made from that patient--this time we can try to score a touchdown but the defense is there to run interference.

By developing this "human-mouse" model, we can test the safety and effectiveness of our CAR-T cell therapy in a more real-world scenario for the benefit of kidney cancer patients, and other patients with aggressive cancers, who might one day receive CAR-T treatment.

Proposal Title:	Development of PROTAC Degraders for VHL Synthetic Lethal Partner FTO in Clear Cell Renal Cell Carcinoma
Log Number:	KC220156
Current PI Name:	James Brooks
Award Number:	HT9425-23-1-0170
Current Contracting Organization:	Leland Stanford Junior University, The
Current Performing Organization:	Leland Stanford Junior University, The
Web Approval Date:	02-09-2023

Scientific Background and Objective: The outcomes for patients with advanced/metastatic clear cell renal cell carcinoma (ccRCC), the most common type of kidney cancer, that cannot be treated with surgery, are very poor because these tumors are not responsive to either radiotherapy, chemotherapy, or targeted therapy. Therefore, new classes of drugs are urgently needed to improve the survival of patients with metastatic ccRCC. Fat mass and obesity-associated (FTO) gene is expressed at high levels in ccRCC cancers compared to normal tissues. More importantly, drugs that block the function of FTO selectively kill ccRCC cells that have mutations in the von Hippel-Lindau (VHL) gene, which occurs in 90% of cases, but not in cells without these mutations, suggesting that ccRCC cells cannot survive when both VHL and FTO are not functional. The same phenomenon has been observed by two independent researcher groups, confirming that targeting FTO may be an effective therapy, particularly in VHL-mutated ccRCC. However, current FTO inhibitors cannot be used in patients because they not only inhibit FTO but also other proteins similar to FTO, causing toxic side effects. In addition, their ability to shut down FTO is insufficient in the dosages that can be given safely to patients. Therefore, we must develop novel potent and selective FTO inhibitors with low toxicity.

Our team of chemists, urologists, and oncologists will use a cutting-edge technology called proteolysis-targeting chimaera (PROTAC), to make a new class of FTO-targeting drugs. PROTAC technology works by creating chemical compounds that hijack the cell's own protein recycling machinery. It works by coupling a chemical that binds to the protein of interest (in this case, FTO) to a chemical that attracts and activates the cell machinery to degrade that protein. Over 1,600 PROTACs have been made in a wide variety of diseases. There are several PROTAC drugs in clinical trials, demonstrating the efficacy of this new technology. However, a PROTAC-based degrader of FTO has not been reported to date. We have all the chemical pieces we need and will find the best combination to effectively and specifically target FTO in kidney cancer cells. We will test new drugs in ccRCC cells derived from human cancers, a unique resource we have developed in our laboratory.

What is the important gap in patient care the study will focus on? There is an unmet need for novel and effective treatment for patients with advanced/ metastatic VHL-deficient ccRCC. Our study will focus on developing a potent and selective PROTAC inhibitor targeting FTO to eliminate VHL-deficient ccRCC cells based on synthetic lethality. Since this inhibitor will be effective only in VHL-mutated cancer cells, the treatment will be highly specific for the cancer cells, making it less likely to cause side-effects. As a result, we anticipate effective killing at doses tolerable to patients. Moreover, our drugs are likely to synergize with other therapies to improve clinical outcomes of ccRCC patients. For example, blocking FTO is particularly effective in killing VHL-deficient ccRCC cells with low expression of the protein HIF2alpha. In 2021, the Food and Drug Administration approved belzutifan, a drug that inhibits HIF2alpha. Very likely, combining our PROTAC degraders of FTO with belzutifan will be a new, highly effective therapy for VHL-deficient ccRCC patients.

What are the innovative aspects of the proposed research project? Our proposal is designed around a number of innovative elements including concept and technology, which will lead to new areas of research in

ccRCC. First, the concept of FTO inhibition for VHL-deficient ccRCC is novel and impactful, given the morbidities caused by advanced ccRCC. We are the first to hypothesize that FTO inhibition induced by its PROTAC degraders will kill VHL-deficient kidney cancer cells, but not normal cells since they do not have VHL mutations. Second, PROTAC-based technology for FTO inhibition is innovative. It is exciting to see that one PROTAC-tracking database now lists more than 1,600 publicly disclosed heterobifunctional degraders, acting on more than 100 targets. However, a PROTAC-based degrader of FTO has not been reported to date. Our study will represent the first effort to develop a PROTAC inhibitor of FTO. Third, using kidney cancer cells derived from kidney cancers removed from patients and grown in animals to determine the effects of PROTAC degraders of FTO on cell growth and survival is original. These cells are authentic models for developing novel therapeutic targets in ccRCC, which will facilitate the translation of our results into clinics.

What is the likely impact of this study on the field of kidney cancer patient care? Our study addresses one of the overarching strategic goals of KCRP in 2022, which is to develop novel therapeutic strategies for the treatment of kidney cancer. The short-term impact of our study is the demonstration that PROTAC-based FTO degraders can be produced that will eliminate FTO protein in ccRCC cells. In the long term, the impact will be to pave the way for developing a new class of PROTAC drugs that target FTO specifically in cancer cells for development for clinical use, either alone or in combination with other therapeutic strategies to improve outcomes of cancer patients.

Proposal Title:	Increasing Efficacy of EZH2 Inhibitors in Kidney Cancer
Log Number:	KC220163
Current PI Name:	Ju-Seog Lee
Award Number:	HT9425-23-1-0290
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	09-02-2023

EZH2 is a frequently activated oncogene in kidney cancer, and its high expression is observed in advanced kidney cancer and associated with poor prognosis of kidney cancer. Ferroptosis is a newly discovered programmed cell death mechanism that can prevent tumor development by killing precancerous or cancer cells by oxidizing the cell membrane. By analyzing genomic data from mouse models and human cancer cell lines, we discovered that EZH2 alters cellular metabolism to make cancer cells resistant to ferroptotic cell death. Therefore, we seek to evaluate the possibility of novel treatments by characterizing previously unrecognized roles of EZH2 in regulation of ferroptosis in kidney cancer. This research primarily addresses the FY22 Kidney Cancer Research Program Focus Area "Develop novel therapeutic strategies for the treatment of kidney cancer, such as novel drug targets, therapeutic modalities and agents, treatment combinations, and drug delivery systems."

Our near-term goals are to determine the molecular mechanisms of EZH2-mediated resistance to ferroptosis in kidney cancer and to examine the therapeutic efficacy of cotreatment of EZH2 inhibitors with ferroptosis inducers. If successful, this work will yield insights about the mechanisms of EZH2-mediated ferroptosis resistance in kidney cancer that can be translated into novel treatments for kidney cancer patients. Our long-term goal is to establish a novel treatment option for these patients within 5 years after completing the proposed study.

Proposal Title:	Tumor-Intrinsic and Tumor-Extrinsic Determinants of Immune Responsiveness in MiT/TFE Translocation Renal Cell Carcinoma
Log Number:	KC220168
Current PI Name:	Prathyusha Konda
Award Number:	HT9425-23-1-0066
Current Contracting Organization:	Dana-Farber Cancer Institute
Current Performing Organization:	Dana-Farber Cancer Institute
Web Approval Date:	02-08-2023

Objective and Rationale: Kidney cancer is one of the five most common cancers diagnosed amongst United States Veterans and accounts for over 79,000 new cases and 14,000 deaths each year in the United States. There are many different types of kidney cancer. About 70% of kidney cancers are of "clear cell" kidney cancer variety. The other 30% of kidney cancers are called "non-clear cell" kidney cancer, of which there are multiple subtypes. One specific subtype of non-clear cell kidney (renal) cancer is called "translocation renal cell carcinoma." This subtype accounts for 1%-5% of all kidney cancers in adults and over 40% of kidney cancers in children. Translocation kidney cancer is rare, aggressive, and usually occurs in younger, female patients.

Translocation kidney cancer is so named because on the molecular level, it has a joining (translocation) between two genes. One of the genes in the translocation is a transcription factor (protein that binds DNA) in a family of genes named "MiT/TFE". The second gene in the translocation can be any one of several possible partner genes. The protein created by the joining of the MiT/TFE gene and the partner gene is known as an "MiT/TFE fusion." Previous studies, including our prior work, support the notion that the MiT /TFE fusion is the key mutation in translocation renal cell carcinoma, and hence the primary therapeutic target in this disease.

Over the past several years, there have been many promising treatments tested and approved for clear cell kidney cancer, including various types of immunotherapies, which use our body's own immune system to attack and destroy cancer. However, these therapies approved for clear cell kidney cancer are not effective in patients with translocation renal cell carcinoma. This is because clear cell kidney cancer and translocation kidney cancer have significant molecular and immunological differences, which alter how the immune system recognizes these cancers. As a result, treatments developed for clear cell kidney cancer cannot be readily used to treat patients with translocation kidney cancer.

Because translocation kidney cancer is rare and recently described, it has not been extensively studied. Therefore, there are currently no therapies designed specifically for this subtype of kidney cancer, which remains a major unmet need in the field. This proposal seeks to answer several fundamental questions about translocation kidney cancer, with the goal of developing immunotherapies tailored to this subtype. First, how exactly does translocation kidney cancer differ from clear cell kidney cancer, and what are the immune cells involved in detecting these two subtypes of kidney cancer? Second, how are the immune cells detecting translocation kidney cancer? Here, we will specifically test the hypothesis that immune cells recognize translocation kidney cancer through MiT/TFE fusions, which can then be targeted for cancer immunotherapies. Third, what are the specific characteristics of the immune cells that recognize MiT/TFE fusions in translocation kidney cancer? Answering these questions will enhance our understanding of translocation renal cell carcinoma and help in the development of new therapies for this disease.

Clinical applicability/Impact: This research is designed to improve the lives of patients with translocation renal cell carcinoma, a rare and aggressive subtype of kidney cancer, that has major molecular and

immunological differences from clear cell kidney cancer. The studies proposed here are necessary to gain a complete picture of the immune cells and immune responses involved in translocation kidney cancer, important to fully understand this subtype. Our hope is that this proposal will identify new targets for translocation kidney cancer within the next 3 years. Longer term (~5-8 years), this will allow us to develop personalized immunotherapies and combination therapies that are specifically tailored to translocation kidney cancer. Such therapies will likely be far more effective than current therapies used in the clinics, which have been designed for clear cell kidney cancer.

PI's career goals: My long-term career goal is to become an independent investigator in the field of kidney cancer, focused on developing personalized cancer immunotherapies for this cancer. During this award, I will receive extensive training from two physician-scientists at the forefront of kidney cancer research. Under their mentorship, this work helps me to gain a deeper understanding of the clinical challenges associated with translocation kidney cancer while also using my prior knowledge and expertise in immunology to therapeutic discovery in this cancer. Through this project and the collaborations that emerge from it, I hope to expand my scientific skills and develop a strong expertise in kidney cancer research, which will help me transition from a postdoctoral fellow to a successful independent investigator. I envision an ultimate career with successful research program that contributes significantly to the clinical progress of kidney cancer therapeutics while also training the future generation of scientists.

Proposal Title:	Role of Uridine Phosphorylase 1 (UPP1) in Chromophobe Renal Cell Carcinoma
Log Number:	KC220200
Current PI Name:	Carmen Priolo
Award Number:	HT9425-23-1-0291
Current Contracting Organization:	Brigham and Women's Hospital, Inc.
Current Performing Organization:	Brigham and Women's Hospital, Inc.
Web Approval Date:	04-17-2023

Chromophobe renal cell carcinoma is a rare form of kidney cancer that can occur as a sporadic cancer or in patients with genetic syndromes such as Tuberous Sclerosis Complex and Birt-Hogg-Dubé. There are no proven therapies for patients with metastatic chromophobe renal cell carcinoma, most of whom will die of their disease, representing a significant unmet clinical need.

The KCRP Focus Areas addressed by this proposal are: (1) Define the biology of rare kidney cancers and develop treatments to improve outcomes and reduce death. (2) Conduct basic biology research to better understand etiology and cancer progression, metastatic disease, refractory disease and therapeutic resistance, genetic and environmental risk factors and the prevention of kidney cancer.

The proposed research focuses on Uridine Phosphorylase 1 (UPP1), a protein involved in key cellular metabolic processes that is upregulated in chromophobe renal cell carcinoma. We hypothesize that UPP1 regulates the proliferation and survival of chromophobe renal cell carcinoma cells by modulating their bioenergetic metabolism.

The proposed studies may lead to a new area of research for chromophobe renal cell carcinoma: the identification of clinically relevant UPP1 inhibitors or inhibitors of other key enzymes in the UPP1 pathway.

In summary, this project will uncover novel mechanisms in the pathogenesis of chromophobe renal cell carcinoma and provide insights into therapies for this rare kidney cancer.

Proposal Title:	Investigating the Role of Noncanonical Tricarboxylic Acid Metabolism in Kidney Cancer
Log Number:	KC220206
Current PI Name:	Justin Cross
Award Number:	HT9425-23-1-0986
Current Contracting Organization:	Sloan Kettering Institute for Cancer Research
Current Performing Organization:	Sloan Kettering Institute for Cancer Research
Web Approval Date:	09-02-2023

Background: Despite recent advances in kidney cancer treatment, kidney cancer remains the 8th most common cancer with approximately 63,000 new cases and approximately 14,000 fatalities each year in the United States alone, devastating patients, their families and friends, and communities, among whom many are current or former military personnel. Renal cell carcinoma (RCC) accounts for greater than 90% of cancers detected in the kidney. This encompasses a large heterogeneous group of cancers all derived from renal tubular epithelial cells, but of these, clear cell renal cell carcinoma (ccRCC) is the most common (70% -75% of kidney cancer) and aggressive subtype of kidney cancer. In patients impacted by ccRCC, metastasis, the process by which cancer cells spread from the primary tumor to distant locations in the body, is responsible for most deaths.

Despite this long-standing observation, the mechanisms that cause kidney cancer to spread to distant locations in the body remains largely unknown. In this grant application, we focus on SETD2, a gene that is commonly mutated in ccRCC and associated with kidney cancer metastasizing to distant organs. Our goal is to investigate the mechanisms by which SETD2 mutations promote kidney cancer metastasis and specifically, we will investigate whether SETD2 mutations alter cell metabolism in ways that make it easier for kidney cancer cells to spread and colonize these metastatic sites.

Areas of Emphasis: Cancer metabolism; cancer genetics.

Impact and applications: Metabolism is fundamental to tumor cell growth. To meet the biosynthetic challenges of continued cell proliferation, cancer cells must accumulate biomass either through biosynthetic reactions or by scavenging nutrients from the environment. When cells that leave the primary tumor site and disseminate for tumor metastases in other parts of the body cells it marks a critical transition into more aggressive disease. Recently, understanding the nutrient acquisition and metabolism of cancer cells in these settings has become an area of intense focus as this represents one of the major challenges these cells overcome.

In ccRCC, SETD2 mutations are associated with more aggressive and metastatic cancers our preliminary data indicates that loss of SETD2 also results in dramatic alterations in cellular metabolism. We believe these metabolic changes may be required to support the metastatic dissemination of cells from the primary to distant sites. Our interest in studying this metabolic transition is therefore to identify pathways that tumor cells are particularly reliant upon, more so than other non-tumor cells in the body. Such pathways then become targets which could be inhibited therapeutically.

Our research will pave the way for the application of new therapeutic strategies aimed at targeting nutrient utilization and metabolic enzymes in cancer. To generate these insights, we will leverage several complementary approaches to assess kidney cancer metabolism in cells with and without mutant SETD2 protein.

Our study is designed to provide mechanistic insights which will have broad impact on cancer biology beyond kidney cancer. In the long term, we expect our elucidation of SETD2-driven metabolic changes will pave the way for the future discovery of therapeutic strategies for SETD2-mutated cancers.

Proposal Title:	Serine-Induced Glycosylation in Progression and Metastasis of ccRCC
Log Number:	KC220211
Current PI Name:	Suman Karki
Award Number:	HT9425-23-1-0341
Current Contracting Organization:	Alabama, University of, at Birmingham
Current Performing Organization:	Alabama, University of, at Birmingham
Web Approval Date:	04-25-2023

Background: Kidney cancer is the seventh most common type of cancer in the United States with over 81,000 new cases and 14,000 deaths estimated in the year 2023. Metastatic clear cell renal cell carcinoma (ccRCC) is the most common type of renal carcinoma (85% of all kidney cancer), with a median survival rate of about 13 months, and is refractory to chemotherapy. Cancer cells are armored with specialized metabolic pathways to support enhanced proliferation, metastasis, and immune evasion. Aberrant metabolic reprogramming in the uptake and metabolism of amino acids is one of the hallmarks of cancer cells. Serine is the second most nonessential amino acid utilized next to glutamine by cancer cells and is produced by the serine biosynthesis pathway. The first enzyme in the serine biosynthesis pathway, PHGDH, is reduced in ccRCC. Hence, unlike many other cancers, ccRCC depends on the exogenous serine availability for their proliferation and survival. Interestingly, this dependency makes serine a conditionally essential amino acid in ccRCC, but also presents opportunities to target serine-related metabolism for a therapeutic target against ccRCC. So far, the role of serine in the development of primary tumors has been extensively studied; however, it is not clear if availability of serine impacts metastasis. Metastasis is, so far, the major driver of cancer-related deaths. In this proposal, we aim to further expand our understanding of metabolic vulnerability due to the loss of PHGDH in ccRCC. This study aims to test if serine restriction can be augmented with existing therapy to enhance the efficacy of primary treatments.

State the KCRP Area(s) of Emphasis the project addresses: Basic Biology Research - Tumor metabolism, tumor progression and metastasis

Describe the ultimate applicability of the research: Understanding gained from the successful completion of this proposal will enable us to strategize the targeting of specific pathways and factors mediated through serine usage and serine-induced glycosylation for therapeutic approaches.

What types of patients will it help, and how will it help them? In general, this study will help to target cancers that are dependent on exogenous serine for proliferation and survival. Restriction of serine usage will cause reduction in the metastatic potential of ccRCCs.

What are the potential clinical applications, benefits, and risks? If the research is too basic for clinical applicability, describe the interim outcomes expected and their applicability to the field. Dietary restriction augmented with other therapeutic options is the ultimate clinical application envisioned. The understanding of the serine, glycosylation, metastatic potential is the expected interim outcome.

What are the likely contributions of this study to advancing the field of kidney cancer research? Glycosylation is a biologically necessary modification of proteins with implications in both normal development and disease. It is one of the abundant and complex modifications that are frequently demonstrated during neoplastic transformation. However, the impact of serine-induced glycosylation has not yet been explored. Cancer-specific glyco-diversification supports neoplastic progression and unique alterations in tumor biology and metabolism. However, there is no comprehensive information on the glycosylation status in ccRCC. Our study potentially leads to identification of serine-dependent glycan

signatures as prognostic markers. Furthermore, serine-induced aberrant glycosylation may generate ccRCC-specific neoantigens, which can be targeted for therapeutic purposes.

How is the project relevant to military Service Members, Veterans, and their families? The etiology of the kidney cancer is not well understood, however, our Service Members and Veterans are constantly exposed to some of the known risk factors including smoking tobacco, environmental and occupational exposures to toxic chemicals, ionizing radiation, and hazardous materials. Knowledge gained from the completion of this proposal will help to build our understanding of kidney cancer to strategize the therapeutic interventions.

Proposal Title:	Increase Clinical Trial Enrollment of Underrepresented Kidney Cancer Patients by Improving Access to Participation
Log Number:	KC220215
Current PI Name:	Susan Rux
Award Number:	HT9425-23-1-0873
Current Contracting Organization:	Institute for Cancer Research
Current Performing Organization:	Institute for Cancer Research
Web Approval Date:	09-02-2023

Scientific Objective and Rationale: In the world of cancer, clinical trials are the foundation for the approval of new drugs. Most patients who participate in cancer trials are non-Hispanic white, male, and younger than 65 years of age. This is no exception for trials specifically for kidney cancer. Kidney cancer is more common in men than women, and more prevalent in the African American, American Indian, and Alaska Native populations. However, we see fewer racial and ethnic minorities enrolled onto trials. Additionally, patients with limited financial resources are also less likely to participate in a trial for their cancer. It is well-studied that multiple barriers exist to clinical trial participation, especially among groups such as racial and ethnic minorities, women, older adults, and patients from lower-income households. For example, concerns about cost and limited or no education about clinical trials are two identified barriers that factor into a patient's decision not to enroll in a clinical trial. By offering educational programs and financial assistance, our goal is to increase enrollment of underrepresented patients on kidney cancer trials at Fox Chase Cancer Center (FCCC) and throughout Temple University Health System (TUHS).

Applicability: Our project will help all kidney cancer patients at FCCC and throughout TUHS, who are hesitant to enroll in a clinical trial. The educational program will review topics such as the basics of a clinical trial, where a trial fits in a patient's cancer journey, and trials offered at FCCC and TUHS. To accommodate all patients, the program will be offered as a live in-person session and as a shortened video available for viewing during clinic visits.

Information learned from the program will increase patient knowledge and improve preparation for decision making about clinical trials. A financial support plan will benefit patients who are worried about the indirect costs of participating in a clinical trial, such as food, lodging, and travel expenses. Clinical research nurses (CRNs) will advocate for patient assistance during the study start-up process and help patients apply for funding from non-profit organizations.

The benefit of focused education on clinical trials allows the patient to play a more active role in their care and make an informed decision about participation. Clinical trial participation often times comes with additional indirect costs. Securing financial support for such costs will positively affect a patient’s decision to participate in a clinical trial. Potential risks exist with both goals of the project. The educational session may further increase patient concerns about participating in a clinical trial. Some patients may not qualify for financial assistance or may not receive adequate funds to cover costs associated with participation.

Immediately after the education session or watching the video, a patient will have more knowledge about clinical trials and their kidney cancer. Patients can use this information at their next physician visit or when talking with friends and family about treatment options for themselves or others they may know with cancer. Patients can receive financial assistance for indirect costs from non-profit organizations after enrolling onto a trial. If a study offers a patient stipend or reimbursement, the patient typically receives reimbursement on a monthly basis.

It is not well understood how race and ethnicity play a part in both the safety and the process by which a drug affects a patient's cancer. By including a broader population of kidney cancer patients in clinical trials, the results become more applicable to all kidney cancer patients. This will have a long-lasting impact on kidney cancer research and will positively affect patient outcomes.

Proposal Title:	Targeting Stemlike CD8 T Cells in Immunotherapy Against Kidney Cancer
Log Number:	KC220216
Current PI Name:	Chen Yao
Award Number:	HT9425-23-1-0801
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	09-02-2023

Hypothesis, Evidence, and Rationale: CD8 T cells are essential for controlling tumor growth. However, in Renal Cell Carcinoma (RCC) and most other cancers, antitumor CD8 T cells become exhausted. Exhausted CD8 T cells are impaired in their function, proliferation and persistence due in part to upregulation of inhibitory receptors (i.e., immune checkpoints) including PD1. Immunotherapies with immune checkpoint inhibitors (ICI) aim at reinvigorating exhausted T cells. Yet, less than 30% of RCC patients responded to ICI treatment, only a minority of whom experienced complete responses. The molecular program of T cell exhaustion is largely unaffected by ICIs and contribute to the lack of durable control of RCC in ICI-treated patients. CD8 T cells with stem cell characteristics were recently found in tumors including RCC. Unlike terminally exhausted CD8 T cells, stem-like CD8 T cells resist exhaustion and respond potently to ICIs. In addition, the abundance of stem-like T cells predicted favorable clinical outcomes in ICI therapy for melanoma patients. Thus, enhancing the immune response by stem-like CD8 T cells may improve the efficacy of ICIs in RCC patients. My recent studies revealed the transcriptional and epigenetic mechanisms underlying T cell stemness and T cell exhaustion. However, how the antitumor response of stem-like CD8 T cells is regulated in RCC and strategies to improve the efficacy of ICCs in RCC via targeting stem-like CD8 T cells remain elusive. In addition, the prognostic value of stem-like CD8 T cells in RCC patients treated with ICIs is unclear.

In this study, I will test the hypothesis that the therapeutic efficacy of ICIs against RCC is determined by the quantity, metabolic fitness and tumor microenvironment (TME) of stem-like CD8 T cells. I will conduct this study under the supervision of Drs. Simon and Brugarolas and leverage the rich resources in patient samples and mouse RCC models in University of Texas Southwestern Medical Center at Dallas Kidney Cancer Program (KCP) and expertise of my mentors/collaborators in single-cell multi-omics, machine learning of pMHC-TCR interaction, metabolism, TME, and image mass cytometry. In Aim 1, I will determine the antigen specificity and differentiation status of antitumor CD8 T cells in human RCC and evaluate whether the abundance and transcriptome of antitumor stem-like CD8 T cells predict clinical outcomes of PD1 blockade in RCC patients. I will also evaluate strategies to potentiate the therapeutic efficacy of ICI against RCC using a therapeutic vaccine that induces robust expansion of stem-like CD8 T cells. In Aim 2, I will investigate the mechanism underlying the synergistic effect between tyrosine Kinase Inhibitor (TKI) and ICI. I will leverage single-cell metabolism assay to determine how TKI modulates the metabolism of antitumor stem-like CD8 T cells and whether TKI promotes formation of immunostimulatory intra-tumoral niche for stem-like CD8 T cells in mouse and human RCC.

Career Goals in Kidney Cancer Research: My overarching career goal is to establish a sustainable and robust research program focusing on the molecular and cellular mechanisms governing the immune response of T cell against RCC and apply the knowledge gained from basic research to developing novel immunotherapies for RCC patients. I plan to discover pathways involved in T cell exhaustion and pathways that endow stem-like T cells the ability to resist exhaustion in RCC. In collaboration with Dr. Brugarolas, I hope to develop animal models that better recapitulate human RCC and use these models to evaluate combination therapies that target pathways governing T cell stemness and/or exhaustion to enhance control of RCC. In addition, I

will leverage the immense resources including clinical and omics data and samples from RCC patients treated in KCP and develop decision-guiding biomarkers, especially immune biomarkers. The Academy of Kidney Cancer Investigators (AKCI) funding will help me establish a foothold in kidney cancer research, pursue research directions outline above, and generate data that will be used to secure further funding to sustain a research program with national reputation.

AKCI Participation: My mentors, Drs. Simon and Brugarolas, are well-known for their kidney cancer research and outstanding mentorships. I will interact with them in regular one-on-one meetings for guidance on research, lab management, and career development. I am committed to all AKCI organized activities including webinars and workshops. During these activities, I will seek to broaden my horizon on kidney cancer research, gain feedbacks from the leaders in the kidney cancer field, and establish my own network. As a member of AKCI, I will contribute my expertise in immunology, cutting-edge sequencing technology, and bioinformatics analysis. Within the AKCI framework, I will initiate collaborations with other AKCI members, including other early career scholars, mentors, and the Dean. These future collaborations will help me initiate new research directions and secure further funding to expand my research portfolio in kidney cancer research.

Applicability, Contributions, and Impact: Although immunotherapies including ICI therapy have revolutionized treatment of RCC, only a fraction of patients benefited from immunotherapies. Understanding the limiting factors for the efficacy of immunotherapies against RCC will help develop future treatments that cure a greater population of patients. Here, I will test the hypothesis that the efficacy of ICIs in RCC is determined by stem-like CD8 T cells. This study will reveal the antitumor response of stem-like CD8 T cells in RCC, develop novel prognostic T cell biomarkers for RCC patients treated with ICI, and evaluate strategies to potentiate T cell immunity induced by ICI. My results will build the scientific foundation for future combination therapies that harness stem-like CD8 T cells to benefit a greater population of RCC patients.

Proposal Title:	Elucidating the Role and Therapeutic Vulnerability of Sema5B in RCC
Log Number:	KC220219
Current PI Name:	Anirban Kundu
Award Number:	HT9425-23-1-0783
Current Contracting Organization:	Alabama, University of, at Birmingham
Current Performing Organization:	Alabama, University of, at Birmingham
Web Approval Date:	09-02-2023

The kidney removes wastes from our body and maintains a healthy balance of water, salts, and minerals. When we are exposed to toxins, our two kidneys help to eliminate them from our bodies as well. Hence, the toxins one point in time, travel through the kidneys that increasing the chance of kidney cancer. Exposure to toxic chemicals while on a military base or during deployment can lead to kidney cancer among a great deal of Veterans, and not only Veterans but anyone can be diagnosed with kidney cancer in their lifetime for some known and unknown reasons. In the United States, kidney cancer is among the top 10 malignancies in both men and women. The American Cancer Society's most recent estimates for kidney cancer in the United States for 2022 note that about 79,000 new cases of kidney cancer (50,290 in men and 28,710 in women) will be diagnosed and about 13,920 people (8,960 men and 4,960 women) will die from this disease. Clear cell renal cell carcinoma histology (ccRCC) is the most frequent form of this cancer. Although outcomes are excellent for patients with tumors confined to the kidney, patients with the advanced disease face unfavorable outcomes despite several approved therapies. This underscores the need for newer therapies. Semaphorin 5B (Sema5B) protein is a factor that becomes abundant in RCC patient tumors compared to adjacent normal kidney tissues. Our group had already reported that Sema5B promotes renal cancer growth, but its mechanism is unclear. Moreover, Sema5B's role in RCC metastasis as well as its therapeutic vulnerabilities remain unexplored. Based on our solid preliminary data, I will deal with these possibilities during the Kidney Cancer Research Program Academy of Kidney Cancer Investigators Early Career Scholar Award.

The short-term impact of this proposal is that we will likely establish a therapeutic vulnerability for renal cancers that have high Sema5B content. As a result, those patients with high Sema5B can have a customized regimen of therapies for a better outcome.

The long-term impact of the proposal is huge, as Sema5B is high in most of the RCC tumors (greater than 50%) and, therefore, the proposal will impact the majority of RCC patients.

Proposal Title:	Drug Development of ONC392, a Novel CTLA-4 Inhibitor in Advanced Renal Cancer
Log Number:	KC220225
Current PI Name:	Ulka Vaishampayan
Award Number:	HT9425-23-1-0926
Current Contracting Organization:	Michigan, University of
Current Performing Organization:	Michigan, University of
Web Approval Date:	09-02-2023

A clinical trial will be conducted in advanced (stage IV) clear cell kidney cancer patients after they have progressed on at least one previous therapy. The study utilizes a new medication called ONC392 that appears to be a more effective and less toxic immune therapy.

Background: Immune checkpoint inhibitor therapy has demonstrated remarkable efficacy in advanced renal cancer and has become the standard of care. The combination of ipilimumab and nivolumab is one of the immunotherapies used. The regimen of ipilimumab+nivolumab was evaluated in the Checkmate-214 trial and showed robust efficacy with an average life expectancy of more than 4 years. Multiple other combination regimens of VEGFR-TKI and PD-1 inhibitor have been established in the frontline therapy of advanced renal cell carcinoma (RCC). Despite the promising responses, more than 50% of the patients progress on this regimen. The treatment of these patients is a large unmet need. Novel drug development is needed to improve the outcomes of metastatic RCC, a lethal disease. The toxicities of the combination of ipilimumab and nivolumab are also overwhelming with a high incidence of severe diarrhea requiring hospitalization and steroid therapy. Treatment-related adverse events led to discontinuation of therapy in 22% and severe (grade 3 and 4) toxicities were noted in 46% of patients. The toxicity led to limitations in drug delivery with the dose of ipilimumab being only 1 mg/kg and the number of doses being limited to a maximum of four. The mechanism of action of ipilimumab is inhibition of a receptor on immune cells called CTLA-4, which results in activation of immunity against cancer.

We propose a clinical trial of a novel CTLA-4 inhibitor, ONC392 in advanced pretreated kidney cancer. ONC-392 is a highly selective, humanized monoclonal immunoglobulin G1 (IgG1)-kappa isotype antibody against CTLA-4. Laboratory research has shown that ONC392 is a more efficient CTLA-4 inhibitor than ipilimumab. It decreases the toxicity and improves immune cell activation making it an attractive agent for drug development in advanced RCC. A clinical study of ONC-392 (NCT04140526) has shown promising efficacy, tolerability and safety in patients with advanced solid tumors such as lung cancer, melanoma and ovarian cancer.

Study Design: We propose a Phase 2 clinical trial testing ONC-392 as a monotherapy and in combination with tivozanib for advanced or metastatic kidney cancer patients who have progressed after at least one line of prior therapy. Eligible patients will be randomized to receive ONC-392 monotherapy or in combination with tivozanib. Tivozanib is a U.S. Food and Drug Administration-approved agent in advanced RCC for patients who have progressed after two prior therapies. A safety run in will be conducted within the first six patients enrolled on the combination. ONC-392 will be dosed at 10 mg/kg intravenously every 4 weeks; this dose and schedule has been well established. The patients will be continuously followed for tumor shrinkage or response, overall survival, and progression-free survival. A sample size of about 30 patients per arm will be planned.

Applicability, Impact and Projected Timeline: The clinical trial grant proposes to add two cohorts in kidney cancer to the current ongoing ONC-392 trial (NCT04140526). The cohorts will be added as an amendment

to the existing ongoing study and the RCC cohorts will be opened at selected sites, with investigators experienced in treating RCC and who have adequate volume of RCC patients to anticipate accrual. This strategy has multiple advantages: (1) The timing for regulatory submissions and approvals will be reduced; (2) The grant and contracting time will be expedited as the study is already open and active at these sites; (3) The drug delivery and processing to the sites is already established; (4) The participating investigators will have experience with the novel agent through their prior enrollment on the study. Quality of life surveys, and correlative biomarker testing will be conducted. The anticipated timeline is about 2 years for completion of enrollment. Phase 3 trial planning will start if efficacy and safety are confirmed and will enable bringing access to this product within the next 5-7 years.

This clinical trial of monotherapy ONC-392, and in combination with tivozanib, promises the expedited development of a regimen for advanced RCC with better efficacy and improved safety. Development of this therapy in RCC will meet the fiscal year Kidney Cancer Research Program priority Focus Areas of novel therapeutic strategies for the treatment of kidney cancer, and the identification of a strategy to improve the quality of life and survivorship for patient

Proposal Title:	The Neural Regulation of Small Cell Lung Cancer Progression
Log Number:	LC220010
Current PI Name:	Humsa Venkatesh
Award Number:	HT9425-23-1-0475
Current Contracting Organization:	Brigham and Women's Hospital, Inc.
Current Performing Organization:	Brigham and Women's Hospital, Inc.
Web Approval Date:	09-27-2023

Lung cancers are the leading cause of cancer-related death. Small cell lung cancer (SCLC) is a particularly aggressive subtype accounting for about ~15% of all lung cancers. Compared with civilians, military personnel are at higher risk for being diagnosed with this type of lung cancer in part due to occupational exposure to inhaled carcinogens. Yet, due to their limited occurrence and stigmatic association with tobacco and cigarette use, SCLC is often not prioritized in research. As treatment options are currently limited due to the large metastatic burden and advanced stage at the time of diagnosis, SCLC remains one of the most intractable human cancers, demanding a fundamental change in our approach to therapy. Thus, defining how these malignant cells interact with their microenvironment is crucial to understanding the fundamental factors contributing to this disease pathology.

My past work has illustrated that one critical microenvironmental dependency of cancer cells is their direct integration into neuronal circuitry. We have found that neuronal activity promotes brain cancer progression, which highlights the previously unexplored potential to target neuron-cancer circuit dynamics for therapy of these lethal cancers. My work has led us to the startling realization that cancers can functionally integrate into electrically active neuronal circuits, which has already led to an ongoing clinical trial. We have thus uncovered that cancers are electrically active – a new and fundamental property of cancer pathophysiology that has largely shifted our view of malignant tissue. Effective therapy for these cancers may thus require targeting not only molecular mechanisms of cell proliferation, but also functional/structural neuron-cancer interactions. As small cell lung cancer (SCLC) originates from neuroendocrine cells in the lung and exhibit several neural-like properties, it stands to reason that these cancers similarly benefit from direct neuronal input. This has been hypothesized in the literature, but has been a vastly understudied area of research due to communication barriers between cancer biologists, neuroscientists, and lung biologists. Understanding the neurobiological aspects of SCLC pathophysiology would create a more comprehensive understanding of this disease with vast therapeutic implications. By appreciating these paradigm-shifting insights, this proposal seeks to uncover the detailed mechanisms by which SCLCs rely upon these powerful neuron-malignant cancer interactions for progression and may uncover innovative angles for therapeutic strategies. Using novel imaging and neuroscience tools uniquely applied in the context of cancer, this proposal aims to further clarify which neurons innervate lung tumors, alter the activity of these neurons to determine the effects on overall SCLC malignant outgrowth, and reciprocally detect changes in these distinct neuronal subpopulations over the course of disease progression to understand the dynamic vulnerabilities of SCLC circuit integration. This proposal makes large conceptual advances, pushing forward our understanding of neuron-cancer interactions by utilizing technological innovation never used in the context of lung cancer. We aim to reframe our perspective of SCLC by investigating how tumor cells in the lung integrate electrical inputs and hijack mechanisms of neural plasticity. A comprehensive understanding of these dynamic malignant-network interactions is necessary to clarify outstanding questions in this emerging field of cancer neuroscience and identify new targets with the potential to change how we treat this devastating disease.

Proposal Title:	Novel Driver in Lung Adenocarcinoma and Its Therapeutic Potential
Log Number:	LC220031
Current PI Name:	Ju-Seog Lee
Award Number:	HT9425-23-1-0033
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	11-04-2022

Lung cancer is one of the most common cancers worldwide. Compared with other major lethal cancers, patients with lung cancer suffer from lack of effective therapeutic intervention. Thus, new approaches for the rapid identification and functional validation of the genetic and/or epigenetic alterations associated with lung cancer development are urgently needed. The major goals of this proposal are to characterize a newly identified potential driver gene of lung cancer using genomic studies as well as to test the therapeutic potential of targeting this novel driver gene.

Our proposal is highly relevant to focused areas of research, as PEA15 is highly associated with poor survival and early recurrence due to metastasis and regulates the oncogene that is most significantly activated in multiple cancers, including lung cancer. Therefore, our proposal covers the following Areas of Emphasis: (1) understand the molecular mechanisms of initiation and progression to lung cancer" by identifying novel oncogene PEA15 and uncovering molecular mechanism of PEA15-mediated regulation of the oncogene and (2) identify innovative strategies for treatment of lung cancer" by identifying PEA15 as potential therapeutic target.

In our analysis identifying significantly amplified genes in all cancers, we found that PEA15 is highly amplified throughout many cancers, including lung cancer. Its amplification is significantly associated with its mRNA expression and patient survival. Further analysis showed that it regulates the oncogenes that are frequently activated in lung cancer. Therefore, we hypothesize that PEA15 promotes tumor development by activating the oncogene in lung cancer. We will test the hypothesis by using several complementary experimental approaches in cellular and mouse models.

Our near-term goals are to determine the molecular mechanisms of a PEA15 in regulation of the oncogene in lung cancer and to examine the therapeutic efficacy of targeting the oncogene. If successful, this work will yield insights about the mechanisms of PEA15-mediated oncogenesis in lung cancer that can be translated into novel treatments for lung cancer patients. Our long-term goal is to establish a novel treatment option for these patients within 5 years after completing the proposed study.

Proposal Title:	Development of a First-in-Class Therapeutic to Treat Non-Small Cell Lung Cancer (NSCLC)
Log Number:	LC220039
Current PI Name:	Keith Robinson
Award Number:	HT9425-23-1-0397
Current Contracting Organization:	MicroQuin
Current Performing Organization:	MicroQuin
Web Approval Date:	08-10-2023

Lung cancer (LC) is the leading cause of cancer deaths in the United States. Non-small cell lung cancer (NSCLC) is the most common type of LC, comprising 82% of all LC. Despite significant advances in cancer therapeutics, there has been only a ~10% increase in survival rates since 1975, with an average 5-year survival rate of 21.7%. Additionally, compared to the general population, military Service Members are 25% more likely to receive a lung cancer diagnosis. The survival rate among Veterans has been historically lower than the general population. It is evident that there is an urgent need to develop new, more-effective treatments for NSCLC.

Addressing the Area of Emphasis: Identify innovative strategies for the treatment of lung cancer. MicroQuin developed therapeutics focusing on a novel target, TMBIM6, to treat NSCLC effectively and safely. TMBIM6 is shown to be a critical regulator of LC cellular environment, metabolism, stress, metastasis, and survival. NSCLC patient clinical isolates have shown high levels of TMBIM6 expression both in the primary and metastatic tumors. High levels of TMBIM6 expression correlated with rapid tumor growth, tumor survival, chemoresistance, and spread (metastasis). When assessing patient populations, clinicians found a three- to fivefold reduction in patient survival in those whose tumors express high levels of TMBIM6. Inversely, the reduction of TMBIM6 expression significantly inhibits tumor growth, and in several cases, causes cancer to “commit suicide.” Data also indicate that ALL NSCLC patients would likely benefit from drugs that target TMIBM6, and that treatment response is not predicated by the varying levels of TMBIM6 expression within the different types of NSCLC.

To take advantage of the significant role TMBIM6 plays in lung cancer, MicroQuin worked with NASA on the International Space Station to develop unparalleled understanding of the structure of TMBIM6. This detailed, structural information enabled MicroQuin to be the first in the world to create drugs (MQPs) that can modulate TMBIM6’s activity without having consequence on healthy cells. To date, our data have shown that, regardless of the types of NSCLC, our drugs can cause over 50% of cancer cells to die within 48 hours and over 90% within 96 hours. And more importantly, there have been no signs of toxicity.

Optimizing MQPs has been piloted prior to this proposal, and excellent results were obtained in improving their drug-like properties. This is a 12-month project, after which we can enter formulation and IND-enabling studies for progression into clinical trials in a further 18 months. If MQP potential is realized clinically, this would create a revolutionary treatment that significantly reduces the mortality associated with NSCLC. At a minimum, our data indicate that MQPs will expand the available treatment options to patients and improving the survival and quality of life to both civilians and military Service Members alike.

Proposal Title:	Development of First-in-Class Gene Therapies to Treat Non-Small Cell Lung Cancer (NSCLC)
Log Number:	LC220040
Current PI Name:	Keith Robinson
Award Number:	HT9425-23-1-0001
Current Contracting Organization:	MicroQuin
Current Performing Organization:	MicroQuin
Web Approval Date:	11-04-2022

Rationale: Gene therapy transfers genetic material into cells, enabling them to ‘manufacture’ proteins of interest to treat diseases, like cystic fibrosis, but also non-genetic diseases, such as age-related macular degeneration; whereby cells are instructed to manufacture and secrete anti-inflammatory proteins. Gene therapy approaches can be used to convert cells into manufacturing units for therapeutic peptides. Peptides with good drug-like properties but shortcomings in absorption, posology, and dose-scheduling would significantly benefit from this approach. We developed peptide drugs (PEPDRGs) which target the novel, untouched, intracellular protein TMBIM6. TMBIM6 plays an integral role in many diseases; in oncology, TMBIM6 is overexpressed in many cancers with its knockdown inducing rapid cancerous cell death. Prior assessment of PEPDRG on treating cancers showed >50% paraptotic cell death in 24 hours of treatment, rising to >92% in 96 hours across all cancer cell lines tested. PEPDRGs are highly soluble in solvents; blood and urine and do not precipitate/aggregate even at high concentrations (>100 mg/ml) or under physiological stress conditions. PEPDRGs follow a two-compartment pharmacokinetic model and have excellent in vivo stability (85-93% remaining in the body after 24 hours), low clearance (4.1-9.3 ml/min) and efficacy. In vivo breast and ovarian cancer models showed PEPDRG induces >90% tumor shrinkage within 25 days with no measurable/observable toxicity or immunogenicity. However, the distribution phase in the two-compartment PK model reduces PEPDRG blood concentrations below its EC50, requiring frequent, daily dosing to reach an effective concentration. We propose using gene therapy approaches to convert epithelial/muscle cells, via direct DNA transfer, into PEPDRG manufacturing and secreting cells. We piloted this approach by transfecting cultured epithelial cells (IEC-6) with a PEPDRG “gene therapy” vector, which showed PEPDRGs could indeed be manufactured and secreted, without toxicity or deleterious effect; furthermore, secreted PEPDRG transferred to wells containing cancerous cells (MCF-7)-induced paraptotic cell death. We believe this novel approach would generate breakthrough technology for NSCLCs, which overexpress TMBIM6, and TMBIM6 is shown to be important in NSCLC survival, growth, and spread. This approach could negate burdensome multi-dosing, intravenous treatment schedules; minimize hospitalization; improve survivability; and enable round-the-clock cancer treatment.

Objectives:

Aim 1 (1 month): Produce 20 PEPDRG constructs.

Aim 2 (1.5 months): Assess PEPDRG production, secretion, and impact on cell viability. Transfect PEPDRG constructs into epithelial or muscle cells to assess levels of PEPDRG production, secretion, extra-cellular stability, and impact on manufacturing cell viability.

Aim 3 (3 months): In vitro assessment of secreted PEPDRG on NSCLC cell viability over a 7-day period. Identify secreted PEPDRGs that induce cell death in NSCLC cell lines and assess MOA for paraptosis.

Methods:

Aim 1: Manufacture 20 gene therapy constructs with different PEPDRGs and N-terminal secretion signals for delivery using non-viral vector approaches. Potential Pitfalls: None expected.

Aim 2: PEPDRG constructs will be transfected into epithelial (bronchial/tracheal ATCC: PCS-300-010) and intestinal (ATCC: CCD 841 CoN and HIEC-6) or skeletal muscle (ATCC: HSkMC) cells, and a time series analysis (7 days) will be performed to determine PEPDRG production (secreted vs. un-secreted), extra-cellular stability, and cytotoxicity. Potential Pitfalls: Secretion levels may be too low or production too high, resulting in aggregation; both can be overcome by modifications to the promoter region or secretion signal. Pilot data indicated cytotoxicity was not an issue.

Aim 3: ATCC lung cancer panel TCP-1016 will be seeded grown until 70-80% confluent. PEPDRG efficacy at inducing NSCLC death will be assessed first via media transfer from manufacturing cells, where a defined concentration of secreted PEPDRG is transferred into wells containing NSCLC cells. Second, addition of NSCLC cells into wells containing manufacturing cells (48hrs after transfection) to assess NSCLC response to PEPDRG concentrations over time. As described in the Statement of Work, NSCLC cell viability will be assessed over a 7-day period using numerous viability assays, with efficacious PEPDRGs having their MOA assessed using numerous kits. Potential Pitfalls/Alternative Approaches: PEPDRGs may not induce NSCLC cell death, in which case we will assess a longer timeframe for treatment (up to 200 hours). In some cases, paraptotic events such as ER vacuolation may not occur. However, provided NSCLC cell death is induced, this will be deemed a positive outcome. If a new MOA for PEPDRGs treating NSCLC is identified and this does not occur in non-cancerous cells, we consider this a positive outcome.

Impact: This proposal's proof-of-concept approach is carefully designed to establish a foundation on which to develop peptide therapeutic gene therapies while remaining open to both non-viral and viral-based delivery or pivoting to alternative approaches (i.e., mRNA). If successful, we will perform a rationalization of which delivery technology is optimal before progressing to in vivo assessment. This project could reimagine the use of existing and future peptide therapeutics as well as provide an extremely novel treatment approach that could improve NSCLC survivability and patient access to treatment and redefine the treatment paradigm for NSCLC.

Proposal Title:	Application of Multifunctional RNA Nanoparticle for Lung Cancer Therapy
Log Number:	LC220046
Current PI Name:	Jiukuan Hao
Award Number:	HT9425-23-1-0014
Current Contracting Organization:	Houston, University of
Current Performing Organization:	Houston, University of
Web Approval Date:	11-04-2022

Lung cancer is the leading cause of cancer-related deaths in the U.S. Although chemotherapy has been used for lung cancer, its effectiveness and safety are not satisfactory. The Area of Emphasis the project addresses is to identify innovative strategies for the treatment of lung cancer. The objective of the project is to design multifunctional RNA nanoparticles for lung cancer therapy with a goal to identify innovative strategies for the treatment of lung cancer.

RNA therapeutics can effectively knock-down the target gene expression involved in lung cancer development and hold an exciting promise for lung cancer treatment. However, the challenge of RNA therapeutics is their instability in biological fluids and low distribution in cancer cells. The application of RNA therapeutics is not successful because of their delivery problem. The proposed tumor-targeted multifunctional RNA-nanoparticles will improve their stability and pharmacokinetic profile and specifically target lung cancer cells to treat lung cancer.

Moreover, multifunctional drugs like the proposed multifunctional RNA-NPs could be superior in therapeutic effect and possibly reduce unwanted effects in comparison to conventional chemotherapy with monospecific drugs or polypharmaceutic combinations of different agents. The proposed approach is significant and innovative because applications of multifunctional RNA-NP for lung cancer-targeted delivery and therapy have never been explored. It will create an entirely new avenue for tumor-targeted delivery and therapy. The project will eventually contribute to eradicating deaths from lung cancer to better the health and welfare of the military and the American public.

Proposal Title:	A Novel Approach to Exploit Metabolic Vulnerability in Metastatic Lung Cancer
Log Number:	LC220053
Current PI Name:	Shengyu Yang
Award Number:	HT9425-23-1-0348
Current Contracting Organization:	Pennsylvania State University, Milton S. Hershey Medical Center
Current Performing Organization:	Pennsylvania State University, Milton S. Hershey Medical Center
Web Approval Date:	07-24-2023

This proposal will address two areas of emphasis for the FY22 LCRP:

1. Identify innovative strategies for the treatment of lung cancer.

2. Identify innovative strategies for the prevention of recurrence of or metastases from lung cancer.

Lung cancer is by far the leading cause of cancer-related death in the United States. Veterans and military personnel have high incidence of lung cancer and poorer survival compared to civilians due to the prevalence of tobacco smoking and the exposure to other risk factors such as Agent Orange, radon, asbestos, and depleted uranium. Most lung cancer patients eventually succumb to local and/or metastatic recurrence, including many patients with complete removal of primary tumor and no detectable metastasis at the time of surgery. Understanding molecular mechanisms underlying metastatic recurrence and developing novel anti-metastasis therapies is crucial to prolong the overall and metastasis-free survival of lung cancer patients. Recently, there is preclinical and epidemiology evidence suggesting that the biguanide family of metabolic drugs, such as metformin and phenformin, holds promise in the prevention and treatment of lung cancer. Biguanide drugs have been widely used in the treatment of type II diabetes and have been shown to have outstanding safety profile. However, the clinical trials using biguanides such as metformin for the treatment of cancer resulted in mixed success. This signify the importance to identify patients resistant to biguanides and to develop novel approaches ton increase sensitivities. Lung cancer cells with mitochondrial abnormalities, such as cancer cells with LKB1 mutation or mtDNA mutations are hypersensitive to biguanides, which suggested that reprograming lung cancer metabolism might be a viable approach to increase biguanide sensitivities. However, the challenge is how to specifically target mitochondrial metabolism without affecting other tissues when mitochondria plays a critical role (e.g., the heart).

Fascin is a pro-metastasis protein that is highly upregulated in metastatic tumor while absent in most normal tissues. Our lab previously discovered that fascin promotes lung cancer metastasis in part by aberrantly promoting two branches of lung cancer cell metabolism, namely mitochondrial metabolism and glycolytic metabolism. Since the ability to promote lung cancer metabolism depends on its canonical actin-bundling activity and there is a fascin inhibitor currently in clinical trial, we hypothesize that this inhibitor could be employed to selectively target the aberrant metabolism in metastatic lung cancer and could be used to increase the efficacy of biguanide treatment. Notably, since fascin expression is very low or absent in most adult tissue (with the exception of the brain), the metabolic effect of G2-44 will likely be specific to lung cancer cells while sparing normal tissues. Our preliminary studies have provided excellent support for such hypothesis. In this proposal, we will further use patient-derived organoid models and patient-derived xenograft models to examine our hypothesis. The success of this proposal will provide strong rationale to use clinical-grade fascin inhibitor G2-44 to exploit metabolic vulnerability in lung cancer, alone or in combination with biguanide metabolic drugs.

Proposal Title:	Monocarboxylate Transporter 1 as a Novel Therapeutic Target Against Small Cell Lung Cancer
Log Number:	LC220065
Current PI Name:	Ramesh Ganju
Award Number:	HT9425-23-1-0187
Current Contracting Organization:	Ohio State University, The
Current Performing Organization:	Ohio State University, The
Web Approval Date:	11-07-2022

No public abstract provided.

Proposal Title:	KRAS G12C-Specific Immunotherapy
Log Number:	LC220079
Current PI Name:	Gerald Linette
Award Number:	HT9425-23-1-0366
Current Contracting Organization:	Pennsylvania, University of
Current Performing Organization:	Pennsylvania, University of
Web Approval Date:	09-27-2023

Lung cancer is a leading cause of cancer-related death among U.S. active-duty Service Members, retired military personnel, and civilians. Tobacco use is a primary cause of lung cancer promoting DNA damage in the normal lung epithelial cells that results in the accumulation of mutations. In the past decade, translational research has revealed that a patient's immune system (of special interest, is a particular type of white blood cell called T cells) can identify the mutations that are presented on the tumor cell surface. Scientists now refer to these mutated proteins present on the tumor cell surface as neoantigens. Similar to an infectious pathogen (virus or bacteria), the tumor neoantigens can activate the body's T cells which may serve as a defense mechanism to protect the individual against cancer. In many instances however, the immune response fails to control the tumor resulting in cancer progression and uncontrolled spread throughout the body.

In the past decade, immunotherapy has revolutionized cancer treatment resulting in tumor shrinkage and in certain diseases such as melanoma, durable long-term remissions. In lung cancer, addition of anti-PD-1/PD-L1, an immune checkpoint inhibitor, to standard chemotherapy has resulted in improved survival providing definitive evidence for the role of the immune system. The challenge facing scientists is how to build on this success by designing more effective therapies that can harness the patient's own immune system. In parallel, dramatic progress in the development of new targeted agents that inhibit signaling pathways active in certain subtypes of lung cancer has resulted in improved survival. One recent example is the introduction of new oral inhibitors specific for the KRAS G12C mutation; however, many patients relapse and develop drug resistant disease.

Adoptive T cell therapy (ACT) is a novel therapeutic approach that uses the patient's own T cells to target and kill tumor cells. T cell therapies show great promise for the treatment of several blood cancers; however, progress against non-small cell lung cancer (NSCLC) has been hampered by the paucity of tumor neoantigens. Our current work is focused on developing ACT for the treatment of advanced NSCLC using a strategy that genetically modifies a patient's T cells to express a receptor (T cell receptor, TCR) that recognizes a genomic alteration in a protein, KRAS, that controls cancer growth and survival. A mutation in KRAS (mKRAS) called G12C is found in 13% of all NSCLC cases in the U.S. Our group recently identified mutated KRAS G12C as a neoantigen in lung cancer. We have isolated a new TCR that is specific for mKRAS G12C neoantigen and fails to react against non-mutated KRAS or other normal proteins. We propose to develop a new T cell therapy which will redirect the patient's T cells to target mKRAS G12C expressed in lung cancer. In this grant proposal, we seek funding that will allow us to perform key pre-clinical experiments that are required to support the regulatory filing for an Investigational New Drug (IND) application to the United States Food and Drug Administration (FDA). This filling will lead to the initiation of a phase 1 clinical trial of adoptive T cell therapy targeting mKRAS G12C. Our proposal will bring the first T cell therapy targeting mKRAS G12C to the clinic and is responsive to the FY22 LCRP Area of Emphasis, Identify innovative strategies for the treatment of lung cancer.

Proposal Title:	Role of Subtype-Specific Essential Factor Networks in Small Cell Lung Cancer
Log Number:	LC220088
Current PI Name:	Lu Wang
Award Number:	HT9425-23-1-0360
Current Contracting Organization:	Northwestern University, Chicago, Illinois
Current Performing Organization:	Northwestern University, Chicago, Illinois
Web Approval Date:	07-18-2023

The fight to eradicate the deaths of those suffering from lung cancer is still an ongoing battle. Lung cancer remains the leading cause of cancer mortality in the United States, with approximately 131,880 deaths in 2021. More importantly, there is a higher prevalence of lung cancer risk for military Service Members and Veterans versus civilians. Higher exposures to smoking and other environmental factors (carcinogens, hazardous chemicals, toxic materials, etc.) can contribute significantly to developing lung cancer during and after a military service obligation. Despite improved screenings and medical advances in lung cancer treatment, lung cancer is still mainly diagnosed at later stages when the tumor has spread to other parts of the body, leading to difficulties in finding an effective treatment option at this point.

This dilemma is especially the case for small-cell lung cancer, an aggressive form of lung cancer diagnosed at late stages for 70% of cases. Consequently, the emotional burdens of disclosing a poor prognosis can negatively impact both members of the military service and their families as they face challenges in battling a disease with limited treatment options. To overcome this barrier, discoveries in classifying small-cell lung cancer into molecular subtypes, based on the high expression of specific genes driving this cancer, can serve as a molecular fingerprint to develop more precise, target-based therapeutics. Our ultimate goal is to implement the need for a more personalized approach to small-cell lung cancer clinical treatment by targeting mechanisms that contribute to tumor growth based on the degree of molecular subtypes present.

In recent decades, small-cell lung cancer has been classified into four molecular subtypes based on their distinctive gene expression patterns. Our previous research focused on identifying a factor known as the BAP1/ASXL3/BRD4 complex, which regulates one of the most predominant molecular subtypes of small-cell lung cancer (known as ASCL1-subtype or A-subtype) by controlling its gene expression patterns. As a result, we screened and optimized small-molecule drugs that could target this complex by inhibiting its function and promoting its breakdown through degradation. Using this approach, we saw a significant reduction in tumor progression in cells and animal studies targeting the A-subtype of small-cell lung cancer. Now, we are working towards expanding this methodology to discover another target for a different molecular subtype of small-cell lung cancer, known as POU2F3-subtype (or P-subtype). In this case, we found a promising target, known as C11orf53, which is an essential, highly expressed factor that may serve as a potent therapeutic target for patients expressing the P-subtype of small-cell lung cancer.

One of the main challenges in treating small-cell lung cancer comes from the complex nature of tumors mixed with different molecular subtypes. By conducting biochemical and genetic studies, we can further identify contributing factors to each subtype, investigate the mechanistic role they play in cancer development, and create a synergistic combination of fast-acting drug therapeutics that can specifically target this mechanism by changing gene expression to reduce tumor growth. We hope our research contributions will help advance small-cell lung cancer treatment by discovering new biomarkers for specific molecular subtypes and categorizing treatment plans for patients based on their tumor gene expression profiles in the coming years.

Proposal Title:	Redirecting Viral Immunity to Eradicate Low Tumor Mutation Burden Lung Cancers
Log Number:	LC220099
Current PI Name:	Aaron Hata
Award Number:	HT9425-23-1-1000
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	09-27-2023

Immunotherapies that stimulate the immune system to attack tumors have revolutionized the treatment of lung cancer. However, while these therapies work well in some patients, not all tumors respond. Lung cancers that harbor low numbers of gene mutations (low tumor mutation burden, or TMB), such as those with EGFR mutations or ALK gene fusions, or those that occur in the absence of cigarette smoking, are especially difficult for the immune system to recognize. As a result, these patients have very few T cells that are capable of attacking the cancer. In contrast, most patients have large numbers of T cells that are capable of recognizing viruses such as CMV, EBV, or Flu that are commonly encountered in the context of normal life. These “bystander” T cells are functional but unable to participate in anti-tumor responses because the specificity of their T-cell receptor does not allow them to recognize tumor cells. In this project, we will develop a novel therapy that is capable of reprogramming tumor cells to look like they are infected by virus so that viral-specific T cells will attack them. These molecules, called Antibody Epitope Peptide Conjugates (APEC), use antibodies to deliver viral peptide antigens to the surface of the tumor cell, where they are displayed to nearby viral T cells. APECs can be tuned to match the specific viral T cells and tumor characteristics of each patient. In this project, we will develop APECs specifically designed for non-small cell lung cancers. Using patient-derived models of low mutation burden tumors and tumor explant cultures, we will test whether APECs can activate viral T cells to selectively kill cancer cells, while sparing normal tissue. We will also investigate how cancer-associated fibroblasts in the tumor microenvironment may enhance or suppress the ability for APECs to redirect bystander viral T cells. This approach of using antibodies to deliver viral antigens to tumor cells to render them immunogenic is a departure from other immunotherapy approaches that typically seek to reinvigorate existing anti-tumor T cells and has the potential to open up broad new therapeutic opportunities lung cancer patients with that do not respond to current immunotherapies.

Proposal Title:	Decreasing Lung Tumor Progression Through Transcriptional Reprogramming of Tumor-Associated Endothelial Cells
Log Number:	LC220104
Current PI Name:	Tatiana Kalin
Award Number:	HT9425-23-1-0660
Current Contracting Organization:	Arizona, University of, College of Medicine, Phoenix
Current Performing Organization:	Arizona, University of, College of Medicine, Phoenix
Web Approval Date:	09-27-2023

Compared to other organs, lung has a robust blood capillary network, providing unique microenvironment for highly metastatic lung cancers and frequent metastases from other cancers. Lung endothelial cells form tumor vessels that are essential to provide tumor cells with nutrients and oxygen to support tumor growth and metastases. Existing treatments to inhibit growth of tumor-associated vessels (anti-angiogenic treatments) have not significantly improved cancer patients' survival; there is a critical need for new approaches.

There is a general agreement that tumor blood vessels are abnormal, the endothelial layer is disorganized and leaky, making it easier for tumor cells to enter the blood circulation and to invade secondary organs during tumor metastasis. Also, the tumor vessels have decreased perfusion, preventing efficient delivery of anti-cancer drugs. "Normalization" of tumor vessel will prevent vessel leakiness and improve delivery of anti-cancer drugs.

Presence of tumor cells in the lung induces the changes in the normal lung endothelial cells (EC) and they become tumor-associated endothelial cells (TEC). The key regulators that control EC-to-TEC transition are poorly understood. Based on genome-wide comparison of EC and TEC expression profiles from human lung adenocarcinomas (LUAD) we have identified the FoxF1 gene as a critical regulator of EC-to-TEC transition. FOXF1 protein is present in normal EC but is rapidly decreased in TEC of mouse and human LUAD. Low FoxF1 predicts poor survival in LUAD patients. Our preliminary data using mouse lung cancer models show that increasing FoxF1 levels in endothelial cells inhibits lung cancer progression and metastases.

Our proposed studies will validate FOXF1 as a therapeutic target and yield important insights into the mechanisms of FoxF1-regulated lung tumorigenesis. Moreover, these studies will expand our understanding on the contribution of lung endothelial cells to tumor progression in non-small cell lung cancers and directly lead to the development of new clinical strategies to inhibit tumor progression and metastases in human lung cancers. We also propose studies to test the efficacy of nanoparticle FoxF1 delivery in mouse models of lung cancer. We will determine whether FoxF1 gene therapy will be an efficient therapeutic approach to inhibit lung adenocarcinoma progression and metastasis.

This proposal addresses following Areas of Emphasis: (1) Identify innovative strategies for the treatment of lung cancer and (2) Identify innovative strategies for the prevention of the recurrence of or metastasis from lung cancer.

The nanoparticle polymers used in this application are non-toxic and some of them (PEI, PEG) are currently used in phase 2 clinical trials in patients with advanced ovarian cancers. The minicircle vector is designed for gene therapy and can be used in humans. It consists of human DNA sequence but lacks bacterial and viral sequences as required by FDA guidelines. If our preclinical studies are successful, they can directly lead to clinical trials in patients with advanced lung adenocarcinomas within 3-5 years.

Results from the proposed studies will benefit patients with non-small cell lung cancers including lung adenocarcinomas with K-Ras mutations. NSCLC with K-ras mutations are among the most aggressive and treatment-resistant lung tumors.

Since military personnel and Veterans have twice the incidence of lung cancer and have poorer outcomes compared to the general population, the new treatment targets and therapeutics approaches proposed to be tested in this application may ultimately benefit and lead to the improved outcomes for military patients with lung cancers.

Proposal Title:	Replication Stress in Lung Cancer
Log Number:	LC220107
Current PI Name:	Ann Kirchmaier
Award Number:	HT9425-23-1-0356
Current Contracting Organization:	Purdue University
Current Performing Organization:	Purdue University
Web Approval Date:	11-09-2022

Innovation: Results will, for the first time, identify at base pair resolution where replication fork progression is compromised genome-wide in a genotype-specific manner in lung cancer lines. Results will reveal classes of fork impediments susceptible to FDA-approved or candidate drugs for treating lung cancer or that become “hotspots” for toxicity in normal cells. This fundamental work will provide new frameworks for understanding normal functions of DNA replication and repair machinery and where and why different responses to replication stress occur. This work will provide insight into how replication fork-related defects contribute to lung cancer plus provide novel replication integrity defect-related biomarkers predictive of drug susceptibility in individual lung cancers. The long-term goal is to guide custom treatments that take advantage of creating “synthetic defects” in fork integrity at impediments that become enriched in a tumor-dependent manner.

Rationale: During replication stress, forks stall or collapse (the latter resulting single or double stranded DNA breaks) at non-random sites across the genome and must be restarted or repaired using mechanisms that are distinct for different kinds of impediments. This project’s working hypothesis is that genetic defects in lung cancers lead to DNA replication stress and cause stalled or broken replication forks to accumulate at cancer- specific “hotspots” in addition to sites found in normal cells. Such “hotspots” could arise from either a defect in repair/restart of forks or a defect in another biological process that results in increased frequency of formation of certain types of impediments (e.g., R loops) within the genome. Such “hotspots” are predicted to reduce replication fidelity, contribute to tumorigenesis via errors during fork restart, as well as influence sensitivity to distinct classes of therapeutic drugs that compromise fork restart and/or impediment removal/prevention. Inhibition of pathways required for replication through identified impediments is predicted to disrupt proliferation and promote cancer cell death or senescence. The rationale for mapping stalled and collapsed forks in lung cancer lines from the NCI-60 panel is that data can be leveraged by comparing to extensive publicly available NCI-60 datasets in CellMiner and DTP databases (NCI). Genome-wide mRNA or miRNA expression, gene copy numbers, protein levels, and whole exome sequencing data (which identified normal and cancer-specific genetic variants such as insertion, deletion, missense mutations, splice site defects, etc.) is available. Similarly, thousands of compounds have been screened in these lines and their 50% growth inhibition (GI50) are available for cross- comparison. Comparing mutations unique to each cancer to responses to drugs targeting DNA repair or chromatin biology machinery and to tumor-specific stalled fork “hot spots” will facilitate identifying pathways critical for responding to different impediments. Paired tumor/normal lines will also reveal tumor-specific defects.

Objectives:

Objective 1. To map genome-wide sites where replication forks stall or collapse in lung cancer cells by TrAELseq in the nine NCI-60 lung cancer cell lines (A549, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-322M, NCI-H460, and NCI-H522) +/- FDA-approved or candidate drugs used to treat lung cancer.

Objective 2. To map genome-wide sites where forks stall or collapse in matched lung tumor/normal cells by TrAELseq using two pairs of matched cell lines (NCI-H1395/NCI-BL1395, NCI-H1437/NCI-BL1437) +/- FDA-approved or candidate drugs used to treat lung cancer.

Methods for Both Objectives: To identify stalled and collapsed forks, genomic locations where 3' ends of DNA strands accumulate (e.g., sites of partial or full fork reversal, breaks) will be determined to base pair resolution by a new NGS strategy called TrAELseq. For example, accumulation of 3' end peaks in certain mutant genetic backgrounds could reflect novel sites of fork stalling resulting from defects in the ability of the replication fork to transverse regions with impediments. A change in the width of 3' end peaks in the absence a functional gene product could indicate a defect in a step during resection needed for restart of forks stalled at certain genomic structures. To illustrate, lung adenocarcinomas that lack the tumor suppressor and SWI/SNF homolog SMARCA4 exhibit increased facultative heterochromatin formation and stalled forks by microscopy and DNA fiber analyses. Cells lacking SMARCA4 are hypersensitive to ATR inhibition, and treatment results in hyper-resection of forks, replication catastrophe, and reduced tumor growth in nude mice.

Expected Result: The proposed analysis will reveal genomic locations where forks stall or collapse based on genotype (e.g., SMARCA4 is deleted or not expressed in A549, EKVX, and NCI-H522 cells). Comparing this pattern to one, e.g., after inhibiting ATR, will reveal "hotspots" where ATR is required in the absence of SMARCA4. Comparing patterns across cell lines (taking into consideration functions in replication/repair /chromatin biology of mutated genes in a given cell line) will aid in distinguishing mutant-specific (e.g. SMARCA4-dependent) "hotspots" for replication integrity defects from sites shared across cell lines. Detailing biological mechanism(s) behind these patterns will be the subject of future studies. Changes in levels of accumulated 3' ends (peak height) or footprints (peak width) in response to two chemotherapeutic agents will be evaluated to identify loci with fork impediments that are "hypersensitive" to the drugs. This will aid future work in identifying factors that promote replication through these impediments, with the long-term goal of targeting these factors for synergistic therapeutic treatments for lung cancer.

The locations of stalled or collapsed DNA replication forks in NCI-60 lung cancer lines +/- FDA-approved or candidate drugs to treat lung cancer (Objective 1) or in ATCC matched lung tumor/normal cell lines derived from single individuals (Objective 2) will be determined. Cells will be grown +/- drug, e.g., topotecan, to inhibit Topoisomerase I (Topo1) prior to isolating DNA for TrAELseq. Topo1 creates ssDNA breaks to reduce torsional strain at, e.g., R-loops. Forks stalled at R-loops either normally or due to a mutation in a cancer that causes accumulation of R-loops, are anticipated to be (hyper)susceptible to topotecan as stalled forks would collapse.

In TrAELseq, an adenosine tail is added to 3' ends of DNAs (broken forks) with terminal deoxynucleotidyl transferase to enable addition of a biotinylated adaptor. This hairpin adaptor primes extension by Bst 2.0 polymerase (NEB), to create un-nicked dsDNAs. After fragmentation, DNAs are isolated by affinity purification using streptavidin magnetic beads (Thermo), the ends of DNAs are blunted and ligated to a second adaptor containing deoxyuracil (NEXT Ultra II, NEB), and cleaved with USER (NEB) to elute dsDNA for library amplification (Ultra II DNA Library Prep Kit for Illumina). For each condition, two biological replicates will be subjected to 150 base paired-end strand-specific sequencing of ~35 million reads per library (54 libraries for Objective 1, 24 libraries for Objective 2) (NovaSeq 6000, Novogene). Bioinformatic analyses will be conducted with a collaborator (Bioinformatics Core). Raw data will be processed and mapped to the reference genome; peak calling and annotation will be conducted using established open-source scripts; and functional analyses of peak-associated standard genomic landmarks (promoters, etc.) plus those known to stall replication forks (trinucleotide repeats, poly(dA:dT) tracts common fragile sites, G quadruplexes, palindromic DNA, R loops, etc.) will be conducted. Differential peak analyses across cell lines or conditions will enable identification of genotype-specific "hotspots" for replication integrity defects and classes of fork impediments hypersensitive to drug treatment. Select peaks of stalled or collapsed forks and ones that change in response to drug (Objectives 1 and 2) or in normal vs. tumor tissue (Objective 2) (as funding allows) will be confirmed by assessing accumulation of ssDNA binding protein (anti-RPA2, Novus) or replicative helicase (anti-MCM2, Cell Signaling Tech) by ChIP qPCR. Overlap of collapsed forks with select sequence motifs will establish class(es) of impediments that preferentially lead to stalled/collapse forks in specific genetic backgrounds or in response to certain drugs. To illustrate, TDP1 phosphodiesterase cleaves between tyrosyl and a 3' DNA end to promote removal of

TopoI-DNA complexes associated with transcription. Lung cancer lines HOP-62 and NCI-H522 contain silenced TDP1 or homozygous mutations in TDP1.

Expected Result: In these cells (but not cells with wild-type TDP1), stalled and collapsed forks will be hyper-enriched at highly transcribed sites containing R-loops, and such sites will be hypersensitive to fork collapse in the presence of topotecan.

Additional Analyses: Findings will be leveraged by comparing to public data (see Rationale) in CellMiner, DTP database, and Ingenuity Pathway Analysis (Qiagen). A second drug, a Topoisomerase II inhibitor (doxorubicin), alkylating agent (cisplatin), or ATR inhibitor (Berzosertib) will be tested in duplicate, depending on cell line-specific genotypes.

Alternatives: If unanticipated issues arise, ENDseq can alternatively be used to identify and quantify forks that stall or collapse during replication stress.

Impact: This proposed work will (a) reveal genome-wide which classes of replication fork impediments serve as inherent “hot spots” based on unique mutant genotypes of the lung cancer lines, (b) illustrate the intrinsic nature of replication-related defects caused by these mutations, (c) identify which loci and types of impediments become “hyper-susceptible” to DNA damage upon exposure to cancer drugs, given the pre-existing mutations in each lung cancer line, and (d) determine cancer-specific vs. normal replication impediments occurring in cell lines derived from individuals plus highlight potential toxic effects of drugs in normal cells. This approach could be applied broadly to other lung cancer cell lines, any proliferating tumor, and the whole NCI-60 panel +/- compounds. Long-term, this strategy should aid developing drugs for lung cancer that promote fork impediment formation or function synergistically with drugs inhibiting factors involved in repair or restart of replication forks (e.g., inhibitor of a factor involved in preventing R loop formation for use with topotecan).

Proposal Title:	Targeting Metastatic Lung Tumors with Gene-Edited and Engineered Stem Cells
Log Number:	LC220109
Current PI Name:	Khalid Shah
Award Number:	HT9425-23-1-0550
Current Contracting Organization:	Brigham and Women's Hospital, Inc.
Current Performing Organization:	Brigham and Women's Hospital, Inc.
Web Approval Date:	09-27-2023

Lung cancers are the second most common form of cancer in the United States with roughly about a 250,000 new cases each year. Non-small cell lung cancer (NSCLC) is an aggressive cancer and the 5-year relative survival rate for NSCLC patients in 25%. Owing to occupational exposure to agent orange, asbestos, diesel exhaust, etc., combined with an increased prevalence of tobacco exposure in active-duty military personnel and veterans, the risk of non-small cell lung cancer (NSCLC) is higher by 50% in these populations as compared to civilians. Despite considerable progress made in the management of advanced NSCLC over the past two decades, improvement in overall survival has been elusive, with brain metastases contributing to half of all NSCLC-related deaths. Brain metastatic-NSCLC (BMLC) tumors in the brain have multiple tumor deposits at the time of diagnosis making surgery an inadequate therapeutic option. In addition, conventional systematic therapies to treat NSCLC brain metastasis are ineffective mainly due to poor delivery of available drugs to the tumor deposits in the brain. Therefore, new therapies are urgently needed for patients with NSCLC brain metastasis.

In the ongoing search for therapeutics that can eliminate metastatic tumor deposits, oncolytic viruses have shown great potential in preclinical studies. Among therapeutic viruses, oncolytic Herpes Simplex Virus (oHSV) is one of the most promising candidates for therapy of tumors in the brain as it is an inherently neurotropic virus. oHSV has shown promising efficacy in treating various types of cancers in several animal studies. This has led to the FDA approval for talimogene laherparepvec (T-VEC; recombinant oHSV) which has shown anti-tumor immune response for distant un-injected tumor lesions. Although these studies are promising for primary tumors, there are currently no oHSV based therapeutics/strategies focused on brain metastasis, which is the major cause for NSCLC-related mortality. In our previously published studies, we have shown that mesenchymal stem cell (MSC) mediated delivery of oHSV (MSC-oHSV) extensively targets brain metastasis and single application of MSC-OHSV has therapeutic efficacy in mouse models of brain metastasis. These results although promising, have raised fundamental question for our MSC-oHSV strategy to treat BMLCs: how to boost MSC-oHSV mediated oncolytic virus mediated tumor cell killing and how to boost the immune responses to prevent tumor recurrence? Our ongoing and recent studies on the (1) creation of an in vivo animal model that authentically reproduces tumor growth and tumor progression seen in NSCLC patients and (2) the ability of MSC to track tumors in the brain and to efficiently deliver oHSV and immune system promoting biological agents provide a unique platform to develop and test new therapeutic approaches for advanced melanomas.

In this proposal, two underlying principles will be employed to develop therapies that will directly influence the future of advanced NSCLC metastatic tumors in the brain: (1) development of MSC releasing regulatable immunomodulators that selectively target metastatic NSCLC tumors in the brain and (2) testing their ability to only kill tumor cells specifically in the brain in mouse models. The specific aims of the proposed study are to evaluate the fate and therapeutic efficacy of MSC-oHSV and MSC releasing regulatable immunomodulators and immune check point inhibitor in syngeneic and humanized mouse BMLC tumor models. Once validated, these studies can be easily translated into clinics using patients own MSC or reprogrammed cells loaded with oHSV and engineered to release immunomodulators. In next 3-5 years, we

envision a therapeutic modality in which at the time of lung metastatic tumor detection in the brain, therapeutic stem cells will be systemically injected into patients to target the metastatic tumor deposits. This will have a major impact in saving the lives of many metastatic NSCLC patients, particularly military personnel who are at a higher risk of developing such cancers.

Proposal Title:	Metabolic Targets to Overcome Chemoradiation Resistance in Genotype-Defined Non-Small Cell Lung Carcinoma
Log Number:	LC220117
Current PI Name:	Henning Willers
Award Number:	HT9425-23-1-0466
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	07-25-2023

Scientific Objective and Rationale: Non-small cell lung carcinoma (NSCLC) can cause death through uncontrolled local tumor growth within the chest or metastatic spread. For tumors without metastases that cannot be surgically removed, radiation therapy (RT) is administered in curative intent. However, large tumors (= stage III) recur in the chest in up to 50% of patients after RT and chemotherapy (chemoRT), highlighting the need to identify more effective therapies. Improving chemoRT by combining it with drugs that specifically target the behavior of cancer cells may lead to better tumor eradication. A particular problem are NSCLCs that are resistant to RT at the outset; often those that carry mutations in genes such as KRAS, STK11, or KEAP1. Thus, these genes could serve as biomarkers to identify patients who would benefit from a more intensified treatment course of chemoRT plus a biological drug that is aimed at decreasing tumor recurrence rates and improving cures.

There exists currently considerable excitement surrounding the possibility that altered metabolism in cancers could be exploited for improving therapies. Findings suggest that in NSCLC there can be increased production of lipids, for example, to generate energy or make cell membranes for tumor growth. We find that blocking the production of monounsaturated fatty acids (known as “healthy type of fat”) in NSCLC cells with mutated KRAS renders these more sensitive to RT. We propose to leverage a unique institutional screening platform to examine drugs that affect cancer metabolism together with chemoRT. We will test about 2,000 combinations of drugs with NSCLC genetic features using tumor models that are grown in the lab under 3D conditions that are more physiologic than traditional cell growth on flat plastic surfaces. We seek to acquire unprecedented insight into the variation of treatment responses of NSCLC with common mutations (including KRAS and others) and how we may overcome radiation resistance by, for example, manipulating lipid metabolism in specific cancers.

Area(s) of Emphasis: This research will help us understand mechanisms of resistance to chemoRT, develop innovative strategies for the treatment of lung cancer through RT/drug combinations, and potentially identify predictive markers to assist with therapeutic decision making.

Ultimate Applicability of the Research: There currently exist no biological drugs that are combined with chemoRT in principally curable, but radioresistant lung cancers. Our research may inform strategies to modify cancer lipid metabolism which may reduce radioresistance. This would be tested in clinical trials.

What Types of Patients Will It Help and How Will It Help Them? Clinical trials informed by our research, and ultimately changes in clinical care, may benefit patients with locally advanced but non-metastatic NSCLC that are resistant to chemoRT. As a result, tumor regrowth would be less likely, patients may live longer and may have a higher chance to be cured. Interventions to overcome radioresistance could involve drugs that interfere with cancer lipid metabolism or may involve dietary supplements/modifications to achieve similar results.

What Are the Potential Clinical Applications, Benefits, and Risks? Our foundational research will provide unique insight into the ability of a broad range of drugs to manipulate metabolism in laboratory models of NSCLC before it can then be tested in clinical trials. Clinical trials with novel drugs may benefit some patients but could also increase the toxicity of treatment. Therefore, it will be critical to identify biomarkers, such as KRAS mutations, to identify patients most likely to benefit from more aggressive treatments adding metabolic drugs to chemoRT.

What Is the Projected Time Anticipated to Achieve a Clinically Relevant Outcome? We anticipate that a clinical trial based on the results of this research could be designed within about 3 years.

What Are the Likely Contributions of This Study to Advancing the Field of Lung Cancer Research? Using a unique screening platform, we will provide unparalleled insight into variations in treatment response across genomically characterized NSCLC models. Identifying new weaknesses in cancer metabolism to overcome resistance to chemoRT may inspire other researchers to pursue similar investigations. Ultimately our research will help advance precision oncology concepts in the treatment of non-metastatic, curable NSCLC – a disease that to date has been dominated by generic treatment approaches that are not tailored to the individual patient and tumor.

How Is the Project Relevant to Military Service Members, Veterans, and Their Families? Lung cancer remains a major problem for members of the military and Veterans, for example, due to increased smoking rates compared to the civilian population. Smoking is associated with genetic changes in tumors that can lead to increased rates of recurrence after radiation. Thus, novel approaches to improve the effectiveness of radiation are needed and may disproportionally benefit the military. Identifying and exploiting weaknesses in the metabolism of lipids in lung cancers will benefit U.S. military members, Veterans, and their family members suffering from this deadly disease.

Proposal Title:	Overcoming Jumonji KDM4A Oncogenic Function in SCLC
Log Number:	LC220118
Current PI Name:	Elisabeth Martinez
Award Number:	HT9425-23-1-0012
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	11-07-2022

Small cell lung cancer (SCLC) is a recalcitrant disease that rapidly acquires drug resistance and for which no mechanistically novel drug therapies have been developed over the last several decades. SCLC responds poorly to second-line therapy or immunotherapy. There is therefore a challenging yet impactful opportunity to discover new therapeutic strategies against this recalcitrant tumor type. A major obstacle to accomplishing this goal is that over 500 drugs under clinical development have shown no benefit over current first-line etoposide treatment. We have highly promising preliminary data in cell culture and in vivo mouse models that strongly support that epigenetic enzymes of the Jumonji lysine demethylase family constitute viable druggable targets in SCLC and even in disease that has acquired chemotherapy resistance. We therefore propose a new concept: that Jumonji demethylases, and KDM4A in particular, are key contributors to SCLC tumorigenesis and to its fast-acquired resistance to therapy. We have novel biological tools to probe this concept molecularly and genetically. If Jumonji enzymes do indeed play a role, then this would mean that this cancer can be targeted and tumors killed using existing Jumonji enzyme inhibitors. Indeed, the first clinical trials for Jumonji inhibitors have just been posted in gastrointestinal tumors, so translation of our work to the lung cancer clinic is feasible in the near future. This will contribute to keeping the American public healthy and will provide potential cures for Service Members who develop lung cancer from environmental and other exposures during the course of duty.

Proposal Title:	Targeting KRAS-Mutant Lung Cancers Using Novel Biologics
Log Number:	LC220157
Current PI Name:	John O'Bryan
Award Number:	HT9425-23-1-0774
Current Contracting Organization:	Medical University of South Carolina
Current Performing Organization:	Medical University of South Carolina
Web Approval Date:	09-27-2023

Background: Lung cancer is the 3rd most common cancer in the United States (U.S.) and the leading cause of cancer-related deaths in the U.S. and worldwide. Nearly 8,000 Veterans are diagnosed with lung cancer each year, and approximately 5,000 succumb to this disease. Thus, devising new and more effective therapies for lung cancer is a critical unmet need for veterans as well as the larger U.S. population.

Area of Emphasis: This proposal will specifically address the FY22 LCRP Area of Emphasis to identify innovative strategies for the treatment of lung cancer as well as identify innovative strategies for the prevention of recurrence of or metastases from lung cancer.

Objectives/Hypothesis: We hypothesize that AAV delivery of specific RAS inhibitory monobodies will provide a powerful approach to inhibit KRAS-mutant lung tumor cells and reduce the mortality of patients harboring such tumors. We also propose that this novel gene therapy method will provide an effective approach to reduce resistance that emerges upon treatment of KRASG12C mutant lung cancers with sotorasib.

Specific Aims: We propose two specific aims to address this hypothesis: (1) obtain proof-of-concept data that AAV delivery of KRAS-inhibitory monobodies suppresses KRAS-mediated signaling and transformation in vitro and (2) obtain preclinical data on the efficacy of AAV-monobody vectors for reducing tumor burden and mortality in KRAS-mutant lung cancer.

Study Design: We will develop AAV-monobody vectors to deliver expression constructs to tumor cells to inhibit the mutant KRAS. As negative controls we will also assess the effects of AAV encoding GFP alone or a GFP-tagged negative control monobody termed Mb(Neg). These reagents will be used to infect NSCLC cell lines in vitro to assess the ability to inhibit these cells. Next, we will assess their ability to inhibit lung tumor development in a genetically engineered mouse model in which and oncogenic KRAS allele drives lung tumor development. AAV vectors will be delivered to the lungs of the GEMMs to assess their ability to inhibit tumor development and progression in vivo. In addition, we will utilize patient derived tumor organoids to assess the inhibitory activity of the AAV vectors on human tumor samples. Together these studies will determine the translatability of this approach to human lung cancer patients.

Innovation: Our proposal combines several innovative technologies (AAV gene therapy and RAS inhibitory biologics) to establish an entirely new therapeutic approach for treating primary and metastatic lung cancers. These technologies include unique KRAS inhibitory monobodies developed by the O'Bryan lab and the AAV gene therapy vectors generated from the Meyer lab that will enable delivery of each of these powerful anti-KRAS biologics to lung tumors and metastatic lesions in vivo. Given the incidence and mortality of lung cancer cases, the deadly nature of this disease, and the higher incidence of lung cancer in the Veteran population, successful development of this technology will benefit service members, Veterans, their families and the American public.

Impact: We anticipate that this research will have a significant impact on the treatment of lung cancer patients, for both Veterans and the general U.S. population. Although sotorasib was recently approved for treatment of KRASG12C mutant lung cancers in early 2021, this drug is limited to only those patients

harboring a KRASG12C mutant protein. Furthermore, resistance inevitably develops following treatment with this targeted therapy, often times due to emergence of additional RAS mutations . The beauty of our proposed therapy is that NS1 inhibits all KRAS mutants and R15 inhibits >50% of mutant KRAS alleles observed in human cancer and thus both monobodies would be effective against a larger number of KRAS-mutant lung cancers and not limited to just KRASG12C mutant tumors. In addition, it would also likely be effective in reducing the emergence of non-G12C mutations as a mechanism of resistance to G12C-specific inhibitors (such as sotorasib and adagrasib).

Relevance to Military Health: Veterans are at a higher risk for lung cancer due to their use of tobacco products and their exposure to environmental carcinogens. Given the large number of deaths in veterans from lung cancer, our proposed studies are highly relevant to the development of new approaches to treat these patients. It is our hope that these studies can be rapidly translated to the clinic, resulting in better outcomes for Veterans and their families, as well as the general population affected by lung cancer.

Proposal Title:	The Use of Microfluidic Lung Cancer on a Chip in Investigating Novel Drug Resistance
Log Number:	LC220159
Current PI Name:	Takeshi Shimamura
Award Number:	HT9425-23-1-0497
Current Contracting Organization:	Illinois, University of, at Chicago
Current Performing Organization:	Illinois, University of, at Chicago
Web Approval Date:	07-25-2023

LCRP Areas of Emphasis and Military Relevance: This proposal addresses three of the FY22 LCRP Areas of Emphasis: (1) identification of innovative strategies for the treatment of lung cancer, (2) understanding mechanisms of resistance to treatment, (3) understanding predictive markers to assist with therapeutic decision-making. The incidence of lung cancer among Veterans is higher and the survival rate is alarmingly lower compared to civilian populations. Since lung cancers are often diagnosed at an inoperable late stage, developing effective therapies is necessary to improve the survival of this particularly vulnerable group. This study will help develop a novel strategy and device to ensure effective drug delivery to all types of lung tumors regardless of mutation status.

In lung cancer treatments, the therapeutic efficacy is evaluated radiographically using CT assuming that the drug concentration in the tumors has reached to its optimal level. This is due to the fact that frequent lung tumor biopsies to assess the drug concentration are not feasible as they are a burden to the patients. This poses an interesting yet important question regarding the tumors that do not respond to therapy: did the tumors not respond as the drugs failed to reach the optimal concentration or are the tumors resistant to the drugs?

Alarmingly, we have published that a subset of lung cancers reacts to drug treatment by secreting endothelin-1 (EDN1), which shrinks the diameter of tumor feeding blood vessels. The consequence of this response is the reduction of blood flow that carries drugs to the tumors. We genetically engineered lung cancer cells that are unable to express EDN1. Compared to the control cells that express EDN1 in response to drug treatment, tumors with the engineered cells with no EDN1 production maintained the blood flow to the tumors and increased the concentration of the drug in the tumors.

We propose a novel concept that tumors secrete EDN1 to shrink blood vessels around the tumors to reduce the blood flow carrying drug to the tumor, which will create a unique niche for the therapy-resistant tumors. In this proposal, we test the hypothesis that inhibiting the EDN1 binding to the EDN receptors (EDNR) on the tumor blood vessels will improve the tumor blood flow increasing the drug penetrance to the tumors. To test this hypothesis, we will evaluate whether inhibiting the EDN1–EDNR axis using FDA-approved pulmonary hypertension drugs improves the drug delivery in NSCLC cells. We will test the drug combination using conventional mouse model and unique tumor and tumor supporting cells on a chip technology. The efficacy of drugs or drug combination have been tested using mouse models, which has been laborious, time consuming and expensive. The “tumors on chip” is scalable with individual cells that associate with tumors in human so that we could analyze which supporting cells are impacting drug resistance. The chip has small channels simulating blood vessels to carry nutrients and drugs to the cells. The chip uses significantly less NSCLC patient-derived cells compared to the usual tissue culture method and it take much less time than animal models to evaluate drugs on patient tumors.

We will also investigate mechanisms by which a subset of lung cancer cell promotes secretion of EDN1. Results collected from this proposal will facilitate the discovery of prognostic and therapeutic tools to inhibit

EDN1 activity that promotes drug resistance due to poor drug delivery, and to provide a rationale to stratify NSCLC patients whose tumors stop responding to therapies for EDN1-EDNR targeted therapeutics. EDNR inhibitors have never been accessed for their therapeutic potentials to modulate blood flow and drug delivery to NSCLC tumors. Consequently, through studying the EDN1-mediated drug resistance, we will develop a new therapy to improve drug delivery and help develop tumor on a chip device to accelerate the evaluation of drug response using clinically relevant patient-derived tumors. The benefits of this radical concept include the wide applicability to patients with any types of lung cancer undergoing drug therapies. We also expect our efforts will yield a validated microfluidic platform for precision modes of NSCLC.

Proposal Title:	Investigating the Role of Nitric Oxide in the Immune Regulation of Non-Small Cell Lung Cancer
Log Number:	LC220164
Current PI Name:	Marie-Liesse Asselin-Labat
Award Number:	HT9425-23-1-0099
Current Contracting Organization:	Walter and Eliza Hall Institute of Medical Research
Current Performing Organization:	Walter and Eliza Hall Institute of Medical Research
Web Approval Date:	11-09-2022

Lung cancer remains the leading cause of cancer deaths globally, causing over 1.8 million deaths worldwide per year (Siegel et al., Cancer Statistics 2022). In the United States, lung cancer is estimated to cause 130,180 deaths in 2022, and 236,740 new cases will be diagnosed in the same year (SEER database). In the last 10 years, immunotherapy – a type of cancer treatment that activates the immune system against cancer cells – has become the first therapeutic approach for lung cancer patients. While it has improved outcomes for lung cancer patients, only 20% of patients respond to these treatments demonstrating the dire need for increasing the visibility of tumor cells to the immune system.

Tumor cells accumulate changes that allow them to multiply in an uncontrolled manner. Yet these changes also result in the presentation of “tags” on their cell surface, making them visible to the immune system as “non-self” cells to be destroyed. Significant research efforts are ongoing to identify tumor tags for developing cancer vaccines or therapies. Our proposal takes an innovative approach to identify specific characteristics in these tags, which are induced by enzymes activated in tumor cells upon exposure to toxic fumes. We will then determine how these characteristics impact the visibility of tumor cells to the immune system. This new knowledge will constitute preliminary data for the scientific and medical community to explore novel ways to improve response to immunotherapy. It will accelerate the discovery of new therapeutic targets for lung cancer, leading to the development of novel therapies in the long term.

A large contributor to lung cancer is the constant exposure to air pollutants during the deployment of military personnel, such as diesel exhaust, sandstorms, firearms fumes, and smoke from burn pits. Inhalation of combustion fumes alters the expression of different metabolic enzymes. Our proposal will address the role of these enzymes in regulating the visibility of tumor cells to the immune system, addressing an issue of huge importance to military personnel exposed to combustion fumes.

Proposal Title:	Modulating Metabolism to Sensitize Lung Tumors to Proton Therapy
Log Number:	LC220177
Current PI Name:	Gabriel Sawakuchi
Award Number:	HT9425-23-1-0430
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	07-25-2023

Background: Glutamine is the most abundant amino acid in the body. Within cells, glutamine is metabolized by the enzyme glutaminase-1 (GLS1) to produce glutamate, which is essential for producing antioxidants and energy and for synthesizing amino acids, lipids and nucleotides. An essential antioxidant generated from glutamine metabolism is glutathione (GSH). Cancer cells have high metabolic rates that generate high levels of free radicals, which can react with cellular structures with detrimental effects and cell death if not managed. To maintain balanced levels of free radicals, cancer cells often develop mutations that increase their reliance on high levels of free radicals. One such mutation is in the protein KEAP1, which in cell metabolism pushes more glutamate to form antioxidants such as GSH. Thus, KEAP1-mutant cells rely on glutamine metabolism through GLS1 to maintain the glutamate levels needed to produce antioxidants.

Notably, 15% of non-small cell lung cancer (NSCLC) tumors have mutations in KEAP1. NSCLC patients with KEAP1 mutations are prone to local recurrence after standard-of-care treatments such as radiotherapy (RT), emphasizing the need for new strategies to improve outcomes for NSCLC patients with KEAP-mutated tumors. GLS1 is a promising target that should act specifically on KEAP1-mutated tumors but not affect normal tissues. GLS1 inhibitors (GLS1i) are being tested in clinical trials as potential therapy, either alone or in combination with, for example, RT. GLS1 products (like GSH or proteins and nucleotides) are crucial in the cell response to radiation by neutralizing radiation-induced free radicals or repairing radiation-induced DNA damage. Our own work indicates that a novel GLS1 inhibitor (IACS-6274) currently being tested in a clinical trial led by Dr Yap (co-investigator of this proposal) profoundly sensitizes KEAP1-mutant lung cancer cells to proton therapy, a form of RT that specifically targets tumors while sparing normal tissues and that generates many more clustered DNA lesions and free radicals than conventional photon RT. Thus, protons+IACS-6274 hold promise for strengthening the effects of RT and could reduce local recurrence in patients with KEAP1-mutated NSCLC.

Area of Emphasis: This project proposes an innovative strategy to treat lung cancer by establishing KEAP1 as a biomarker to identify tumors that will respond to the novel combination of protons+IACS-6274.

Patient Population: Patients with KEAP1-mutated NSCLC have poor overall survival and high recurrence rates after RT. Because about 236,000 patients are diagnosed with lung cancer per year in the United States (85% of which will be NSCLC), and because 15% of those patients will have KEAP1 mutations, we expect our research to be relevant to more than 30,000 lung cancer patients per year.

Clinical Application, Benefits, and Risks: Successful completion of this project will benefit a significant proportion of patients with KEAP1-mutated lung cancer with minimal risks, because proton therapy is part of standard treatment for lung cancer patients and because IACS-6274 is only mildly toxic in humans.

Projected Time to Achieve Clinical Translation: Our team is uniquely qualified to accomplish the objectives of this project, with experts in radiobiology and proton therapy (Sawakuchi, Shaitelman, Lin, Bright), ferroptosis and metabolism (Gan), and the clinical use of IACS-6274 (Yap). We also have expertise in radiation oncology (Sawakuchi, Shaitelman, Lin), medical oncology (Yap), and medical physics (Sawakuchi). The Principal Investigator (Sawakuchi) is a board-certified medical physicist with experience in treating patients with proton therapy. Dr. Lin is an experienced radiation oncologist who treats lung cancer

patients with proton therapy. Dr. Yap is an experienced medical oncologist who led a clinical trial on IACS-6274. Our combined expertise positions us well to ultimately translate the results of this work into a clinical trial. With positive findings from this project, we will apply for funds from the DoD and other agencies to support a clinical trial to start soon after this project ends.

Advancement in the Field of Lung Cancer Research: This project will advance our understanding of how modulating cancer cell metabolism can greatly amplify the effectiveness of proton therapy, a standard of care for lung cancer treatment.

Relevance to Military Service Members, Veterans, and Their Families: Lung cancer risk is significantly higher among military Service Members and Veterans than in civilians (up to 38% vs. 14%). Our research is relevant because it aims to develop a new strategy to treat lung cancer by establishing KEAP1 as a biomarker to identify which tumors will respond to the novel combination of protons+IACS-6274. This research has the potential to significantly improve outcomes for patients with lung cancer, including military Service Members, Veterans, their family members, and the general public.

Proposal Title:	Development of O'PROTACs-Based Novel EIF4G1 Degraders for Treatment of Non-Small Cell Lung Cancer
Log Number:	LC220178
Current PI Name:	Zhiqiang Qin
Award Number:	HT9425-23-1-0083
Current Contracting Organization:	Arkansas, University of, for Medical Sciences
Current Performing Organization:	Arkansas, University of, for Medical Sciences
Web Approval Date:	11-09-2022

Lung cancer is the number one killer among cancers in the United States with an estimated 154,050 deaths (83,550 in men and 70,500 in women) expected to occur in 2018. It is also the second most diagnosed cancer in the U.S. and is responsible for approximate 234,030 new cases in 2018. As a highly heterogeneous cancer, the majority of lung cancer patients doesn't respond well to conventional therapies, thus having a poor survival rate. More importantly, many studies have indicated higher rates of lung cancer incidence and mortality among Veterans than non-Veterans, probably because the active-duty military can have more chances to be exposure to many risk factors associated with lung carcinogenesis. As one component of the translation initiation complex, although EIF4G1 has been found overexpressed in a variety of cancers, its functional roles and therapeutic application in lung cancer remain largely unknown.

Our studies will address two of FY22 LCRP Areas of Emphasis, “Understand the molecular mechanisms of initiation and progression to clinically significant lung cancer” and “Identify innovative strategies for treatment of lung cancer.” In the first Area, we plan to identify the signature of EIF4G1-controlled proteins in non-small cell lung cancer (NSCLC) cells, their individual function and clinical relevance in NSCLC patients; in the second Area, we plan to use newly developed O’PROTACs technology to design and synthesize EIF4G1 specific degraders, then screen and test the growth-inhibition effects of new degraders on a panel of NSCLC cell lines.

Our final goal is to develop clinical trials of EIF4G1 targeted therapy (alone or combination with other therapies such as chemotherapy and immunotherapy) for NSCLC patients, prolonging their survival or improving their life quality. Based on high risks for developing lung cancer in military personnel and the size of lung cancer patients in VA population, we believe that a lot of military Service Members, Veterans, and their family members (as well as public population) suffering lung cancer will benefit from our studies eventually. Although our current project focuses on NSCLC, we will test the efficacy of our new compounds to other types of lung cancer in future studies. Also, based on the results from this project, we will further modify EIF4G1 degraders to create new compounds with better specificity and efficacy.

To reach the goal of clinical application, in the current project, we will screen and identify the most effective EIF4G1 degraders against NSCLC. Moreover, we will determine which subtypes of NSCLC cells are more sensitive to our compounds. Considering lung cancer is a highly heterogeneous cancer, precision medicine is important to lung cancer treatment for achieving maximal therapeutic effects on every patient. The most effective EIF4G1 degraders from this study will be determined concerning their dosing safety, pharmacokinetics, and the in vivo efficacy of suppressing tumor progression in NSCLC xenograft mice models. These data are critically helpful to design future clinical trial for development of EIF4G1 targeted therapy in lung cancer patients.

Proposal Title:	Targeting Tinagl1 to Enhance Immunotherapy Response in Lung Cancer
Log Number:	LC220184
Current PI Name:	Minhong Shen
Award Number:	HT9425-23-1-0297
Current Contracting Organization:	Wayne State University
Current Performing Organization:	Wayne State University
Web Approval Date:	07-24-2023

Lung cancer is the leading cause of cancer-related death and accounts for more than 20% of all cancer deaths. Non-small cell lung cancer (NSCLC), which consists of 80-85% of lung cancers, is one of the most devastating diseases that threaten our health. Recently, immune checkpoint inhibitor (ICI) therapy, such as anti-PD-1/PD- L1, which boosts our own cytolytic immune cells (e.g., CD8+ T cells) to enhance immune surveillance to against tumor cells, has revolutionized lung cancer treatment. Unfortunately, the vast majority of NSCLC patients do not response to this attractive therapy. It is urgent to identify an effective biomarker to precisely identify the NSCLC patients who could benefit from ICI therapy. More importantly, it is imperative to uncover the mechanisms through which NSCLC patients develop ICI treatment resistance.

Previous study indicated that Tubulointerstitial nephritis antigen-like 1 (Tinagl1) is involved in cancer progression; however, its oncological function in lung cancer is largely unknown. Our preliminary analysis with publicly available datasets suggests that Tinagl1 is correlated with worse clinical outcomes in NSCLC patients. Moreover, Tinagl1 expression is negatively correlated with CD8 expression in patient tumor samples, and NSCLC patients who response to ICI therapy have lower Tinagl1 expression levels. These results suggest that Tinagl1 may serve as a biomarker to predict the ICI treatment response in NSCLC patients. In addition to these correlation studies, our mouse model-based in vivo experiments revealed that Tinag1 inhibits CD8+ T cell infiltration into the lung tumors, and therefore, enhances immune evasion to promote NSCLC progression.

Mechanistically, Tinagl1 inhibits tumor antigen processing and presentation to help tumor cells to escape from CD8+ T cell's recognition and killing. Encouraged by these promising preliminary data, in this proposed study, we aim to uncover the mechanism underlying Tinagl1-mediated tumor antigen presentation-suppression and the consequent immune evasion and lung cancer promoting. Given the functional importance of Tinagl1 in lung cancer promoting, we speculate that targeting this gene may have therapeutic potential. In this regard, we have developed Tinagl1 antisense oligonucleotides (ASOs). These Tinagl1-ASOs exhibited remarkable tolerability in our mouse models and can effectively knockdown endogenous Tinagl1 in vivo. Next, we will evaluate the therapeutic potential of these Tinagl1-ASOs. Considering that Tinagl1 induces immune evasion to promote NSCLC progression, we will test if Tinagl1-ASOs treatment restores tumor immune surveillance, and more importantly, we will investigate whether Tinagl1-ASOs sensitize NSCLC to CD8+ T cell-targeted therapy, such as anti-PD-1.

The outcomes of this proposed study will address the following three areas of emphasis: (1) understand the molecular mechanisms of initiation and progression to lung cancer; (2) identify innovative strategies for treatment of lung cancer; and (3) develop or optimize biomarkers to assist with therapeutic decision-making.

In addition to the exciting preliminary results we have obtained and the in vivo lung cancer mouse models we have generated, this study is also supported by an interdisciplinary team of scientists from both preclinical and clinical filed with extensive lung cancer research experience. Moreover, the proposed study is fully supported by our state-of-the-art facilities, such as an animal facility, which is essential for us to

evaluate the therapeutic potential of Tinagl1-ASOs in mouse models, as well as the Biobanking and Correlative Sciences Core, which will enable us to validate our findings in NSCLC patient samples. With these collaboration and support, we are confident in our ability to successfully carry out the proposed research project. We believe that the proposed project will provide insight into the mechanisms underlying immune evasion during NSCLC progression and further our understanding of lung cancer development in patients. Meanwhile, we expect that it will open a new avenue to develop Tinagl1 as a better biomarker to recognize NSCLC patients who could benefit from ICI treatment and to establish Tinagl1-ASOs as a novel therapeutic strategy to enhance anti-PD-1 treatment response in NSCLC patients.

Due to the higher incidence and lower survival than in civilian population, treating lung cancer is an urgent priority for military personnel and Veterans. We expect that the outcomes of this proposed study can be translated into developing more effective treatments to increase the overall survival of NSCLC patients of military Service Members, Veterans, and their families within 10 years.

Proposal Title:	Prevention of Peripheral Neuropathy Side Effect in Chemotherapy Treatment of Lung Cancer
Log Number:	LC220190
Current PI Name:	Xiangxi Xu
Award Number:	HT9425-23-1-0049
Current Contracting Organization:	Miami, University of, Coral Gables
Current Performing Organization:	Miami, University of, Coral Gables
Web Approval Date:	11-07-2022

This proposal is to test the use of low intensity ultrasonic/ultrasound treatment to counter taxane chemotherapy-induced peripheral neuropathy (a major side effect of the cancer treatment), based on our initial laboratory discovery. The removal of the dose limiting side effect will enable the optimal use of taxane to treat primary and recurrent metastatic lung cancer. Potentially, a higher dosage of taxanes can be used to counter the problem of drug resistance. The ultrasound treatment will also improve quality of life for cancer patients.

Taxanes (Taxol/paclitaxel, Taxotere/docetaxel, Jevtana/cabazitaxel) are a key group of drugs in the current treatment of several major solid tumors, including both small cell and non-small cell lung cancer, used as a single agent or in combination with additional drugs. Currently, Taxane/platinum combinations still remain the foundation in the management of advanced or metastatic non-small cell lung cancer. Docetaxel and paclitaxel as single agents are also effective in the second-line setting in advanced/metastatic lung cancer. Although generally taxanes are highly effective with tolerable side effects, sensory neuropathy, presenting as numbness and pain of feet and hands, is often the dose-limiting toxicity of the agents. Currently, cooling gloves and socks are used to reduce drug exposure during chemotherapy with uncertain or limited success, but no satisfactory methods are available to prevent or reverse this side effect of taxanes.

We have made a surprising discovery that low-intensity ultrasound treatment can effectively and completely neutralize the cytotoxic effect of taxanes on cells in culture. To apply this discovery, our proposed study is to develop the use of ultrasound to prevent the side effect (sensory neuron toxicity) of taxanes in treating patients. This approach may be especially attractive as ultrasound treatment can be targeted and limited to local regions, such as the hands and feet, instead of systematically affecting the whole body. Thus, our ultrasound treatment may overcome taxane toxicity only in hands and feet to prevent peripheral neuropathy without affecting the efficacy of the taxane drug to kill cancer cells elsewhere.

The biological basis is that taxane kills cancer cells and causes neuronal toxicity by stabilizing cellular microtubule bundles, and local ultrasound shock waves break the microtubule bundles so to eliminate the effect of the drug in the targeted neuronal cells without affecting the killing of cancer cells. In this research proposal, we plan to understand further the science underlying our discovery and to explore questions and gain information to plan a procedure for a practical clinical intervention. The work includes the further study of ultrasound effects on primary neuronal cells in culture, in animal models, and the evaluation of suitable ultrasound devices for optimal efficacy. Our goal includes the formulation of a clinical protocol and consideration of the details of a clinical trial based on the study. The goal is that by the end of this 1-year laboratory study, or even before, we will be able to immediately carry out a clinical trial to use low-intensity ultrasound to prevent taxane side effect in chemotherapy treatment of lung cancer patients.

This research project will likely impact on common chemotherapy using taxane: removing the dose-limiting side effect of the therapy and enabling effective treatment of patients with primary and recurrent metastatic breast cancer. The overcoming of the side effect will enable an increased dose or prolong treatment with

taxanes, either in the initial treatment or salvage therapy, which will surely extend the survival and improve the quality of life of lung cancer patients. The results of the proposed study may be able to guide the costumed design and manufacture of an ultrasound device that is optimal in the treatment of taxane-induced peripheral neuropathy. If the project is supported to proceed and will be successful, I believe that our findings and study will have clinical application and impacts within a few years. If found effective, ultrasonic treatment may become a common procedure to counter the side effect of peripheral neuropathy following chemotherapy.

In summary, the ultrasound treatment is common and is known to be safe, and our goal of a clinical study is very feasible. We have developed this idea based on our laboratory study and findings. The potential outcomes are that we may be able to prevent the taxane-induced side effect of peripheral neuropathy. Removal of the dose-limiting side effect will enable the optimal use of taxane to treat metastatic and recurrent lung cancer and an increased drug dosage to counter the expected common issue of drug resistance. By the end of the project, we will produce a practical plan ready to carry out a clinical study. We are excited and believe that our proposed research, if supported, will have an immediate clinical impact.

Proposal Title:	Targeting Small Cell Lung Cancer by Systemic Delivery of an Oncolytic Virus Via a Stem Cell Carrier
Log Number:	LC220226
Current PI Name:	Mohamed Hammad
Award Number:	HT9425-23-1-0045
Current Contracting Organization:	City of Hope Beckman Research Institute
Current Performing Organization:	City of Hope Beckman Research Institute
Web Approval Date:	12-27-2022

Lung cancer is one of the most lethal cancers in the United States. Most patients become resistant to chemo- and radiotherapy, leading to cancer progression and death. Killing cancer cells using viruses (virotherapy) is a promising approach that enhances the immune system's recognition and destruction of cancer cells. Clinical trials have shown that this approach is safe, but it is limited by the rapid inactivation of the virus by the immune system. Our goal is to overcome this barrier by delivering cancer cell-killing viruses using clinically safe neural stem cells, which we have determined can selectively migrate to tumors throughout the body. We have demonstrated that our cells can protect viruses from inactivation by the immune system and improve delivery to tumors. Once delivered, the viruses infect tumor cells and continue to multiply, with greater tumor-killing effects until normal tissue is reached. We successfully developed and are conducting clinical trials using these cells to deliver an adenovirus to selectively kill brain cancer cells. We have tested another novel oncolytic virus against different cancer cell lines and found it potent against lung cancer cell lines. In the proposed study, we will modify these cells to express this novel oncolytic virus and then test it in mouse models of lung cancer. We hope our novel approach will improve clinical outcomes, minimize toxic treatment side effects, and improve the quality of life for patients with advanced lung cancer.

Proposal Title:	RNA Medicine-Enhanced Immunotherapy and Targeted Therapy for Non-Small Cell Lung Cancer
Log Number:	LC220240
Current PI Name:	Yan Tang
Award Number:	HT9425-23-1-0069
Current Contracting Organization:	Brigham and Women's Hospital, Inc.
Current Performing Organization:	Brigham and Women's Hospital, Inc.
Web Approval Date:	11-09-2022

Scientific Objective and Rationale: Tumor relapse and spreading to secondary organs (metastasis) may occur after initial treatments, arising from minimal residual tumor cells that can survive these treatments. Treatments targeting specific genes that drive tumorigenesis and tumor progression are called targeted therapy, which is more efficient in killing fast growing tumor cells. However, a small percentage of tumor cells can transform themselves into a cell state that mimics hibernation to evade drug treatment; these are called dormant persisters. After cessation of treatment, tumor persisters regain normal growth, repopulate tumor, and metastasize. A co-mentor of this project and colleagues have identified that, after targeted therapy, tumor persisters turn on YAP1 expression to enable entering the dormant cell state to survive the treatment, which leads to tumor relapse. However, how YAP1 drives entering dormancy and eventually regrowing of persisters is not known. Downstream effector genes of YAP1 need to be identified to fully understand this cell state transition. DNA is tightly wound into a complex called chromatin, which must be opened up to enable gene expression. This process is called chromatin (epigenomic) remodeling. How YAP1 affect chromatin remodeling during the development of persisters is not clear.

Immunotherapy is developed to use the power of the body’s own immune system to prevent, control, and eliminate cancer. Our preliminary single cell analyses of resected tumors from NSCLC patients who have received immunotherapy identified tumor persisters after immunotherapy that are also characterized with high expression of YAP, which is associated with suppressed immune system and diminished therapeutic efficacy. These persisters coexist and interact with the immune system in the tumor ecosystem, which is called the tumor microenvironment (TME), in a way that favors the growth of tumor cells and suppresses the immune system. In addition, tumor cells create favorable microenvironment for proliferation by continuously crosstalk with surrounding normal cells. Therefore, it is important to understand the spatial structure of TME.

Most of genes regulating expression and chromatin remodeling, called transcription factors, are undruggable due to the lack of small molecule binding pocket or cellular localization. We have developed nanoparticles that can deliver small interfering RNA (siRNA) molecules to lung and tumor cells to regulate the expression of these transcription factors and thus re-sensitize tumor cells to therapy. This is call RNA therapy. Theoretically, any gene can be targeted using siRNAs for therapeutic intervention. siRNA therapy represents one of the most recent breakthroughs in medicine. FDA approval, in 2018 and 2019, of two siRNA-based therapies (Patisiran and Givosiran) for genetic diseases represents a landmark for RNA medicine. One of advantage of siRNA therapy is its long-lasting therapeutic effect. For instance, twice-yearly administration of siRNA therapeutics has been shown sufficient to achieve optimal therapeutic effect, an unprecedent achievement in the pharmaceutical history.

In this study, we aim to reveal the role of YAP in the development of tumor persisters, to identify YAP-mediated alterations in gene expression and chromatin remodeling that enable tumor evasion from targeted treatment and immunotherapy, and to examine how YAP-high persisters educate immune system to avoid attacking tumor cells. We will also evaluate the therapeutic efficacy of transformative targeted therapy and

immunotherapy which are enhanced by RNA medicine. This project directly addresses the FY22 LCRP Areas of Emphasis to (1) identify innovative strategies for the prevention of recurrence of or metastases from lung cancer and (2) understand mechanisms of resistance to treatment (primary and secondary).

Career goals of the PI: Dr. Tang is dedicated to delineating multi-layer regulatory mechanisms that drive relapse of tumor from minimal residual disease after targeted treatment or immunotherapy, using cutting-edge single cell technologies. In this project, the PI aims to reveal alterations in gene expression and chromatin remodeling essential for tumor persisters to survive targeted/immunotherapy, to identify potential therapeutic targets that will block transition from drug-sensitive to drug-resistant cell state, and to develop a transformative RNA medicine that will eliminate tumor persisters and enhance targeted/immunotherapy. This award will protect her time and funding to complete proposed research, a critical step toward her career goals.

Ultimate Applicability of the Research: The outcome of this project will benefit all patients with NSCLC, including military Service Members and Veterans. Scientifically, this project will generate a list of potential therapeutic targets that affect gene expression and chromatin remodeling to sensitize tumor persisters to targeted/immunotherapy. Translationally, the RNA therapy developed in this project has the potential of rapid clinical translation.

Proposal Title:	Evaluation of In Silico-Designed mutP53 Anti-Misfolding Small Molecules in Lung Cancer
Log Number:	LC220257
Current PI Name:	Donald Weaver
Award Number:	HT9425-23-1-0426
Current Contracting Organization:	Treventis Corporation
Current Performing Organization:	Treventis Corporation
Web Approval Date:	07-25-2023

Rationale: One of the reasons lung cancers are so dangerous is because, in such cancers, a gene called p53 is often modified in such a way as to affect the body's ability to fight it (e.g., trigger cell death or growth arrest of tumors). Instead of being an "anti-cancer" gene, mutated p53 (mutP53) turns into a "pro-cancer" gene that does a variety of things to negatively affect treatment, one of which is to hijack various proteins to promote cancer progression. In fact, mutP53 protein has a distinct, misfolded shape that enables it to "clump" with itself and other cancer inhibitive proteins. These clumps of protein have additional unhealthful affects in fighting cancer.

Objective: This project is attempting to discover and test new drugs able to stop mutP53 from clumping. Building on innovative work in designing anti-clumping drugs for other diseases that involve misfolded proteins (e.g., Alzheimer's disease, ALS), we have used a computer-based model of misfolding proteins and some laboratory systems for looking at misfolding of mutP53 to discover starting points for drug discovery. We have discovered nine such starting points so far that can stop mutP53 from clumping and kill cancer cells carrying mutP53 on the one hand, while ignoring cells that have normal p53 on the other.

Aims:

1. We will begin by testing our nine starting points, as well as some molecules that resemble them, to determine how effective they are at halting the clumping of mutP53 in a cell-free system. We will use a number of different methods to confirm that the anti-clumping effect is true.

2. We will then test the best molecules from Aim 1 in cell-based systems to show that they indeed work on cellular mutP53, as well as have an inhibitory effect on lung cancer cell growth, and that these anti-cancer effects are through mutP53 (as opposed to being just a coincidence).

3. We will, finally, test the best molecules from Aim 2 in animal models of cancer to confirm that anti-clumping molecules for mutP53 can indeed halt the growth of lung cancer cells in a living animal.

Applicability:

Overarching Challenge: There is an unmet medical need to develop a new therapy for lung cancer. The proposed study will revolutionize lung cancer treatment regimens by providing an innovative drug that will have the ability to slow the progression of the disease and improve treatment outcomes by minimizing the effects of mutP53 on lung cancer cells.

Military Impact: While the Department of Defense spends over $1.6 billion per year in health-related outcomes due to tobacco use, the 5-year survival rate of those suffering from non-small cell lung cancer

(NSCLC) remains despairingly low. Upon a successful completion of the proposed project, we expect to produce one or more small anti-cancer molecules with the ability to drastically improve survival and standard of living of those suffering from the disease.

Translatability: Ultimately, a compound that treats misfolded mutP53 in cancer could be of value in other cancers that have misfolded mutP53. It could be added to current therapy with the potential to improve outcomes including longer progression-free survival and reduced metastatic potential. It is not a traditional “chemotherapy” and would not be expected to have the same kinds of serious side effects as most chemotherapy. It is, however, a new approach and will have to be carefully tested for safety.

Translational Timeline: The potential drugs discovered in this project will still require several years of optimization post-project and animal testing before putting into clinical trials; but if successful, this work will establish a new way to attack cancer using anti-mutP53 misfolding agents, which is a significant advance that is directly on the pathway to new therapies.

Proposal Title:	Targeting Notch Signaling to Augment Immunotherapy in Small Cell Lung Cancer
Log Number:	LC220270
Current PI Name:	Nitin Roper
Award Number:	HT9425-23-1-0093
Current Contracting Organization:	The Geneva Foundation
Current Performing Organization:	National Cancer Institute
Web Approval Date:	11-07-2022

Immunotherapy has revolutionized the treatment of cancer, particularly cancers of the lung. Today, because of immunotherapy, many patients with metastatic lung cancer have a chance for long-term remission. However, the greatest benefit with immunotherapy is among patients with non-small cell lung cancer (NSCLC). For patients with small cell lung cancer (SCLC), the benefit of immunotherapy has been modest and long-term remissions are rare. In the United States, 34,000 people are diagnosed yearly with SCLC with a mortality rate of 95%. Moreover, SCLC is a significant cause of morbidity and mortality within the Veteran and military Service populations.

Until recently, very little has been known about why response to immunotherapy in SCLC is modest. Last year we discovered that activation of a particular pathway known to be suppressed in SCLC, the Notch pathway, is enriched in SCLC patients with clinical benefit to immunotherapy. This work is among the first to show a specific mechanism that may be altering immune response in SCLC.

In our proposal, we seek to further understand why patients with SCLC with activation of the Notch pathway have the greatest benefit to immunotherapy. The project will focus on the following LCRP Areas of Emphasis: (1) identify innovative strategies for the treatment of lung cancer and (2) develop or optimize prognostic or predictive markers to assist with therapeutic decision making. Through experiments with SCLC preclinical models, we will decipher whether the Notch pathway boosts activity of a key immunomodulatory protein, known to be important for anti-tumor immune response. Moreover, we will use animal models to assess whether clinical stage pharmacologic drugs that activate the Notch pathway can boost the anti-tumor immune response. We will also assess whether high expression of a critical Notch pathway gene in SCLC patient tumors is associated with better survival with the addition of immunotherapy to chemotherapy.

The studies in this proposal are anticipated to lead to new clinical trials designed to augment immunotherapy in SCLC within 2-3 years. Such clinical trials will be of great importance to improving outcomes of SCLC patients, including those within the Veteran and military Service populations. The goal of these studies and future clinical trials is to develop immune based therapies that will lead to long-term remissions for all patients with SCLC.

My career goal is to become a physician-scientist studying and developing new therapies for patients with SCLC. This award will provide the means to study clinically relevant questions and provide a strong basis for me to continue studying SCLC in my own independent laboratory.

Proposal Title:	PP2A: A Promising Biomarker and Therapeutic Target in Small Cell Lung Cancer
Log Number:	LC220287
Current PI Name:	Ravi Salgia
Award Number:	HT9425-23-1-0581
Current Contracting Organization:	City of Hope Beckman Research Institute
Current Performing Organization:	City of Hope Beckman Research Institute
Web Approval Date:	09-27-2023

Objective and Rationale: Lung cancer (LC) is the number one cause of cancer death in Veterans and military Service members in the United States. Surgery is the most effective therapy for solid tumors, and ~700,000 cancer patients have tumors removed each year. However, despite removal of the primary tumor, up to 20% of patients develop local recurrences and ultimately die. Local recurrences occur due to tumor cells that are not removed at the time of surgery and ultimately regrow. If human tumor tissue could be more readily visualized and better differentiated from normal tissue during surgery, cancerous lesions could be completely removed, reducing the probability of local tumor recurrence.

Although several agents and strategies have been identified to prevent LC, an optimal strategy that does not cause drastic side effects has not been achieved. Therefore, there is an urgent need for more effective agents that can safely prevent LC development and spread without causing unwanted toxic effects. One approach to produce novel compounds is through the rational modification of well-established preventive agents. The resultant compounds may target cancers through mechanisms of action similar to those of the original agent, but with enhanced potency, reduced toxicity, and lower dose requirements. We have developed one such agent, LB100 (a PP2A inhibitor), which has shown promising effects in our preliminary studies. Our long-term goal is to develop this rationally designed, effective, and safe agent to treat and prevent SCLC.

Areas of Emphasis: Our proposal is directly relevant to LCRP Area of Emphasis, “To understand the molecular mechanisms of initiation and progression to lung cancer.”

Ultimate Applicability: What Types of Patients Will It Help and How Will It Help Them? Treatment with the novel small molecule LB100 will protect members of the general public from developing SCLC. LB100 will also help to control already existing tumors and reduce the recurrence of SCLC, thereby contributing to the treatment of all SCLC. What are the potential clinical applications, benefits, and risks? As per our preliminary studies, LB100 has been effective against SCLC in vitro and in vivo. The proposed studies will assess the bioavailability of LB100 in mice and assess its protective effects against SCLC in mouse models. In addition, this project will also assess and confirm the ability of miRNA and proteomic biomarkers, identified in our preliminary studies, to guide future clinical SCLC prevention trials. The overall results of the proposed studies will help us to determine how effective and generally applicable LB100 treatment may be to prevent the development of SCLC and to control aggressive, existing SCLC. Our preliminary studies also show that LB100 is effective and well tolerated in vivo without any overt toxicity.

What Is the Projected Time Anticipated to Achieve a Clinically Relevant Outcome? We expect to complete the proposed studies within 3 years. Phase 1 and 2 trials could follow within a reasonable time frame, after approval from our Institutional Review Board (IRB) and an Investigational New Drug (IND) submission to the FDA. Public funding through foundation grants for preclinical and clinical studies is feasible if our results are promising.

What Are the Likely Contributions of this Study to Advancing the Field of LC Research? The overall impact will be to reduce the incidence of SCLC and to improve the length and quality of life of patients with metastatic disease. The influence of this work will be enhanced by City of Hope's status as a "Comprehensive Cancer Center" with unique facilities for clinical trials, enabling us to rapidly translate our findings for further on-site validation in preventive care. The efficacy and broad spectrum of the effects of LB100 in SCLC provides a strong rationale for its applicability to a wide range of SCLC tumor types. As described above, we believe that the direct clinical application of LB100 to prevent or treat SCLC is quite feasible given its fundamental role in regulating critical LC processes and pathways. These studies will also provide a range of important information regarding the efficacy, mechanisms of action, and response milestones to lead to clinical trials of LB100.

How Is the Project Relevant to Military Service Members, Veterans, and Their Families? Military Service Members and Veterans may be more prone to LC than the general public due to a higher likelihood of smoking and smoking more heavily. They also have a higher likelihood of exposure to cancer-causing agents during their service that may translate into a higher incidence of LC. Therefore, if successful, the proposed therapeutic approach will potentially have the greatest effect in active military Service Members and Veterans but will also provide their families and the general public with significant health and economic benefits.

Proposal Title:	The Oral Periodontal Pathogens and Checkpoint Blockade in NSCLC
Log Number:	LC220290
Current PI Name:	Qingsheng Li
Award Number:	HT9425-23-1-0667
Current Contracting Organization:	New York University Langone Health
Current Performing Organization:	New York University Langone Health
Web Approval Date:	09-27-2023

Lung cancer causes more death than colorectal, breast, and prostate cancers combined. Therapies targeting T cell inhibitory checkpoint pathways can provide durable responses to common cancer including lung cancer at unprecedented rates. Yet only a minority of patients respond to these treatments. New evidence suggests that gut bacteria affect immunotherapy response. Our oral cavity harbors the second number of bacteria after our gut. Studying requires studying whether oral bacteria behave like gut bacteria to influence immunotherapy response in lung cancer patients.

Based on LSL-Kras mouse lung cancer model we have found that some oral bacteria that drive periodontitis greatly promote lung cancer development while some oral bacteria associated with healthy gum didn't influence lung cancer development. P gingivalis, one of the major oral bacteria found in periodontitis patients not only promoted lung tumor development but also reduced the power of checkpoint inhibitors on lung cancer treatment. Thus, our work has revealed functional interconnections between oral bacteria, spontaneous lung tumor development, and anti-tumor reaction to checkpoint inhibitors. In this proposed project, we will work to address the following questions: What are the mechanisms that underline the link between the oral pathogen bacteria and lung tumor resistance to checkpoint inhibitors therapy? Can we determine oral bacteria that affect the resistance of lung cancer to neoadjuvant checkpoint inhibitors in NSCLC patients? Can we modify oral bacteria to sensitize them to checkpoint inhibitor treatment? We think that oral periodontal pathogens such as P gingivalis can modulate lung cancer development and/or the therapeutic response of lung tumor to checkpoint inhibitor therapy.

This project will emphasize one of the important LCRP Areas, Understanding mechanisms of resistance to treatment (primary and secondary). Suppose we can find specific oral bacteria commonly seen in periodontitis linked to lung cancer development and/or the outcomes of immunotherapy; in that case, we should have better ways to target those bacteria and will have immediate implications in immunotherapy for lung cancer patients. This project is also useful for scientists to learn fundamentally important information about how oral bacteria function is related to lung cancer development. This project will help early-stage lung cancer patients receive checkpoint inhibitors new adjuvant therapy and may extend to all lung cancer patients, in particular Veteran patients, to develop and maintain good oral care habits, and eventually increase the survival rate in patients with lung cancer.

Proposal Title:	Early Lung Cancer Detection Using Plasmonic Nano-Aperture Label-Free Imaging of Cancer Exosomes
Log Number:	LC220312
Current PI Name:	Steven Lin
Award Number:	HT9425-23-1-0639
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	09-27-2023

Although lung cancers still carry high mortality, early detection of lung cancer at the earliest stages allows for curative management of the disease. Patients who have are at risk of developing lung cancers, particularly those with more than 20-pack-per-year history of smoking, are currently being screened for lung cancer using yearly low dose computed tomography (CT) scans. However, a CT scan is highly sensitive but often not specific enough to render a definitive diagnosis. Not knowing ahead of time which nodules are benign or malignant obligates the need for additional procedures such as a biopsy or surgery can subject the patient to potential harms if the nodule reflects a benign process. There is a strong need for complementary testing to provide additional specificity of the LDCT for lung cancer. An ideal test will be a blood test, also known as “liquid biopsies,” that would be sensitive enough to detect cancer specific elements even at early stages of disease. The most commonly used liquid biopsies to detect the presence of cancer is to measure levels of cancer-specific DNA released in the blood. In advanced cancers, sufficient levels of spilled DNA is found in the blood to make a liquid biopsy a very convenient way to identify cancer-specific genetic alterations that could help direct the use of specific drugs targeting the specific mutated proteins. However, early-stage cancers fill very little DNA in the blood, and therefore makes cancer detection extremely difficult. Because of the high level of false negativity, liquid biopsy looking for cancer-specific DNA is not a very sensitive test to be very informative about the nature of a lung nodule found on LDCT.

Exosomes are small cell bodies or vesicles actively secreted by cells as a way to discharge excessive amounts of proteins, lipids, DNA, and RNA and as a form of communications between cells locally and at distance. Compared to normal cells, cancer cells tend to secrete high levels of exosomes due to the relatively higher levels of metabolic activity that generates much more byproducts of cellular activity. We have developed a small device called integrated biochip imaging technology (iBIT) that allows direct measurement of exosomes from a small drop of blood. The contents of the exosomes could be evaluated further using specialized probes to measure the contents of the exosomes at the same time. Because this is a specialized microscope that could count the labeled exosomes directly, we could directly measure and distinguish the presence of cancer exosomes and the proportion of total exosomes in that drop of blood that belongs to cancer. We have found that iBIT is a highly sensitive test that could easily identify the presence of cancers, even stage I cancers of multiple origins, and is able to distinguish cancer patients from healthy subjects with >98% specificity.

Our proposal for the Fiscal Year 2022 Lung Cancer Research Program Area of Emphasis is on identifying innovative strategies for the screening and early detection of lung cancer. We believe iBIT is a highly sensitive and specific test that could be used to complement LDCT screening by rendering the verdict on whether a nodule could be benign or malignant. While the research is still early, we believe iBIT has so far achieved impressive performance for us to take it to the next level to evaluate clinical samples from lung cancer screened patients. These precious samples come from a Department of Defense-funded study that was developed several years ago to collect biospecimen from military personnel at the Veterans Administration hospitals across the United States that were being screened for lung cancer. For this two-year project we will provide the evidence that iBIT could be developed for lung cancer screening purposes. This will benefit all

patients who are currently being screened using LDCT and therefore will have a great impact on military personnel and their family members to affect early lung cancer diagnosis and treatment.

Proposal Title:	Identifying an Innovative Strategy to Enhance Efficacy of the Chimeric Antigen Receptor T-Cell-Based Immunotherapy Against Lung Cancer
Log Number:	LC220315
Current PI Name:	Qin Yu
Award Number:	HT9425-23-1-0013
Current Contracting Organization:	Icahn School of Medicine at Mount Sinai
Current Performing Organization:	Icahn School of Medicine at Mount Sinai
Web Approval Date:	11-07-2022

Lung cancer is the leading cause of cancer related death worldwide, and non-small cell lung cancer (NSCLC) accounts for 80%–85% of lung cancers. The chimeric antigen receptor (CAR)-T cell therapy offers curative potential for lung cancer patients. In the CAR-T therapy, autologous T cells isolated from cancer patients are engineered with the chimeric receptors that recognize surface antigens of cancer cells. The engineered T cells are then infused back to cancer patients to allow the CAR-T cells to track down, bind, and kill cancer cells. The CAR-T cell-based therapy has been successful in patients with B-cell leukemia/lymphoma and is being developed against solid tumors including lung cancer, but with limited success so far.

The mechanisms underlying the insufficient solid cancer responses to the CAR-T therapy include (1) inadequate selections of CAR-T targeted tumor antigens; (2) antigen escape of tumor cells; (3) immuno-suppressive tumor microenvironment (TME); (4) inadequate ability of CAR-T cell trafficking, tumor infiltration, expansion, and persistence; and (5) CAR-T cell exhaustion. This proposal offers an innovative solution for last two major problems.

CD44 is a cell surface receptor for hyaluronan; is up-regulated in many cancer types; and promotes cancer progression including that of lung cancer. CD44 is also referred as lymphocyte homing receptor and is a prominent marker that distinguishes memory and effector T cells from their naïve counterparts. CD44 is known to play important roles in enhancing T cell receptor-signaling and T cell migration, proliferation, and survival. Lymphocytes mainly express hematopoietic form of CD44 (CD44H). The CD44-related cancer studies have been exclusively focused on cancer cells, and little is done to investigate the contributions of CD44+ T/memory T cells to cancer progression and response to immunotherapy.

This proposal plans to identify an innovative strategy for the treatment of lung cancer by establishing that the CD44 armored CAR-T cells represents an innovative strategy to enhance efficacy of the CAR-T cell-based therapy against lung cancer (an Area of Emphasis). The proposal is based on our novel finding that an immune checkpoint inhibitor (ICI) displayed reduced efficacy against lung cancer in CD44-null mice comparing to wild type (wt) mice and the established but unexplored CD44 functions in T cells. We hypothesize that CD44 expressed by T cells plays critical roles in enhancing efficacy of the immune checkpoint inhibitors against lung cancer via promoting T cell infiltration into and expansion/survival in lung cancer and by inhibiting T cell exhaustion and that constitutive CD44H expression in CAR-T cells, such as the ROR1-targeting CAR-T cells, enhances efficacy of the CAR-T cell-based therapy by promoting intra-lung cancer infiltration, expansion, and survival of the CAR-T cells.

Two Specific Aims are proposed. Aim 1 is to establish that CD44 expressed by T cells plays critical roles in enhancing efficacy of the immune checkpoint inhibitors against lung cancer by promoting T cell infiltration

into and expansion/survival in lung cancer and by inhibiting T cell exhaustion. Aim 2 is to establish that constitutive expression of CD44H in the ROR1-targeting CAR-T cells enhances efficacy of the CAR T cell therapy against lung cancer by promoting CAR-T cell expansion, migration, and survival.

Successful accomplishment of this proposal will establish (1) CD44 expressed by T cells as a novel determinant of efficacy of the immune checkpoint inhibitors and the CAR-T cell-based immunotherapy and (2) that CD44 plays critical roles in promoting T/CAR-T cell infiltration into and expansion/survival in lung cancer and in inhibiting T/CAR-T cell exhaustion, as well as (3) that constitutive expression of CD44H in the ROR1-targeting CAR-T cells enhances efficacy of the CAR-T cell therapy (short-term impact). Positive results will lead to rapid clinic translation of the CD44 armored CAR-T cells to improve efficacy of the CAR-T therapy against lung cancer and to improve clinical outcomes and survival rates of lung cancer patients (long-term impact).

This proposal supports mission readiness through filling a gap in cancer treatment that would likely have a profound impact on the health and well-being of Service Members, Veterans, and their families, as well as the health of the civilian population, and therefore, is of high biologic and clinical relevance and significance and high therapeutic potentials and impact.

Proposal Title:	Engaging Retiring Military Personnel in VA Lung Cancer Screening Through a Codesigned Communication Campaign
Log Number:	LC220328
Current PI Name:	Gemmae Fix
Award Number:	HT9425-23-1-0944
Current Contracting Organization:	Bedford VA Research Corporation, Inc.
Current Performing Organization:	Bedford VA Research Corporation, Inc.
Web Approval Date:	09-27-2023

Lung cancer kills 5,000 Veterans per year, or 14 Veterans, on average, every day. Veterans and military Service Members are at increased risk of lung cancer because of their age, smoking history, and possible military exposures to Agent Orange, asbestos or burn pits. Lung cancer screening saves lives. It can reduce lung cancer deaths by 20%, which would translate to ~1000 fewer Veteran deaths per year. People are eligible for lung cancer screening if they are between the ages of 50-80 years, currently smoke cigarettes or quit within the past 15 years, and have at least 20 pack-years total smoking history. Too many people – including both Veterans and retiring military Service Members – are eligible for lung cancer screening but do not get screened, often because they are not aware lung cancer screening is available for them. The U.S. Department of Veterans Affairs (VA) has excellent lung cancer screening services, but this program typically focused on Veterans who are already enrolled in VA healthcare. Retiring military Service Members are an important but overlooked group to make aware of lung cancer screening. Smoking is widespread throughout the military, with almost 30% of Veterans reporting current tobacco product use. More than 20,000 Service Members of an age eligible for lung cancer screening retire each year. Yet, the VA does not currently have an outreach program to let retiring military Service Members know about VA's national, high-quality lung cancer screening program, and we intend to change that. Our VA-based research has been working to improve lung cancer screening for Veterans. In this study our team will learn from retiring military Service Members what is important to include in a lung cancer screening campaign targeting other retiring military Service Members. We will then work with a group of retiring military Service Members to co-design a lung cancer screening website, social media advertisements, such as on Facebook, and mail information sheets. Finally, we will test these three ways of communicating about lung cancers to see if this is a feasible and acceptable approach to reach retiring military Service Members and, if the campaign changes, how they think about lung cancer. The resulting communication campaign will help the VA tell retiring military Service Members about VA's lung cancer screening program. This project provides an innovative strategy for the screening and early detection of lung cancer.

Proposal Title:	Interpretable Machine Learning for Molecular Discovery in Lung Cancer
Log Number:	LC220330
Current PI Name:	Haitham Elmarakeby
Award Number:	HT9425-23-1-0023
Current Contracting Organization:	Dana-Farber Cancer Institute
Current Performing Organization:	Dana-Farber Cancer Institute
Web Approval Date:	11-07-2022

Scientific Objective and Rationale: Non-small-cell lung cancer (NSCLC) is the leading cause of cancer-related death in the United States and worldwide. Each year, more people die of lung cancer than of colon, breast, and prostate cancers combined, and more than 130,000 people will die from the disease in the United States during 2022. NSCLC is the most common type of lung cancer, accounting for more than 80% of the cases.

Currently, stratification for treatment of NSCLC patients depends on a set of predictive molecular markers that are associated with better outcome. For example, patients with EGFR mutations are candidates for targeted therapy, while patients with high PD-L1 expression or high tumor mutation burden (TMB) are candidates for immunotherapy in the case of lacking known markers of targeted therapy. Despite the advances in identifying actionable alterations, these predictive markers are not perfect, and most NSCLC patients do not respond to treatment and have their tumors progress to the aggressive stage. We hypothesize that no single molecular feature is enough to predict response in NSCLC patients; rather, a coordinated and interacting set of features that may have better predictive utility is needed. In this research, we will develop a novel machine learning model that is guided by known cancer biology to identify a set of features, genes, and known and novel pathways that explain the different responses and manifestations of NSCLC disease. This will help understand mechanisms of resistance in unselected populations and identify novel therapeutic targets opening the door for developing new treatments. Our proposed research directly addresses the FY22 Lung Cancer Research Program Areas of Emphasis of (1) understand the molecular mechanisms of initiation and progression to lung cancer and (2) understand mechanisms of resistance to treatment.

Principal Career Goals in Lung Cancer Research: My goal is to become a leader in the field of intelligent cancer informatics with focus on lung cancer. My research program combines data-driven approaches with intelligent computational modeling to understand mechanism of resistance and progression. This award will give me the resources and protected time to gain skills and training needed to achieve my goals. I have identified a set of training opportunities including formal courses, professional workshops, and international and national meetings that will help me enhance my scientific profile and connect with the professional community. My mentor, Dr. Eliezer Van Allen, is a pioneer in the field of clinical computational oncology and has a unique record of using genomic data to understand treatment resistance in multiple cancer types. In addition, Dr. Kenneth Kehl is an expert in lung cancer with significant contribution to the field of clinical natural language processing. My mentorship team provide valuable training to achieve my career goals.

Ultimate Applicability of the Research: Our goal is to understand the mechanisms of resistance to immunotherapy, targeted therapy, and aggressive disease. Understanding mechanisms of resistance and aggressiveness will help identify novel therapeutic targets opening the door for developing new therapeutic intervention. It will also help in identifying patients with a high risk of recurrence or progression and understanding their unique molecular composition. This can lead to better risk stratification of early-stage NSCLC patients who may have high-risk molecular features. The discovered mechanisms of resistance and aggressiveness will guide therapeutic development, patients' stratification, and clinical decision-making in

NSCLC patients. This applies to affected duty Service Members, Veterans, military beneficiaries, and the American public, leading to better clinical outcomes especially in resistant and aggressive NSCLC patients.

Proposal Title:	Improve the Efficacy of Lung Cancer Therapy by Targeting the EDN1 Axis
Log Number:	LC220380
Current PI Name:	Takeshi Shimamura
Award Number:	HT9425-23-1-0713
Current Contracting Organization:	Illinois, University of, at Chicago
Current Performing Organization:	Illinois, University of, at Chicago
Web Approval Date:	09-27-2023

Background: In lung cancer treatments, therapeutic efficacy is evaluated radiographically using Computed Tomography (CT) with the assumption that the drug concentration in the tumors has reached its optimal level. This is because frequent lung tumor biopsies to assess the drug concentration are not feasible as they are a burden to the patients. This poses an interesting yet important question regarding the tumors that do not respond to therapy: did the tumors not respond as the drugs failed to reach the optimal concentration or are the tumors resistant to the drugs? Alarmingly, our preliminary results suggest that a subset of lung cancers react to drug treatment by secreting endothelin-1 (EDN1), which temporarily shrinks the tumor feeding blood vessels. The consequence of this response is the reduction of blood flow that also delivers drugs to the tumors. We genetically engineered lung cancer cells that are unable to express EDN1. Compared to the control cells that express EDN1 in response to drug treatment, tumors with the engineered cells with no EDN1 secretion maintained the blood flow to the tumors and increased the concentration of the drug in the tumors.

Areas of Emphasis: This proposal addresses the FY22 Lung Cancer Research Program (LCRP) Areas of Emphasis by identifying innovative strategies for treatment and by understanding mechanisms of resistance to treatment. The outcome of this proposal will facilitate the use of FDA-approved Endothelin receptor (EDNR) inhibitors to help mitigate poor drug penetrance. The incidence of lung cancer among Veterans is higher and the survival rate is alarmingly lower compared to civilian populations. Since lung cancers are often diagnosed at an inoperable late stage, developing effective systemic therapies is necessary to improve the survival of this particularly vulnerable group. The study will initially test the utility of ENDR inhibitor to ensure the delivery of the FDA-approved inhibitors targeting mutated Kirsten RAS (KRAS) G12C as the frequency of KRAS G12C mutation among non-small cell lung carcinoma (NSCLC) patients exceeds 30%. The results from the initial study will eventually be applicable to all types of lung tumors regardless of mutation status or drug types.

Objectives: We propose a novel concept that tumors secrete EDN1 to reduce drug delivery through blood flow to the tumor, which will create a unique niche for the therapy-resistant tumors. In this proposal, we test the hypothesis that inhibiting the EDN1 binding to the EDN receptors (EDNR) on the tumor blood vessels will improve the tumor blood flow increasing the drug penetrance to the tumors. To test this hypothesis, we will evaluate whether inhibiting the endothelin-1 – endothelin receptor axis improves the drug delivery in NSCLC cells. We will also investigate mechanisms by which a subset of lung cancer cell promotes secretion of EDN1. Results obtained from this proposal will facilitate the discovery of prognostic and therapeutic tools to inhibit EDN1 activity that promotes drug resistance due to poor drug penetrance, and to provide a rationale to stratify NSCLC patients who become refractory to therapies for EDN1-EDNR targeted therapeutics. EDNR inhibitors have been tested in lung cancer trials in an attempt to inhibit the mitogen activated protein kinase (MAPK) pathway with no success. EDNR inhibitors have never been accessed for their therapeutic potentials to modulate blood flow and drug delivery to NSCLC tumors. Importantly, the EDN1-mediated restriction to the drug delivery to the tumors can be reversed by interrupting drug treatments that is essentially giving tumors a drug holiday. Consequently, the EDN1-mediated drug resistance can be

linked to the mechanisms of re-sensitization of tumors with “drug holiday,” that is frequently observed in clinics yet not fully understood.

Impact: This concept is clinically innovative as the FDA-approved EDNR inhibitors used to treat pulmonary arterial hypertension can be safely repurposed for this study. If other molecular experiments proposed in this application give credence to the translation of the hypothesis, the clinical translation of the idea presented here will be realized in a few years. Historically, researchers have focused on targeting EDNR to inhibit downstream signaling with no success. Our proposal challenges this notion in the cancer research field by the concept that the EDNR inhibition increases the concentration of the drugs in lung tumors by increasing blood flow. The benefits of this radical concept include the wide applicability to patients with any types of lung cancer undergoing drug therapies while the risks may include that the increased blood flow by EDNR inhibitors to the tumors may help tumors to grow if they are insensitive to the drug combined with EDNR inhibitors. Close monitoring of the tumor growth should easily overcome this adverse effect.

Proposal Title:	Exploring Metabolic Vulnerability of KRAS G12C Inhibitor Resistance Using a Microfluidic Multiarray Platform
Log Number:	LC220390
Current PI Name:	Takeshi Shimamura
Award Number:	HT9425-23-1-0486
Current Contracting Organization:	Illinois, University of, at Chicago
Current Performing Organization:	Illinois, University of, at Chicago
Web Approval Date:	07-25-2023

In 2022, in the U.S., 236,740 new cases of lung cancer patients are estimated to be diagnosed. The United States military population has greater exposure to hazardous chemicals and cigarette smoking. These factors lead to a remarkably higher incidence of lung cancer compared to the civilian population. Although great advances have been made, lung cancer has a poor prognosis because it is often diagnosed at advanced stages when patients cannot benefit from surgery and rely on traditional chemotherapy or targeted therapies if possible.

Although KRAS is the most frequent gene mutated in lung adenocarcinoma, traditional chemotherapy has been the primary treatment for NSCLC patients with KRAS mutations. Chemotherapy has only shown promise when combined with immune checkpoint inhibitors, but it does not benefit all patients. Until recently, targeted therapies against KRAS have not been successful, but specific inhibitors of the most frequent KRAS mutation, G12C, have shown promising results in vitro and in clinical trials leading to the FDA approval of sotorasib. Nevertheless, de novo or acquired drug resistance to these types of inhibitors have already been shown; therefore, it is crucial to identify the mechanisms underlying drug resistance, and to develop methods to overcome this issue.

Tumor cells try to evade drug treatment pressure with the activation of countless different mechanisms; thus, resistant cells show a completely different phenotype. All the alterations derived from the acquisition of drug resistance rely on changes in the epigenome to activate or deactivate certain genes. Some of these adaptations that tumor cells engage to survive create additional vulnerabilities that can be targeted pharmacologically, providing new treatment strategies to overcome drug resistance. This proposal aims to understand the changes occurring during acquired G12Ci drug resistance in order to design new therapeutic approaches.

We have found a strong correlation between G12C inhibitors' resistance and NNMT and its nuclear localization, an essential enzyme that can modulate the whole epigenetic landscape affecting a myriad of cellular processes. The result of this proposal is to describe unknown mechanisms of drug resistance and provide an alternative therapeutic approach for the U.S. Veterans that are not responsive to this drug treatment. This two-year study will validate that NNMT inhibition in KRAS G12C resistant tumors is a realistic and promising therapeutic treatment that will benefit Veterans. This proposal addresses three FY22 LCRP Areas of Emphasis by understanding mechanisms of resistance to treatment, developing predictive markers and identifying innovative treatment strategies that will benefit the military population.

Proposal Title:	Integrin-Targeting Transformable Nanomedicine for Treatment of Non-Small Cell Lung Cancer
Log Number:	LC220425
Current PI Name:	Ruiwu Liu
Award Number:	HT9425-23-1-0358
Current Contracting Organization:	California, University of, Davis
Current Performing Organization:	California, University of, Davis
Web Approval Date:	07-05-2023

The objective of this project is to develop novel and highly effective nanotherapeutic agents for treatment of non-small cell lung cancer via increasing tumor-specific targeting and therapeutic effect of chemotherapy, while reducing systemic toxicity. This nanoplatform involves two-component, two-step, intravenous administration. After injection, component 1, which is a nanoparticle, will bind to a protein, named 31 integrin, on the tumor cell surface and enriched at the tumor site, transformed from the nanoparticle to the nanofibrillar network. After clearance of nanofibrils/nanoparticles from normal organs in a couple of days, the second component, which is a chemodrug conjugated with a chemical that can efficiently linked to the surface of the nanofibril at the tumor sites, can be administered, resulting in slow drug release to kill the cancer cells. In this proposal, we will prepare and characterize those smart transformable nanoparticles and evaluate their toxicity and anti-cancer effect in mice bearing non-small cell lung cancer tumors. If successful, the novel nanomedicine to be developed in this application could provide an innovative strategy for treatment of non-small cell lung cancer and benefit patients including military Service Members, Veterans, and their family members.

Proposal Title:	Spatial Profiling of Early, Osimertinib-Resistant Brain Lesions in EGFR-Mutant Lung Cancer Models
Log Number:	LC220427
Current PI Name:	Swarnali Acharyya
Award Number:	HT9425-23-1-0704
Current Contracting Organization:	Columbia University Medical Center
Current Performing Organization:	Columbia University Medical Center
Web Approval Date:	09-27-2023

It is estimated that 40% of cancer patients with EGFR-mutant lung cancer develop brain metastasis during their disease, and the incidence continues to rise in the clinic. Brain metastasis is associated with poor prognosis and a sharp decline in motor and cognitive skills, which compromises day-to-day functioning and accelerates patient death. Fortunately, this dismal scenario has improved with the advent of third- generation EGFR tyrosine kinase inhibitors, such as osimertinib, which show excellent early responses in the clinic, even in patients with brain metastases. However, despite striking initial responses, osimertinib-treated patients eventually develop relapse, often to the brain, and succumb to death. It is therefore important to understand the underlying mechanisms of brain metastasis to improve targeted therapy outcomes, which is an unmet need in the field and can positively impact the lives of lung cancer patients.

The reappearance of cancer after a striking initial response on osimertinib implies that cancer cells continue to reside in organs below the detection limit. This prompts the following questions: How do these cancer cells escape osimertinib? What are the adaptive programs that help them resist osimertinib? Are there cell-intrinsic or cell-extrinsic changes, or a combination of both? Addressing these biological questions is important because residual cancer cells possibly represent the seeds of future relapse. We hypothesize that a combination of cancer-cell-intrinsic and cancer-cell-extrinsic (microenvironment) signaling drives the survival of these early lesions in the brain. Understanding the biology of residual cancer cells could help us design more effective targeting strategies to prevent future brain recurrences and prolong patient survival.

It is challenging to study residual disease in human patients when cancer is below the detection limit of imaging and it is unclear where residual cells are located. In such instances, mouse models that recapitulate the phases of drug response, residual disease, and relapse in the physiological context would enable the profiling and analysis of these cells. However, most studies on osimertinib resistance have relied on in-vitro cultures, short-term subcutaneous tumor implantation models, or sequencing of human tumors to detect genetic mechanisms of osimertinib resistance. To address this deficiency, we recently published a study describing the generation of osimertinib treatment-response-and-relapse mouse models using human lung cancer cells harboring osimertinib-sensitive, EGFR-activating mutations to study the mechanisms of brain relapse (Biswas et al., Cancer Discovery, 2022). Using these two independent mouse models of osimertinib resistance, we defined the distinct phases of osimertinib response, residual disease, and brain relapse. We will further leverage our preclinical models in this proposal to identify and profile osimertinib-refractory residual disease and relapse, which has not been previously studied and represents an unmet need in the field of lung cancer.

We have developed an innovative new platform known as spatially VISBL (Visualize, Interrogate, Small, Brain-Lesions) collaborating with a team of experts in single-cell and spatial transcriptomic profiling. Spatially VISBL enables us to profile cancer cells and their surrounding microenvironment. This new pipeline is modeled after rapid autopsy platforms used in the clinic that allow for the preservation of brain tissues within 3 hours of death for molecular analysis. Spatially VISBL combines rapid whole-brain vibratome slicing from fresh tissue with fluorescence microscopy and spatial and single-cell transcriptomics.

Using this platform, we have designed the following specific aim with associated subaims (a) and (b): Identify cancer-cell-intrinsic and -extrinsic pathways that sustain residual cells in the brain using the spatially VISBL platform; (a) Single-cell and spatial profiling of cancer cells in the brain and their surrounding microenvironment using the spatially VISBL (Visualize, Interrogate, Small, Brain-Lesions) platform; (b) pathway analysis to identify cancer-cell-intrinsic and -extrinsic pathways that sustain residual cells in the brain after osimertinib treatment. These profiles will be analyzed to identify druggable cell signaling pathways that are activated during different stages of brain lesions (from early to late) and can be targeted by pharmacological strategies in the near future. This proposal addresses the following overarching challenges in the FY22 Lung Cancer Research Program (LCRP) mission: (1) identify innovative strategies for the prevention of recurrence of or metastases from lung cancer and (2) understand mechanisms of resistance to treatment (primary and secondary). These studies will be beneficial for preventing brain relapse in patients with lung cancer that affects military personnel and their families, as well as the civilian population.

Proposal Title:	Transforming Behavioral Health Care to Improve the Quality of Life for Individuals with Childhood-Onset Lupus
Log Number:	LR220016
Current PI Name:	Natoshia Cunningham
Award Number:	HT9425-23-1-0937
Current Contracting Organization:	Michigan State University
Current Performing Organization:	Michigan State University
Web Approval Date:	08-25-2023

Childhood-onset systemic lupus erythematosus (childhood-onset lupus) is a chronic autoimmune disease diagnosed prior to 18 years of age. People diagnosed in childhood are at a greater risk of severe illness. People with childhood-onset lupus often experience depression, anxiety, fatigue, and pain. These symptoms can affect their health and reduce their quality of life. Fortunately, these symptoms can be improved with appropriate care and support. Yet people with childhood-onset lupus often fail to receive such care.

Our team has created a brief and tailored 6-session weekly program, Treatment and Education Approach for Childhood-onset lupus [TEACH] to help people with childhood-onset lupus. TEACH uses cognitive behavioral therapy and can be delivered in person or remotely. We have found more than 90% of people who enroll in TEACH complete it. TEACH improves aspects of emotional health and physical health immediately after the program is completed. There is a clear benefit to TEACH with no known risk. Unfortunately, TEACH is not available to most with childhood-onset lupus. We would like to make TEACH available as part of routine medical care.

Our research team includes physicians, scientists, people with lupus and their family. We are working with multiple rheumatology clinics in the US and Canada. At each site, we will train members of the rheumatology team (e.g., nurses, social workers) to serve as mental health champions. The champions will receive training in TEACH and will deliver it to people who need it. Our goal is to test how TEACH works in real world settings. These results will help us to design better programs to reach more people with lupus, to help them to feel better, and to improve the quality of their lives.

The study aims to learn:

(1) How TEACH impacts emotional health and physical health of people with childhood-onset lupus.

(2) How TEACH impacts emotional health in patients with childhood-onset lupus over time (1 year).

(3) How best to incorporate TEACH into routine clinical care.

This proposed study aligns well with the focus areas of the Department of Defense Lupus

Research Program. By training champions in TEACH, we expect to improve the physical and emotional health of people with childhood-onset lupus. We believe this study will transform care and improve quality of life for such patients.

Proposal Title:	Use of Double-Humanized Mice to Study Diverse Manifestations of SLE
Log Number:	LR220019
Current PI Name:	Xin Luo
Award Number:	HT9425-23-1-0343
Current Contracting Organization:	Virginia Polytechnic Institute and State University
Current Performing Organization:	Virginia Polytechnic Institute and State University
Web Approval Date:	05-04-2023

In recent years, we and others have used mouse models to demonstrate that gut microbiota plays important roles in systemic lupus erythematosus (SLE). However, the functions of specific gut bacteria appear to be distinct depending on the mouse model of choice, indicating the need for a better preclinical model for SLE: one that closely resembles not only the disease but also the interactions between gut microbes and the human host. Therefore, in this Idea Award project, our goal is to establish a “Double” humanized SLE mouse model (the DhuSLE model), in which the mouse would both: (1) possess an autoimmune-prone human immune system and (2) harbor gut microbiota originating from SLE patients. We will transplant patient fecal microbiota into the best available mouse model of the human disease, huNSG-pristane, and ensure the absence of endogenous mouse microbiota via two different methods: antibiotics-mediated removal and the use of germ-free mice. More importantly, we will use this novel model to test the hypothesis that SLE patients exhibit different clinical signs due to differences in their gut microbiota. This is an entirely new concept that will direct future investigations into whether and how human gut microbiota affects human SLE pathogenesis. We have the experience, tools, and clinical samples in hand to test this hypothesis. The novel DhuSLE model will become the best preclinical model for testing new prophylactic and therapeutic agents and will enable clinically relevant mechanistic studies into human SLE pathogenesis. Upon completion, the proposed studies could also initiate a new research direction: mapping specific SLE manifestations to signatures in the gut microbiota. Our long-term goal is to use gut microbial biomarkers to predict disease manifestations for newly diagnosed SLE patients, which would provide the basis for personalized medicine and thus benefit drug selection and patient prognosis.

Proposal Title:	Restoration of Macrophage Function as a Strategy to Prevent/Treat Lupus
Log Number:	LR220023
Current PI Name:	Michele Kosiewicz
Award Number:	HT9425-23-1-0377
Current Contracting Organization:	University of Louisville Research Foundation, Inc.
Current Performing Organization:	University of Louisville Research Foundation, Inc.
Web Approval Date:	05-04-2023

Lupus is a highly complex disease, and very difficult to prevent or treat effectively and safely. Women are much more likely to develop lupus than men, but the reasons for this difference are still not completely understood. Hormones clearly play a role; however, more recently accumulating evidence suggests that the gut microbiota (the beneficial bacteria that live in the gut) may have a significant effect on development and progression of lupus. In our preliminary studies, we have found that fecal transplants (i.e., feeding gut microbiota/bacteria) from male (lupus-resistant) mice to female (lupus-susceptible) mice suppress disease (lupus nephritis) and increase survival in the recipients. We would like to understand how this male microbiota protects female mice from disease, and we have identified a molecule (a metabolite) that the male gut bacteria produce that may be involved in the protection. Patients with lupus have problems with many different types of white blood cells. One of type of white blood cells that may not only help cause the disease, but may also help make it worse, is a cell called the macrophage. Macrophages are scavenger (trash-collecting) cells whose job is to remove dead cells in healthy people. However, these cells do not work very well in lupus patients. The result is the accumulation of dead cells that then activate other white blood cells resulting in the production of anti-nuclear antibodies (ANA) and other types of antibodies. ANAs and the other antibodies can get stuck in important organs such as the kidneys and cause dangerous inflammation and damage. Our female mice have a very similar problem with their macrophage function that also results in production of ANAs and ultimately, in fatal lupus nephritis. The male microbiota-associated metabolite that we have identified is able to significantly improve macrophage function in female mice so that they are capable of removing dead cells (trash collecting) very effectively. We believe that improving macrophage function could prevent the development of disease in at-risk individuals and/or control progression of disease in patients with established disease. In this proposal, we plan to study how our metabolite improves macrophage function in our mouse model of lupus, and very importantly, determine whether it has similar effects on white blood cells from patients with active lupus nephritis. We will test these effects with white blood cells from both mice and lupus patients in culture and also directly in the mice themselves, using state-of-the-art technology. This metabolite has low toxicity and is a natural constituent of red meat, dairy products, and some fish. We believe that this metabolite either alone, or possibly in combination with other therapies, has great potential to be developed into a therapy for the prevention and/or treatment of active lupus.

Proposal Title:	Diagnostic Utility of Antibodies to Histone Post-Translational Modifications in Lupus in Children
Log Number:	LR220027
Current PI Name:	Chandra Mohan
Award Number:	HT9425-23-1-0367
Current Contracting Organization:	Houston, University of
Current Performing Organization:	Houston, University of
Web Approval Date:	05-04-2023

Various serum antibodies (Abs) are commonly used for the diagnosis of systemic lupus erythematosus (SLE). Anti-nuclear antibodies (ANA) are used as one of the diagnostic criteria for SLE, but they display poor diagnostic specificity. Anti-DNA and anti-nucleosome antibodies have been reported to fluctuate with renal disease in several studies, but they have suboptimal diagnostic potential. Anti-nucleosome antibodies appear earlier than anti-dsDNA Abs, but it is not known if additional specificities can be detected even earlier. In reality, the antigens that trigger lupus are heavily subjected to a multitude of chemical changes called “post-translational modifications” (PTM). Ab to nucleosome PTMs in SLE have not been comprehensively studied, and their diagnostic significance remains poorly explored.

Current Knowledge Gap

1. The complete repertoire of Abs targeting such chemically modified nucleosomal epitopes is currently a black box. Given that a wide spectrum of such chemical modifications has been reported, it is imperative that we study Abs to these modified nucleosomes in SLE comprehensively because these fine specificities are likely to have diagnostic significance as well as relevance to disease pathogenesis.

2. Unfortunately, previous technological platforms to assay Abs to these modified nucleosomes have suboptimal diagnostic performance. Importantly, a novel 3-dimensional, fluorescent bead-based assay has recently been pioneered by industrial partner, EpiCypher that relieves this bottleneck. This novel platform has enabled the proposed studies.

Fiscal Year 2022 Lupus Research Program Focus Areas addressed: Understanding how lupus disease heterogeneity impacts risk of disease, disease presentation, clinical course, and outcomes; using a diverse range of research disciplines including, but not limited to, biopsychosocial studies, personalized medicine, variation in treatment studies, health economics, socioeconomic studies, environmental studies, and epidemiological studies

We hypothesize that antibodies targeting such chemically modified nucleosomal epitopes could exhibit superior diagnostic potential and pathogenic relevance in lupus. The objective of this academia-industry partnership is to test this hypothesis using a novel, high-throughput, fluorescent bead-based platform bearing a comprehensive battery of chemically modified nucleosomes/histones and several well-annotated SLE cohorts.

Research Strategy: Two Aims Are Proposed

Aim 1: In this Aim, we propose to identify novel autoantibodies to chemically modified nucleosomes, using two newly designed 50-plex nucleosomes antigen panels.

Aim 2: To validate the diagnostic utility of autoantibodies to selected histone/nucleosomal post-translational epitopes using an extended cohort of SLE/LN patients and disease controls. This includes the testing of serum from patients before the diagnosis of lupus.

The impact of this Idea Award include the identification of novel autoantibodies (and custom Luminex test panels) that could be of potential utility in the following clinical settings: (i) antibodies that best discriminate pre-SLE and/or SLE from HC with high sensitivity; potential utility: initial or early diagnosis of SLE in someone who does not yet meet all classification criteria for SLE, (ii) antibodies that best discriminate SLE from rheumatic disease controls with high specificity; potential utility: accurate initial diagnosis of SLE, (iii) antibodies that best discriminate active LN from inactive SLE and active nonrenal SLE; potential utility: identification of renal involvement in a patient with SLE, (iv) autoantibodies that correlate strongly with disease activity; potential utility: monitoring SLE/LN patients during follow-up for oncoming changes in disease activity, including renal flares, and (v) autoantibodies that correlate strongly with concurrent renal pathology; potential utility: non-invasive assessment of renal pathology/damage and assessment of response to induction therapy.

The availability of reliable serum biomarkers that can accurately diagnose (a) SLE and (b) renal involvement in SLE can both prompt earlier treatment in this disease. This is highly significant and impactful, because starting treatment earlier has been shown to improve long-term outcome in lupus nephritis by several independent studies. Hence, the proposed studies may serve to reduce morbidity and mortality in SLE and improve patients' quality of life.

Proposal Title:	Molecular Basis of Blood-Brain Barrier Breach in Lupus
Log Number:	LR220028
Current PI Name:	Chandra Mohan
Award Number:	HT9425-23-1-0408
Current Contracting Organization:	Houston, University of
Current Performing Organization:	Houston, University of
Web Approval Date:	05-04-2023

Cognitive Impairment Is Common in Lupus: The prevalence of neuropsychiatric systemic lupus erythematosus (NPSLE) varies from 40.3% to 91%, and NPSLE represents a major source of morbidity in systemic lupus erythematosus (SLE), and the second leading cause of mortality after lupus nephritis. Cognitive impairment is the most common manifestation of NPSLE, affecting 10%-80% of SLE patients. Although NPSLE and cognitive impairment are common in lupus patients, we have no way of monitoring these manifestations in the clinic and we do not understand their molecular basis.

Breach in Blood Brain Barrier (BBBB) in SLE: In all individuals, a physical barrier exists between the circulating blood and the brain, called the blood brain barrier (BBB). Recent observations show that ~25% of all SLE patients have extensive BBBB (based on neuroimaging), associated with brain grey matter shrinkage and cognitive impairment. This could contribute to disease since blood proteins can readily gain access to the brain if this barrier is breached. Again, our understanding of this barrier breach in lupus is a black box.

Knowledge Gap: We are now faced with several unanswered questions. What proteins are responsible for BBBB in lupus? What proteins are associated with cognitive impairment in SLE? Can these proteins actually cause BBBB or cognitive impairment? Can tracking these proteins help monitor the progression of disease in NPSLE? Can these proteins lead to novel therapeutics for managing NPSLE patients? To sum, we do not have a good understanding of the molecular basis of cognitive impairment and BBBB in NPSLE. Here, we propose to identify serum proteins/autoantibodies that are indicative of extensive BBB breach and/or cognitive impairment in SLE, using two unique patient cohorts characterized for BBBB using brain imaging, with concurrent serum samples, representing the largest such cohorts in the world.

FY22 LRP Focus Areas Addressed

(1) Understanding the biological mechanisms of lupus disease including, but not limited to, studies of informative/rare patients

(2) Determining the pathobiology of end organ injury related to lupus disease in target human tissues

We hypothesize that selected serum proteins may be associated with BBBB, and actively breach the BBB. The objective of this proposal is to test this hypothesis through two Aims. Exploratory serum proteomics will be carried out in a “Test” cohort, followed by validation in a “Validation” cohort in Aim 1. Mechanistic studies are proposed in Aim 2.

Impact: These studies could have a significant impact on the management of NPSLE in several respects:

(1) They could uncover novel serum proteins for the diagnosis and/or follow up of NPSLE (since such proteins are currently non-existent). In a patient diagnosed to have SLE, the emergence of these novel serum proteins or autoantibodies in circulation may signal the onset of NPSLE. Future studies are warranted to examine if fluctuations in the levels of these proteins are indicative of active NP disease, using serially collected samples.

(2) They may identify potential outcome measures for NPSLE trials, for which biomarkers are lacking.

(3) They may shed light on the pathogenesis of BBBB and cognitive decline in NPSLE, which is currently a black box.

(4) They may pave the path toward more selective therapies for NPSLE patients with cognitive deficits. For example, if a particular protein is associated with cognitive deficits in NPSLE patients (based on Aim 1) and is also shown to induce neuronal injury in pre-clinical models (based on Aim 2), these proteins can potentially be therapeutically targeted. Of importance, we do not currently have any therapeutics that are specifically targeted toward disease manifestations associated with NPSLE.

Proposal Title:	Metabolic Target Identification in Age-Associated B Cells
Log Number:	LR220031
Current PI Name:	Michael Cancro
Award Number:	HT9425-23-1-0264
Current Contracting Organization:	Pennsylvania, University of
Current Performing Organization:	Pennsylvania, University of
Web Approval Date:	05-04-2023

B cells are cells of the immune system that make antibodies. Antibodies are proteins that target invading pathogens for destruction. The immune system of a healthy person is self-tolerant; in other words, the immune system normally does not attack the cells and organs of one's own body. However, in lupus and other autoimmune diseases, self-tolerance breaks down, and B cells make antibodies to one or more components of self. These autoantibodies contribute to disease symptoms.

Nobody knows how self-tolerance is breached in autoimmune individuals, or why people with disease continue to produce these pathogenic antibodies. We recently discovered a tolerance checkpoint that raises a barrier to B cells responding to one type of self-molecule. We found ways that the checkpoint could be circumvented, leading directly to a type of B cell that is strongly associated with lupus called T-bet-positive age-associated B cells (Tbet+ ABCs).

The tolerance checkpoint can be envisioned as a network of molecules that are in a resting state inside a mature B cell. When the B cell is "activated" by encounter with a component of self, one of two things can happen: the cell divides briefly and then dies; or the cell survives, and eventually becomes a Tbet+ ABC that can give rise to antibody producing cells. We have so far identified several key molecules in this network that influence this death-versus-survival fate. Now our objective is to characterize the metabolic architecture that underlies formation and survival of ABCs.

Until recently, metabolism was thought to only play a "housekeeping" role that was similar in all cell types. Research of the past decade has shown that instead, different metabolic processes play very different roles, even within the same cell lineage. The studies proposed here are designed to delineate the metabolic pathways that support and promote the development of Tbet+ ABCs. The overarching objective is to identify candidate molecules within these pathways that could be targeted to reduce or eliminate Tbet+ ABCs, thereby preventing their contribution to the build-up of pathogenic autoantibodies.

The ultimate applicability of the work is in the development of new and more precisely targeted therapies to mitigate or prevent some symptoms of lupus, thereby improving patient quality of life. The time to a patient-related outcome is measured in years for several reasons; for example, candidate target molecules identified here would first require validation in cultured human cells and in live animal models of health and lupus.

Proposal Title:	Screening for Autoantibody-Producing Cells
Log Number:	LR220036
Current PI Name:	Carol Webb
Award Number:	HT9425-23-1-0271
Current Contracting Organization:	Oklahoma, University of, Health Sciences Center
Current Performing Organization:	Oklahoma, University of, Health Sciences Center
Web Approval Date:	05-10-2023

Lupus is a devastating autoimmune disease characterized by inappropriate immune responses. There is currently no cure for lupus and available treatments suppress normal healthy immune responses. Although these treatments relieve some symptoms of the disease, they also result in impaired responses to infections without curing the disease. Antibodies are molecules made by white blood cells called B lymphocytes to help fight infectious agents. An underlying cause of lupus is over-production of a subset of antibodies that recognize an individual's own tissues, called autoantibodies. Some treatments for lupus target all B lymphocytes, eliminating autoantibody-producing cells, but they also leave individuals unable to produce antibodies against infectious agents. It is currently not possible to distinguish the B lymphocytes that make autoantibodies from the B lymphocytes that are needed for healthy immune responses. Exposure to the common virus Epstein Barr Virus (EBV) and other environmental triggers are thought to activate B cells and initiate autoantibody production in patients with lupus by unknown mechanisms. In this study, we will determine if the new biomarkers we have developed will specifically identify the activated B cells that are precursors of the cells which will eventually produce autoantibodies. We will use patient blood cells on consecutive visits to determine if increases in this activated B cell type occur before increases in autoantibodies are detectable in a patient's blood. In addition, we will isolate these cells and determine if they produce the same types of autoantibodies that are produced in that patient's blood at the next visit. Our objective is to determine if we can identify precursors of autoantibody-producing cells in individual patients before patients produce autoantibodies. If our studies are successful, patients could be screened using a simple blood test, with no additional risk, to detect increased numbers of these cells before they mature into autoantibody-producing cells. The ability to diagnose upcoming increases in disease activity before they occur could positively impact individual patients' quality of life by allowing personalized alterations of therapies by their physicians. This should be particularly helpful for patients who undergo disease flares with increased autoantibody production. If successful as a screening tool, this outcome could lead to the availability of prescreening tests within 5 years. More importantly, if we can selectively identify the activated B cells that will eventually develop into autoantibody-producing cells, we should be able to develop methods to specifically target this subset of B cells, leaving other healthy B cell subsets intact. This is a long-term goal that would require clinical trials and take approximately 10 years. However, the potential impact of such a treatment is high. The proposed studies lay the groundwork for testing these long-term goals.

Proposal Title:	Estrogen Receptor Alpha Regulation of B-Cell Receptor Signaling, B-Cell Activation, and Peripheral B-Cell Tolerance
Log Number:	LR220040
Current PI Name:	Karen Gould
Award Number:	HT9425-23-1-0599
Current Contracting Organization:	Nebraska, University of, Medical Center
Current Performing Organization:	Nebraska, University of, Medical Center
Web Approval Date:	07-12-2023

Lupus is an autoimmune disease because it is caused when a patient's own immune cells, which normally protect the body by fighting off infection from invaders such as viruses and bacteria, instead attack the patient's own body. Normally, the cells of our immune system are prevented from attacking our own body, but when the processes for preventing self-attack do not function properly, lupus can develop. Symptoms of lupus often include fatigue, joint pain, fevers, and skin rashes. These symptoms have a major impact on the quality of life of lupus patients as well as their ability to work and care for their families. Lupus can also lead to serious, potentially life threatening diseases affecting vital organs such as the heart and kidneys.

Approximately 90% of patients with lupus are women, and the disease is most commonly diagnosed in women of reproductive age. Both the age at which lupus usually develops and the dramatic female sex bias in lupus, are due, in part, to estrogens, one group of female sex hormones. Although we have known for decades that estrogens play a major role in lupus, relatively little is understood about how estrogens promote this disease or what effects estrogens exert on the type of immune cells involved in lupus.

Estrogens exert their effects on cells by binding to cellular proteins, called estrogen receptors. We have shown that one type of estrogen receptor, estrogen receptor (ER) alpha, mediates the effects of estrogens in lupus. We have also shown that ER alpha influences the action some of the genes that control a person's risk of developing lupus. In the absence of ER alpha, the effects of one of these genes, Sle1b, is eliminated or greatly reduced. In females, Sle1b promotes abnormal growth and development of B cells, one type of immune cell that contributes to lupus, and allows the B cells to bypass the normal processes that prevent lupus by stopping the development of B cells that attack our own body. Sle1b influences the action of the B cell receptor, a protein that controls virtually all aspects of B cell development, growth, and function. We found that ability of Sle1b to perturb normal B cell growth and development is strongly reduced when ER alpha function is disrupted. We have also found that ER alpha influences how B cells respond when the B cell receptor is activated. Others have shown that some of the cellular processes initiated by the B cell receptor influence the activity of ER alpha. Thus, not only does ER alpha impact Sle1b and the B cell receptor, but the converse is also likely to be true – Sleb and the B cell receptor impact ER alpha.

The objective of this application is to determine how ER alpha and Sle1b work together to disrupt normal B cell development and contribute to lupus. First, we will determine on a deeper level how ER alpha influences the action of the B cell receptor and Sle1b and thereby regulates B cell growth and development Next, we will determine how the B cell receptor and Sle1b may in turn influence the action ER alpha and how this impacts B cell growth and development.

This research will shed light on how estrogens, acting via ER alpha, promote lupus. Although we have known for decades that estrogens promote lupus, the basis for this effect is not well understood. Thus, this work will help us to gain insight into why most lupus patients are women. A deeper understanding of how estrogens promote lupus has the potential to lead to novel lupus therapies, and there is an urgent need for new lupus therapies that are safe and effective. The pharmaceutical industry has a long-standing interest in

the development of drugs that bind to estrogen receptors and modify some of their actions. Such drugs are approved for the treatment of some diseases in which estrogens play a major role, including breast cancer and osteoporosis. These same drugs can be used to treat lupus in women after menopause, but because of concerns about effects on the reproductive system, they are not used to treat women with lupus during their reproductive years. By gaining a better understanding of how ER alpha promotes the growth and development of abnormal B cells that contribute to lupus, we will drive the development and/or identification of novel drugs that block the effects of ER alpha specifically in B cells and are thus safe to use in premenopausal women. Given that pharmaceutical companies have many selective ER modulator drugs that have never been tested as therapies for lupus, our work could lead to the further testing and rapid approval of existing drugs that could disrupt ER alpha in B cells (but that spare the reproductive system) as a therapy for lupus in premenopausal women.

This proposal involves research that can be categorized under two of the FY21 LRP Focus Areas: (1) “Understanding the biological mechanisms of lupus disease…” and (2) “Understanding… gene-environment interactions of lupus… using functional genomic studies.” The work involves genomics to gain insight into gene-hormone interactions (a form of gene-environment interactions) and also addresses some very fundamental questions regarding the biological mechanisms of lupus.

Proposal Title:	Understanding Glucose Metabolism in the Development and Treatment of Lupus
Log Number:	LR220048
Current PI Name:	Lucas Chang
Award Number:	HT9425-23-1-0308
Current Contracting Organization:	Jackson Laboratory
Current Performing Organization:	Jackson Laboratory
Web Approval Date:	05-04-2023

Rationale, Objective, Aims: Lupus is an autoimmune disease characterized by immune-system cells that are autoreactive, i.e., they attack the body's own tissues rather than foreign pathogens. Currently, lupus lacks a cure, and treatment focuses on alleviating symptoms or on immunosuppression, i.e., suppression of the overall immune system, including normally functioning immune cells. There is a distinct lack of understanding of lupus initiation and the biological changes that take place to support progression of this disease. Emerging evidence points to abnormal glucose metabolism – the use of glucose as an energy source – in the development of lupus and other autoimmune diseases in both humans and mice. Inhibition of glucose metabolism is therefore an attractive approach for attenuating the highly energetic cells that drive lupus disease while potentially avoiding immunosuppression. We have tested the potential of inhibiting glucose metabolism in multiple mouse models that naturally develop a lethal autoimmune disease resembling human lupus. Specifically, we treated the mice with 2-deoxy-D-glucose (2DG), a compound that limits glucose metabolism, and showed that 2DG treatment is highly effective in reversing lupus-like symptoms and extending lifespan in these mice. However, little is known about how 2DG reverses this disease, hindering testing of 2DG in humans. For example, before 2DG can be tested in humans, it is necessary to identify the specific metabolic pathways impacted by 2DG in mice and humans, to ensure that application of this promising therapy in humans inhibits the same types of autoreactive cells in lupus patients that is does in mice with lupus. To address these knowledge gaps, we will, in Aim 1, identify metabolic-pathway changes that develop as disease progresses in mouse models of lupus and in blood from lupus patients, and compare the mouse and human data to find common alterations that can be targeted. In Aim 2 we will analyze the effects of 2DG on different metabolic pathways in treated mice; test the effects of 2DG treatment on patient cells; and compare these mouse and human data. Our work will identify metabolic pathways altered in lupus development and those impacted by 2DG, laying the groundwork for understanding how abnormal metabolism affects lupus development; identifying the key types of immune cells involved; and ascertaining metabolic-pathway components that represent potential targets for development of improved therapies.

Focus Areas: This project addresses two Fiscal Year 2022 Lupus Research Program Focus Areas: (1) Understanding the biological mechanisms of lupus disease…; (2) Improving quality of life for individuals living with lupus including…symptom and disease control, and comparative effectiveness research.

Applicability of the Research: Completion of this project will greatly expand our knowledge of metabolic dysfunctions that occur in lupus progression as well as those that, when normalized via 2DG treatment, offer therapeutic benefits. These data will not only stimulate development of novel metabolism-based therapies for treating lupus, but will also identify potential therapeutic targets related to specific clinically defined characteristics of human lupus patients. The additional information that we will reveal on metabolic aberrations that either lead to or are caused by lupus disease progression may aid in the diagnosis of lupus. These data, paired with information on how 2DG affects metabolic pathways, have potential to greatly improve the health and quality of life of all lupus patients. Should this treatment become available to lupus patients, it has the potential to reverse the disease by specifically targeting autoimmune cells, leaving the

“normal” immune cells unaffected and effectively limiting the immunosuppression that is a side effect of many current lupus therapies. Moreover, in our mouse models, 2DG has been shown to elicit increased therapeutic potential compared to a metabolic inhibitor currently undergoing clinical trials to treat lupus, indicating that 2DG might also offer improved efficacy in lupus patients. 2DG has been used extensively in clinical trials for human cancers, and also in a recent phase 3 trial as a possible treatment for SARS-CoV-2. It has been shown to be well tolerated with few side effects; hence, the risks associated with a clinical trial for use of 2DG to treat lupus are very slight. Because the process of glucose metabolism is highly similar in mice and humans, data from our proposed study has a high probability of being clinically translatable to human patients. The primary impediments to moving 2DG into human trials are that the mechanisms through which it reverses lupus are unknown, and it is not known if this compound also affects human autoimmune cells. Once these questions are answered via completion of our project, 2DG should be able to be moved quickly into human clinical trials. We have high hopes that in such trials, 2DG treatment will demonstrate improved clinical outcome and quality of life for lupus patients. Because 2DG has previously been shown to be safe in clinical trials, this will reduce the time needed to study this compound, and we hope to see full approval within the next decade.

Proposal Title:	Small RNAs and Heterogeneity of Risk for Developing Systemic Lupus Erythematosus
Log Number:	LR220053
Current PI Name:	Michelle Ormseth
Award Number:	HT9425-23-1-0342
Current Contracting Organization:	Vanderbilt University Medical Center
Current Performing Organization:	Vanderbilt University Medical Center
Web Approval Date:	05-04-2023

Systemic lupus erythematosus (SLE) affects more than 70 per 100,000 people in the U.S., increasing the risk of early death 3-fold. SLE is heterogeneous; this means that the disease can affect nearly every organ in the body and patients can have a variety of symptoms ranging from mild rashes to life-threatening kidney disease. SLE has a strong genetic component and relatives of a patient have a much higher chance of developing SLE. However, because SLE has many nonspecific and heterogeneous symptoms it is difficult to diagnose the disease in its earliest and mildest stages, and even more difficult to predict who will get SLE. If we could better identify those people who will develop SLE, we could treat them with well-tolerated medicines to prevent SLE from occurring.

We think we can predict risk of future disease by using small RNAs (sRNA). DNA makes RNA and RNA makes protein, which is a building block of our body. However, investigators discovered short segments of RNA called small RNAs that do not make protein; rather these sRNAs bind to other RNAs to decrease the amount of protein made. In this way they regulate genes. Groups of sRNAs frequently work together to exert a major effect on the function of a cell and have potential for diagnosis, prognosis, and treatment in SLE. There are several Food and Drug Administration-approved sRNA-based tests available for use in other diseases, and there are many sRNA-based drugs in development for diseases such as cancer. None of these are available for SLE.

We found that the sRNAs circulating in blood differ in patients with SLE and rheumatoid arthritis (RA) compared to people without autoimmune disease. Also, we found that there are sRNAs in human blood that come from bacteria and viruses – nonhuman sources, and the composition of these also differs in patients with SLE versus controls, with SLE patients having increased circulating viral sRNAs. These nonhuman sRNAs can enter human cells and affect how the cells function. We know that some bacteria and viruses increase the risk of developing SLE, but it is unclear how they do this. We believe circulating bacterial and viral sRNAs may be the mechanism used by bacteria and viruses to affect disease development in humans.

Many cells of the human immune system are equipped with defense sensors for single-stranded RNA, including some of the sRNAs in human circulation. These defense sensors help our body detect viral RNA, in particular, but also detect some bacterial and human RNAs. The result of activating these sensors is an increase in type 1 interferon, which is an important signaling molecule to fight infection but that can also worsen SLE. Indeed, anifrolumab, a drug that targets type 1 interferon, was recently approved as a treatment for SLE. Further evidence of the importance of this pathway in SLE is that genetic abnormalities that make the defense sensors more active can cause patients to have SLE. Our preliminary studies show that patients with SLE have more circulating sRNAs predicted to activate these defense sensors. We think that high levels of these sRNAs may trigger SLE and help us predict who is more likely to develop SLE.

Our objective is to define the role of human and nonhuman sRNAs in the development of SLE. We hypothesize that human and nonhuman sRNAs can predict development of SLE and that these sRNAs increase type 1 interferon. In our study we will use previously collected blood and clinical information from

individuals who are at risk for SLE because they have a direct family member with SLE and are therefore more likely to develop the disease. Among these at-risk individuals, some went on to develop SLE and others did not. To test our hypothesis, we will define (1) human and (2) nonhuman sRNAs that predict those people who will develop SLE. We will test whether these human and nonhuman sRNAs can activate the RNA defense sensors and induce type 1 interferon and thus affect risk of SLE.

This application is in response to the Fiscal Year 2022 Lupus Research Program Focus Area “Understanding how lupus heterogeneity impacts risk of disease, disease presentation, clinical course, and outcomes.” In this context our emphasis is on the risk of disease development. The heterogeneity of SLE makes early diagnosis and prediction of disease difficult. If we could predict who will develop SLE, we could help identify people who might benefit from preventive therapy (and avoid unnecessary therapy in those who do not need it). Further, studying sRNAs in SLE will provide information on the mechanisms underlying development of disease and how viruses and bacteria influence this; it could also lead to new treatments.

Proposal Title:	Somatic Mutations in Non-Ig Loci in Autoreactive B Cells as Drivers of Lupus Pathogenesis and Disease Heterogeneity
Log Number:	LR220058
Current PI Name:	Karen Gould
Award Number:	HT9425-23-1-0402
Current Contracting Organization:	Nebraska, University of, Medical Center
Current Performing Organization:	Nebraska, University of, Medical Center
Web Approval Date:	06-12-2023

Abnormal activity of B cells, one of the cells of the immune system, is critical to the development and progression of the autoimmune disease lupus. Uncontrolled activity of an enzyme normally found in B cells has been shown to cause gene mutations that lead to B cell cancers. In B cells from lupus patients, this same enzyme also shows uncontrolled activity. Based upon this observation, we hypothesize that uncontrolled activity of this enzyme may also cause gene mutation in B cells that result in abnormal B cell function and thus, contribute to the development and progression of lupus. Furthermore, we speculate that the occurrence of specific gene mutations in B cells in some lupus patients may explain why some patients develop specific symptoms associated with lupus and/or why lupus affects certain organs in some patients, why lupus flares occur, and why the disease progresses more rapidly in some patients than others. We think that B cell mutations impact lupus symptoms and disease progress by altering the expression of various genes that are essential for normal B cell development, growth, and function.

To explore these ideas, we will analyze the DNA sequence of B cells from a mouse model of lupus that is very similar to human lupus and search for mutations in these DNA sequences. We will also examine gene expression and gene mutations in B cells from mice with aggressive lupus and those with mild lupus and correlate these with various lupus symptoms. Using a mouse model allows us to perform our study in a simplified system so we can more easily find potentially important gene mutations in B cells and correlate them with the severity of different symptoms of lupus and differences in the onset and progression of the disease.

Finally, we will also identify mutations in the DNA of B cells from lupus patients experiencing a disease flare.

This proposal is focused on exploring a novel idea that may help to explain the variability that we see in the symptoms and severity of disease in lupus patients. The basis for this variability, as well as how we can better detect it and best care for lupus patients based upon this variability, is one of the Focus Areas for fiscal year 2022 in the Lupus Research Program (LRP). The work proposed here has the potential to identify the cause of some of this variability as well as lead to the development of novel therapies to treat lupus patients based upon new ways to classify patients with respect to their risk for developing certain lupus symptoms and progressive disease. In addition, because the proposed studies will investigate the role gene mutations in B cells as drivers of lupus symptoms and disease progression, this application also focuses on a new potential disease mechanism in lupus. Thus, this proposal also addressed a second Focus Area of the LRP– understanding the biological mechanism of lupus disease.

The idea that such gene mutations occur in B cells and contribute to lupus has never been examined, and thus the idea being studied in this proposal is completely novel. If our hypothesis is correct, and we detect gene mutations in B cells that correlate with clinical features or lupus disease severity, then our finding would have major implications for the study and treatment of lupus. In cancer care, screening for the presence of specific gene mutations is now very common because it allows physicians to more accurately

classify a patient’s cancer type, determine prognosis, and predict response to various types of therapies. In addition, highly effective targeted molecular therapies have even been developed that effectively treat cancers with specific gene mutations. If our hypothesis that gene mutations in B cells contributes to lupus is correct, then our studies could pave the way for completely new methods to classify lupus disease subtypes and to identify patients with these subtypes. Of utmost importance, our work would also pave the way for the development of targeted molecular therapies that would specifically and effectively treat lupus patients based upon these B cell mutations and disease subtypes. Such drugs could be developed and reach the clinical trial stage within 5-7 years. Although these molecular therapies would take some time to develop, cancer care has shown us that such molecular therapies are not only highly effective in patients whose cancers harbor the mutation that is specifically targeted, but also that these therapies have few side effects, as they target only cells that harbor the gene mutations and spare normal, healthy cells from damage. If different gene mutations in B cells correlate with different clinical features in lupus, then many different types of lupus patients have the potential to benefit from this research. However, it is likely that B cell mutations will be more common in the those with more aggressive disease. Thus, this work may disproportionately benefit those with more severe disease and therefore, those whose quality of life is most impacted by lupus.

Proposal Title:	Protection and Recovery from Burn-Associated Inhalation Injury Through Pharmacologic Manipulation of an Endogenous Airway Regenerative Pathway
Log Number:	MB220014
Current PI Name:	Philip Beachy
Award Number:	HT9425-23-1-0923
Current Contracting Organization:	Leland Stanford Junior University, The
Current Performing Organization:	Leland Stanford Junior University, The
Web Approval Date:	10-01-2023

The mammalian respiratory system supplies oxygen for the entire organism by employing millions of gas exchange units, the alveoli. The thin-walled structure of these delicate epithelial sacs ensures a minimal barrier that facilitates adequate gas exchange, but also renders them vulnerable to injury from inhaled particulates and toxins, such as the smoke and other by-products of fires, including military burn pits, or from the military use of chemical weapons and other explosive devices. To minimize such exposure, the airway tree is critically important for the protective functions of trapping and clearing injurious agents before they reach the alveoli. In this protective role the airway employs a mucociliary clearance system to absorb and remove toxic gases and particulates. Should the airway epithelium itself be injured as it engages harmful agents, it can mount a vigorous regenerative program by activating airway stem cell proliferation and differentiation.

Among the most common airway pathologies are those associated with acute injury from inhalation of external toxicants. The rationale of our proposal is based on the discovery of an epithelial-mesenchymal feedback (EMF) circuit that plays a critical and beneficial role in airway response to acute inhalation injury. EMF circuit activity regulates regenerative activity in many adult organs and is generally initiated by epithelial expression of one of the three members of the Hedgehog family of signaling proteins. Response to this epithelial Hedgehog signal occurs in the underlying mesenchyme, and generally includes expression of secreted signals that feed back to the epithelium and regulate activities such as proliferation and differentiation. The airway EMF circuit is characterized by several unique features: First, the epithelial signal is Desert hedgehog, the least-studied member of the Hedgehog family but functionally the most important in adult airway. Second, in airway Desert hedgehog expression is confined to rare pulmonary neuroendocrine cells (PNECs), which comprise

In our preliminary studies we have observed that genetic or pharmacologic disruption of the airway EMF circuit compromises the response to inhalation injury in mice exposed acutely to SO2 gas, a common environmental toxicant used to model acute inhalation injury. Following this injury, these mice with diminished EMF circuit activity display airway damage augmented by increased apoptotic loss of epithelial cells and attenuated proliferation of basal epithelial stem or progenitor cells; these initial acute effects are accompanied by a significant longer-term delay in airway regeneration. Pharmacologic activation of the airway EMF circuit in contrast protects the airway epithelium from cell loss, suggesting a novel therapeutic approach to acute inhalation injury. Our objective is to determine the mechanism of EMF activation (Aim 1), to explore therapeutic modulation of EMF activity in inhalation injury (Aim 2), and to verify that, as in mice, an airway EMF circuit protects and stimulates regeneration of the human airway (Aim 3).

Successful completion of these studies will elucidate the function of this airway regenerative regulatory circuit and will provide the basis for development of pharmacologic intervention(s) to improve prophylactic

and post-injury management of actual or anticipated inhalation injures from burn related complications, or inhalation of toxic gas in the military setting, in this way directly benefiting injured active Service Members, and Veterans. These benefits can also extend to the public in addressing inhalation injuries from urban fires, and volcanic eruptions, and wildfires, which are occurring with increasing frequency and intensity due to climate change. Our proposal could also benefit the underserved inner-city civilian population, especially lower-income families. Air pollution is a major health problem in these urban settings, with lower-income residents of inner cities disproportionately burdened by chronic lung diseases such as asthma and chronic obstructive pulmonary disease (COPD). These diseases are dramatically aggravated and intensified by airway injury, which our research aims to ameliorate.

Proposal Title:	Improving the Robustness and Generalizability of Post-Burn Sepsis Prediction with the Post-Burn Sepsis Digital Twin
Log Number:	MB220047
Current PI Name:	Seth Schobel-Mchugh
Award Number:	HT9425-23-2-0022
Current Contracting Organization:	Henry M. Jackson Foundation
Current Performing Organization:	Henry M. Jackson Foundation
Web Approval Date:	09-29-2023

This project directly addresses the fiscal year 2022 Military Burn Research Program Clinical Translational Research Award Focus Area of post-burn sepsis by developing a real-time prediction tool for post-burn sepsis. Despite advances in initial care of the burn patient, a significant number of patients will progress to the condition known as sepsis. Sepsis often results from infections, which severe burn patients are at greater risk for, and can result in the failure of various organs and eventually death. The optimal care of a septic patient is greatly enhanced by early recognition, but this can be particularly challenging in burn patients, given that many of the features used to diagnose sepsis are already present in these patients. Machine learning has gained popularity in the prediction of sepsis, but the aforementioned similarity between the physiological response to a major burn and the early signs of sepsis can challenge even these machine learning approaches. We propose that the performance of these machine learning systems can be improved by developing and employing "digital twins" of burn patients. A medical digital twin, which is a concept initially developed in industrial settings (for instance, tracking and optimizing the construction process for jet engines), is a simulation of a particular patient that is personalized to the characteristics of that patient and updated over time; simulations of a medical digital twin can be used to forecast how that patient will behave in the future, including whether and when that patient will develop sepsis.

In this project we will develop the Post-Burn Sepsis Digital Twin by extending an existing simulation model of the cellular and molecular mechanisms of acute inflammation to simulate the physiological responses of burn patients, and we will tune and test the Post-Burn Sepsis Digital Twin to real patient data from three nationally recognized burn centers. We propose that the integration of the Post-Burn Sepsis Digital Twin with a state-of-the-art machine learning artificial intelligence prediction algorithm will greatly enhance our ability to predict, and therefore treat, post-burn sepsis. This project has significant potential to benefit both burn-injured Service Members and burn patients in general by enhancing the prediction of sepsis, thereby allowing for earlier and potentially more effective treatment. If we can predict that something bad is going to happen early (for example, sepsis), we can take steps to prevent that condition from occurring or avoid the most significant impacts. One additional benefit of using a digital twin, as opposed to a purely data-driven approach, is that, in addition to the proximate benefit of enhanced sepsis prediction, the post-burn sepsis digital twin can be used for drug/therapy discovery to identify and test, through running simulated clinical trials, novel therapies to treat sepsis. This may lead to more efficient, timely, and less expensive clinical trials if used effectively.

The role of care for the post-burn sepsis prediction system is intended primarily for use in full-service hospitals, though there is a research/therapy discovery role related to the ability of the digital twin to be used in simulated/in silico ("in a computer") clinical trials. We expect that by the end of the project period (4 years) we will have a prediction system able to be tested in a clinical setting. Additionally, we expect that by the end of the project period, we will be able to begin preliminary in-silico drug testing using the digital twin to posit strategies to prevent progression to sepsis.

Proposal Title:	Understanding the Burn Wound Microbiome: Comparing Traditional Wound Cultures to Next-Generation Sequencing Technology
Log Number:	MB220056
Current PI Name:	Steven Wolf
Award Number:	HT9425-23-1-0405
Current Contracting Organization:	Texas Medical Branch, University of, Galveston
Current Performing Organization:	Texas Medical Branch, University of, Galveston
Web Approval Date:	09-13-2023

Burn wound sepsis remains a significant problem in the treatment of burn wounds. This condition is particularly prominent in combat casualties, comprising about 10% of war wounds that are at risk. Clinical care is driven by looking at the wounds, culturing the bacteria that are present, and treating these with antibiotics and surgery. However, sometimes this is unsuccessful, perhaps because we do not know all the organisms that are there, how these interact with each other and with the burned patient's immune system, and/or the real effects of antibiotic treatments and surgery on the bacteria and fungi that are there. New technology using genetic signatures of all microbes within the wound is now available. We intend to use this technology to fully describe what organisms are usually in burn wounds, how different patients might have different ones, how these interact with each other and the patient, and the real effects of treatment on the burn wound itself. With this information, we will have much more knowledge of what is actually going on in the wound and how the bacteria and fungi there can be better controlled. This information will inform medical providers with much better understanding of what is usually in the wound so it can be treated more effectively. This project will take about 3 years to collect the specimens, and another year for analysis. Once completed, the knowledge gained will be used to develop better treatment strategies for the severely burned warfighter. This will be of particular use in far-forward settings and mobile campaigns, and also at Level 5 facilities back home.

Proposal Title:	High-Throughput Screening for Lipid Nanoparticles Efficient for Systemic Delivery to Tissues Affected by DMD
Log Number:	MD220007
Current PI Name:	Baisong Lu
Award Number:	HT9425-23-1-0685
Current Contracting Organization:	Wake Forest University Health Sciences
Current Performing Organization:	Wake Forest University Health Sciences
Web Approval Date:	08-17-2023

Majority of Duchenne muscular dystrophy (DMD) patients carry deletions and duplications in the DMD gene. These mutations abolish dystrophin expression. Loss of dystrophin expression is the fundamental cause of DMD. Recent studies from several laboratories suggest that DMD can be treated using the CRISPR technology in mice and dogs. All these studies have used adeno-associated virus (AAV) as the vector to deliver the CRISPR editing machinery to muscle. AAV is currently being used in systemic microdystrophin trials because AAV vector can (a) reach all muscles in the body and (b) lead to persistent expression of the gene it carries (e.g., the microdystrophin gene).

While these are advantageous for dystrophin gene replacement therapy, persistent expression of Cas9 will cause problems to patients in the context of CRISPR therapy. CRISPR therapy relies on a protein called Cas9 to bypass the mutation in order to restore dystrophin expression. Cas9 is a bacteria protein. Persistent Cas9 protein expression leads to immune responses and untoward cutting at wrong places. A delivery vehicle that can reach all body muscles and yet only express Cas9 transiently will be ideal for DMD CRISPR therapy.

Lipid nanoparticles are emerging RNA delivery vesicles, exemplified by their successful application in the delivery of mRNA vaccines for COVID-19. DMD CRISPR therapy machineries can be delivered in the form of Cas9 mRNA and guide RNA, the former expresses Cas9 protein which forms a complex with guide RNA to be fully functional. Cas9 mRNA and guide RNA cannot enter cells by themselves, and lipid nanoparticles would be ideal to help them get into human cells. A few studies successfully delivered CRISPR/Cas9 machineries to muscle tissues by local delivery, suggesting the potential application of lipid nanoparticles as a delivery tool for DMD CRISPR therapy. However, local delivery is not ideal for treating DMD which affects many muscles.

Current lipid nanoparticles do not efficiently reach muscle tissues affected by DMD when delivered systemically, necessitating the development of new ones. It is found that tuning the components of lipid nanoparticles changes tissue preference, thus it is possible to find lipid nanoparticles with high delivery efficiency for DMD-affected organs. Unfortunately, lipid nanoparticle's in vitro performance cannot predict in vivo performance due to the completely different environments. Although barcode-based high throughput screening methods are developed for in vivo screening of many lipid nanoparticles in one experiment, they cannot assay gene editing activity or provide cell type information. Most importantly, they cannot distinguish the 95% nonfunctional lipid nanoparticles trapped in endosomes from the 5% functional ones successfully escaping from the endosome and entering the cytoplasm, thus suffer from high false-positive results.

Here we developed a novel barcode integration nanoparticle screen (BINS) method, which can assay gene editing activity, efficiently eliminate nonfunctional lipid nanoparticles from the screening, and provide cell type information. We will use BINS to screen for lipid nanoparticles that can deficiently deliver Cas9 mRNA and guide RNA into all muscle tissues affected by DMD.

Aim 1: Will screen for lipid nanoparticles efficient for dystrophin-expressing cells, which will restore dystrophin expression for immediate therapeutic effects.

Aim 2: Will screen for lipid nanoparticles efficient for Pax7-expressing muscle stem cells for sustained therapeutic effects. Restoring the DMD gene in muscle stem cells provide the possibly for the edited stem cells to regenerate the whole muscle tissue.

Aim 3: Will use the best LNPs from preliminary study, Aims 1 and 2 to deliver CRISPR/Cas9 RNA to all muscles of humanized DMD model mice. We will examine how efficient the LNPs can deliver CRISPR /Cas9 RNA to edit DMD gene and restore dystrophin, in differentiated myocytes and muscle stem cells.

Our BINS is the only high-throughput lipid nanoparticle screen method that assays gene editing activity, eliminates false-positive results, and provides efficiency information for a specific cell type. This study is the first one trying to develop lipid nanoparticles for systemic delivery of CRISPR/Cas9 machineries for DMD CRISPR therapy. The data will provide basis for further preclinical studies to examine toxicity, pharmacology, and therapeutic phenotyping of the candidates in the next stage. The development of lipid nanoparticles efficient for systemic muscle delivery will pave the way for clinical application of DMD CRISPR therapy.

Proposal Title:	Single Nuclei Transcriptome Analysis of DMD to Reveal Disease-Modifying Targets
Log Number:	MD220027
Current PI Name:	Stanley Nelson
Award Number:	HT9425-23-1-0597
Current Contracting Organization:	California, University of, Los Angeles
Current Performing Organization:	California, University of, Los Angeles
Web Approval Date:	09-15-2023

Skeletal muscle is a cellularly complex and plastic tissue that responds to local injury by recruiting and expanding immune cells, fibroblasts, and other muscle resident cells to guide satellite cell-mediated repair of damaged multinucleated myofibers. In Duchenne muscular dystrophy (DMD), lack of dystrophin leads to chronic muscle damage, immune infiltration, dysregulation of repair, with loss of muscle function and replacement with fat and fibrosis. Our understanding of the cell and molecular mechanisms regulating dystrophy and repair remain incomplete, especially in humans. Here we propose to study all of the cells within muscle and their gene expression response with dystrophin loss or restoration that occurs naturally or with genetic therapies. We have developed relatively less invasive biopsy methods, coupled with improvements in sample handling that allow us to study about 5,000 nuclei from each person's muscle biopsy and sequence about 20,000 genes from each nucleus. We have analyzed 8 healthy muscles, 9 DMD muscles with some dystrophin expression, and 43 DMD samples from across the disease severity and stage of disease. This has created an unprecedented set of observations of how cells in muscle are responding to the lack of dystrophin or restoration of dystrophin. Here we propose to augment this database by the strategic collection on an additional 60 DMD/BMD muscles to broaden and clarify insights that can be gleaned from an unbiased assessment of gene expression in dystrophic muscle. We infer that genes that are differentially expressed in relation to disease severity or stage of disease highlight molecules that are potentially identifying therapeutic targets of prognostic markers. We will sample muscle from DMD patients as young as 2 years to as old as 27 years, and BMD patients from 5 years to 69 years, and 20 healthy controls. This data in aggregate will provide unprecedented insights into Duchenne and highlight otherwise cryptic pathways for disease mitigation and identify novel biomarkers of disease. All of the collected and organized data will be shared publicly without patient identifiers to permit ongoing investigation and serve as a reference for muscle studies with various DMD therapeutics in development to accelerate novel treatments. All patients with a dystrophinopathy may benefit from the output of this research. Within 1 year, we will develop a sensitive RNA-based method to observe transgene expression or exon skipping of mRNA from small muscle samples, and within 3 years we will identify promising therapeutic candidates most relevant for augmentation/combination/follow on treatment from therapies like exon skipping and gene therapy. This work is a translational study addressing the Duchenne Muscular Dystrophy Research Program Translational Research Award Focus Area of "skeletal muscle" to improve care for DMD by unbiased discovery of novel prognostic, age, and disease severity expression biomarker, which may serve as surrogate clinical markers. Further, this project will augment our understanding of the natural history of DMD and provide clues to the field for novel therapeutic targeting and combinational targeting of pathways.

Proposal Title:	Advancing Efficacy, Longevity, and Safety of Gene Editing Therapies for DMD
Log Number:	MD220031
Current PI Name:	Niclas Bengtsson
Award Number:	HT9425-23-1-0575
Current Contracting Organization:	Washington, University of
Current Performing Organization:	Washington, University of
Web Approval Date:	09-15-2023

Adeno-associated viral (AAV) vector-based gene therapies are generating encouraging results in Duchenne muscular dystrophy (DMD) trials. However, these approaches do not fully restore muscle function and therapeutic benefits may be lost over time due to muscle turnover following maintenance, or trauma. Gene editing (CRISPR) offers a promising alternative to correct dystrophin production from the patient’s own gene. Here, long-term therapeutic outcome could benefit from repairing mutations in both existing muscles and in muscle stem cells (SCs) to ensure that dystrophin continues to be produced in case of muscle injury and regeneration. While early studies have shown that muscle and SCs can be targeted with AAV-CRISPR, efficient and safe approaches for more straightforward clinical translation have yet to be demonstrated. Here we propose developing methods to permanently restore dystrophin expression in striated muscle by correcting DMD-causing mutations in both existing muscle and in SCs. We will also develop sensitive "switches" for turning gene editing On and Off, with the purpose of reduced risks of unintended editing or development of immune responses within treated patient muscles. Overall, these studies are designed to achieve significant long-term therapeutic improvements of DMD symptoms.

Proposal Title:	Vascular-Targeted Therapy for Duchenne Muscular Dystrophy
Log Number:	MD220059
Current PI Name:	Atsushi Asakura
Award Number:	HT9425-23-1-0461
Current Contracting Organization:	Minnesota, University of, Twin Cities
Current Performing Organization:	Minnesota, University of, Twin Cities
Web Approval Date:	09-15-2023

Duchenne muscular dystrophy (DMD) is caused by mutations in a gene called dystrophin, which acts to maintain muscle fiber structure and function in the whole body, preventing it from being damaged by muscle contraction. Presently, there is no definitive treatment for DMD patients, and current therapies focus on prolonging survival and improving quality of life. It is possible to reduce muscle fiber damage by using pro-angiogenic factors, including anti-Flt1 monoclonal antibody and novel Flt1-LNP-mRNA vaccine, to increase the number of blood vessels and observe the resultant effects on the muscular dystrophy phenotype in DMD model mice. Thus, the administration of anti-Flt1 monoclonal antibody or Flt1-LNP-mRNA vaccine will assist the development of new therapies for DMD via increased vascular density in blood-starved dystrophic muscles.

Proposal Title:	Engineering iPS-Derived MSCs with Enhanced Homing and Anti-Inflammatory Properties for the Treatment of DMD
Log Number:	MD220068
Current PI Name:	Christopher Rohde
Award Number:	HT9425-23-1-0787
Current Contracting Organization:	FACTOR BIOSCIENCE INC.
Current Performing Organization:	FACTOR BIOSCIENCE INC.
Web Approval Date:	10-03-2023

Rationale: Duchenne muscular dystrophy (DMD) is a hereditary, X-linked condition caused by a mutation in the dystrophin gene that leads to progressive muscle fiber degeneration and weakness. The diagnosed incidence of DMD is estimated to be 17.24 per 100,000 live male births in the United States, making it the most common and severe type of muscular dystrophy. Currently, no cure exists for DMD, and treatment is primarily supportive.

DMD results in inflammation and muscle damage due to the absence of the structural protein dystrophin. This constant inflammation also prevents muscle repair. It is believed that, by controlling the inflammation, the DMD disease process can be delayed, and muscles can be allowed to repair themselves. Factor Bioscience uses its novel, patent-protected technologies to develop a type of cell known as induced-pluripotent-stem-cell-derived mesenchymal stromal cells (iMSCs). iMSCs outperformed bone marrow-derived mesenchymal stromal cells and demonstrated anti-inflammatory and antibacterial properties when tested in animals. This project will use additional aspects of Factor's core technologies to engineer these cells to better home to damaged muscles and more strongly suppress inflammation, and we will show this in a mouse model of DMD. In DMD patients, these cells will allow muscle repair and improved motor function.

Applicability: The research proposed in this application will help patients with DMD directly and can also be used to enhance other cutting-edge DMD therapies. In addition to being a more effective alternative to current DMD management, Factor Bioscience's proposal presents a more efficacious, robust, well-characterized, and cost-effective cell therapy option. This project will also create a platform to create even more potent engineered induced pluripotent stem cell (iPSC)-derived-MSC (EiMSCs) with additional mechanisms of action for DMD and other muscle pathologies.

Following this research step, Factor Bioscience plans to move to test EiMSCs in additional animal models (in vivo work) to assess safety and effectiveness before moving to first-in-human clinical work. With successful outcomes, Factor Bioscience are aiming for EiMSCs to be a viable DMD treatment option in clinical trials by 2026.

Proposal Title:	Targeting SHP2 to Improve Outcome of Duchenne Muscular Dystrophy-Associated Cardiomyopathy
Log Number:	MD220073
Current PI Name:	Maike Krenz
Award Number:	HT9425-23-1-0711
Current Contracting Organization:	Missouri, University of, The Curators of the
Current Performing Organization:	Missouri, University of, The Curators of the
Web Approval Date:	09-15-2023

In Duchenne muscular dystrophy (DMD), the lack of dystrophin protein leads not only to severe progressive skeletal muscle disease, but also affects the heart muscle. This can lead to heart failure in adult DMD patients. As multidisciplinary therapeutic advances have extended the life span of DMD patients, heart failure has become a major concern. Therefore, new heart failure treatments that are specifically suited for DMD patients are urgently needed.

Based on pilot data, we propose that targeting the protein Src homology region 2 domain-containing phosphatase-2 (SHP2) in the heart can substantially increase the resilience of the heart muscle cells in DMD. SHP2's main function is to regulate protein-based signals in the heart, some of which are known to be highly protective. Whether SHP2 plays a role in DMD-associated heart failure has not been studied yet. The goal of this project is to evaluate whether targeting SHP2 can improve heart function in mouse models of DMD. We will conduct proof-of-principle experiments using either genetic modification to shut off SHP2 or new drugs that have been shown to block SHP2 in laboratory studies. In addition, we will thoroughly investigate which signals are regulated by SHP2 in the heart to better understand how and why blocking SHP2 is protective. This will advance the field of DMD research and will provide new insights that could be applicable not just to DMD, but other forms of heart failure as well.

Drugs that can block SHP2 are already in clinical trials for other diseases; therefore, such new medications are likely to become available in the near future. The studies proposed in this application will be a critical first step to establish that SHP2 is a valid new target in DMD-associated heart failure. This would build a solid foundation for future clinical trials in DMD patients.

We expect that such new medications would benefit all DMD patients, either preventing heart failure or slowing down heart failure progression. We anticipate that a new treatment that blocks SHP2 would work together with all other treatment approaches that are currently available and could enhance the success of other new therapies aiming to restore dystrophin in the heart muscle.

Proposal Title:	Identification of Genetic Mechanisms Driving Transition of Benign Nevi to Malignant Melanoma
Log Number:	ME220036
Current PI Name:	Adam Dupuy
Award Number:	HT9425-23-1-0780
Current Contracting Organization:	Iowa, University of
Current Performing Organization:	Iowa, University of
Web Approval Date:	10-03-2023

The Critical Problem: On average, cutaneous melanomas have among the highest mutation rates among human cancer. Despite this observation, benign melanocytic nevi show a remarkable genetic simplicity during disease initiation. The genetic analysis of moles (i.e., nevi) along with early-stage melanoma shows that the most well-studied drivers of cutaneous melanoma are present in benign nevi. Mouse models of melanoma also show that mutations in Nras or Braf along with inactivation of Cdkn2a efficiently produce benign nevi but don’t efficiently produce malignant melanoma. These findings suggest that additional mutations are required to drive progression from a benign to an invasive state.

Unfortunately, it seems likely that the mechanisms of disease progression are diverse, likely involving many drivers. The most large-scale and comprehensive genetic analysis of melanoma identified 49 genes whose mutation is associated with melanoma. Most of these genes are presumed to be associated with disease progression since their mutation is not commonly seen in early-stage melanomas. The major issue is that these are generally less well characterized in melanoma. This leaves a tremendous knowledge gap regarding the biological mechanisms of melanoma progression. Understanding the mechanisms of progression at this critical transition point between benign and malignant disease could provide new therapeutic targets and/or strategies to treat invasive melanoma.

The Objective of Our Proposal: The underlying premise of our proposal is that diverse genetic mechanisms drive the transition of benign nevi to malignant melanoma and that understanding these mechanisms will reveal new vulnerabilities that can be therapeutically exploited. Here we propose a novel, genetic screen to identify drivers of disease progression using a transposon mutagenesis method we have pioneered to build unique and novel models of the early stages of melanoma when cells convert from a benign state to an invasive melanoma. In addition, we will evaluate two poorly characterized genes (ZNF99, NEDD4L) that have been implicated in melanoma progression. However, the function of these genes has not been explored thoroughly in melanoma. In the case of ZNF99, our work would be the first attempts to study this protein. These experiments are important given that more than 15% of melanomas have mutations in ZNF99.

Impact and Innovation: This proposal represents a new approach for my laboratory. Our prior melanoma research has focused on mechanisms of BRAF and MEK inhibitor resistance, a drug combination that is commonly used to treat approximately 50% of melanoma patients. We recently developed a new method to model melanoma in vivo using Sleeping Beauty transposon mutagenesis to accelerate the genetic characterization of the disease. We propose the use of the CBT melanoma model described this year in "Science." This cell model is a valuable tool that we will use to perform a novel forward genetic screen to identify drivers of melanoma progression.

Our proposal directly addresses the challenge statement by seeking to elucidate the earliest genetic events that drive the transition of melanoma from a benign or noninvasive form to invasive, metastatic disease.

Understanding these mechanisms could provide insight into new therapeutic or prophylactic strategies to prevent or reverse melanoma progression. This application is focused on elucidating the genetic mechanisms that drive the transition of in situ melanoma to invasive disease.

Relevance to the Mission of the Melanoma Research Program: This application is focused on elucidating the genetic mechanisms that drive the transition of in situ melanoma to invasive disease. Therefore, our proposed experiments directly seek to "understand how precursor lesions and nvironmental/endogenous factors influence melanomagenesis." Part of our proposal will conduct a genetic screen to identify drivers of disease progression using a novel model of early-stage melanoma.

Proposal Title:	CTL-Tolerant Melanoma Persister Cells
Log Number:	ME220037
Current PI Name:	Matthew Hangauer
Award Number:	HT9425-23-1-0719
Current Contracting Organization:	California, University of, San Diego
Current Performing Organization:	California, University of, San Diego
Web Approval Date:	09-15-2023

Fiscal year 2022 (FY22) Melanoma Research Program (MRP) Focus Area: We propose to characterize a novel population of melanoma cells which constitute dormant tumors and seed tumor recurrence. Therefore, this proposal addresses the FY22 MRP Focus Area: Delineate the molecular pathways that influence metastatic spread, recurrence, and/or dormancy. Melanoma tumors must escape the immune system in order to progress both during tumor initiation and also in the context of resistance to immunotherapy. If this process could be blocked therapeutically, melanoma progression would be prevented. However, we have limited understanding of how this process occurs. In particular, while we know that melanoma tumors can convert from initially immune reactive tumors into "cold" tumors in which immune cells are excluded or exhausted, we do not understand the mechanisms which govern this fundamental transition. In particular, the intermediate stage of this process, during which residual dormant melanoma cells survive immune attack and subsequently seed tumor regrowth, has proven very difficult to study.

To study the dormant melanoma cells which survive immune attack, we have leveraged novel experimental models, which allow for observation of melanoma and immune cell interactions over prolonged timespans. Using these models, we made a profound discovery. We observed a novel population of dormant melanoma "persister cells," which survive direct immune cell attack through unknown mechanisms. In addition, persister cells directly seed outgrowth of escaped melanoma cells which have acquired overt resistance to immune cells. Therefore, this proposal presents a new paradigm, centered around melanoma persister cells, for how melanoma tumors initially survive and then escape from the immune system. Therapeutic targeting of persister cells has the potential to prevent melanoma progression both during tumor initiation and acquired resistance to immunotherapy. Therefore, this proposal addresses the FY22 MRP Challenge Statement by revealing a new paradigm for melanoma immune evasion and progression which can be exploited to prevent melanoma progression.

We propose to understand how persister cells form and survive immune attack, how persister cells seed outgrowth of immune cell-resistant melanoma cell populations, and to study the functional roles of persister cells in preclinical animal models of melanoma immunotherapy response. Together, these proposed aims will serve to provide the first characterization of this newly discovered melanoma cell population, which is critical for melanoma progression. This basic research has the potential to reveal new therapeutic targets within persister cells which may be utilized to prevent melanoma progression both during initial melanoma formation (e.g., neoadjuvant treatment) as well as during immunotherapy treatment (e.g., combination treatment) to prevent acquired resistance.

Melanoma is among the most commonly diagnosed cancers in Service Members, Veterans, and other military beneficiaries, and while treatments for melanoma have advanced tremendously in the past decade, there remains a very strong need for new approaches to achieve durable treatment responses. This proposed work, by revealing a new population of melanoma cells which underlie immune resistance, has the potential to create a new field of research and an array of new therapeutic opportunities to prevent melanoma progression. Therefore, this proposal is appropriate in scope and impact for the MRP Idea Award.

Proposal Title:	Biomimetic Nanoparticle-Mediated Delivery of Immunomodulating Nucleic Acids as a Strategy to Anticipate Melanoma Drug Resistance
Log Number:	ME220048
Current PI Name:	Gabriele Romano
Award Number:	HT9425-23-1-0576
Current Contracting Organization:	Drexel University
Current Performing Organization:	Drexel University
Web Approval Date:	08-17-2023

Cancer treatment has been revolutionized in the last 2 decades by targeted approaches (drugs that act on specific cancer vulnerabilities) and immunotherapeutic approaches (drugs that take "off the brakes" of the immune system), extending thousands of people's lives every year. Melanoma, a type of skin tumor, has been the poster child for targeted therapy and immunotherapy. Nevertheless, most patients still experience upfront or acquired resistance to treatment. As a result, precision medicine has sharply moved toward combination approaches to exploit multiple tumor vulnerabilities simultaneously. However, the efforts to combine targeted and immunotherapy strategies have often revealed a lack of efficacy. Some of the patients showing worst treatment outcomes have baseline low immune response, a status that is described as a "cold" tumor bed. Interestingly, our work suggests that certain types of targeted therapy can cause the tumors to go "cold" and might explain some lack of response in treatment approaches and recent trial failures.

Our central hypothesis is that the adverse effects of targeted therapy on the immune system must be mitigated to achieve a long-lasting treatment response in melanoma. This project will detail targeted therapy's undesired immunologic effects (Aim 1) and optimize a counteracting strategy (Aim 2).

Melanoma incidence is increasing among active-duty Service Members, with the greatest incidence rates in the Air Force, Navy, and Marines, with the Air Force presenting with the highest incidence rate among the branches. As a result, there is a pressing need to develop novel treatment strategies, especially for patients currently showing low response rates and survival percentages. The proposed approach is paradigm-shifting, as it has the potential to develop innovative therapeutic tools to re-wire the tumor microenvironment from "cold" to "hot" and tilt the balance in favor of a tumor-eradicating immune response (fiscal year 2022 Melanoma Research Program Focus Area, microenvironment impact on response to therapy). Notably, the project has broad potential applicability as this strategy might be applied to multiple melanoma subtypes and therapeutic strategies where "cold" tumors are an obstacle to efficacious cancer treatment.

Proposal Title:	The Importance of NKG2D Signaling-Induced IL-24 Secretion by Tumor-Specific CD8+ T Cells
Log Number:	ME220112
Current PI Name:	Mary Markiewicz
Award Number:	HT9425-23-1-0630
Current Contracting Organization:	Kansas, University of, Medical Center Research Institute, Inc.
Current Performing Organization:	Kansas, University of, Medical Center Research Institute, Inc.
Web Approval Date:	09-15-2023

Fiscal Year 2022 (FY22) Melanoma Research Program (MRP) Focus Area: This proposal is responsive to the FY22 MRP challenge statement in that inducing strong tumor immunity in a patient has the potential to "inhibit the emergence from tumor dormancy and the development of metastases." The primary FY22 MRP Focus Area this proposal addresses is to "identify how the tumor microenvironment (immune) impacts tumor progression and/or dormancy."

Background: Although highly treatable at early stages, once melanoma has spread to distant sites, the survival rate is low. Clearly additional treatment strategies are needed. Recent successes, particularly in melanoma patients, demonstrate the power of using the immune system clinically to destroy cancer. However, this treatment is ineffective in most patients. Our goal is to move forward continued research into how to make a patient’s own immune system destroy their melanoma. One protein on immune cells that we know has an important role in tumor control is called "NKG2D." Like NKG2D, a subset of immune cells called "CD8+ T cells" play an essential role as well. In addition, CD8+ T cells also express NKG2D, along with the proteins that interact with NKG2D to induce tumor cell killing from the cells. The expression of these NKG2D-interacting proteins by CD8+ T cells has largely been ignored by the scientific community. Our preliminary data demonstrate that mouse CD8+ T cells that express these proteins secrete significantly greater amounts of a protein called "interleukin-24" (IL-24) compared with those that do not express these proteins. IL-24 has been shown to be able to cause the death of melanoma cells. However, there are major gaps in knowledge about IL-24, including how its production is regulated and the mechanisms by which it performs its biological functions.

Objective: To determine if NKG2D is critical in making CD8+ T cells that make IL-24, which have enhanced melanoma-killing properties.

Specific Aims

Aim 1: Determine how NKG2D induces IL-24 production by mouse CD8+ T cells. Aim 2: Determine the importance of IL-24 production in melanoma control in mouse tumor models. Aim 3: Determine if NKG2D generates human CD8+ T cells that secrete IL-24.

Innovation: Although there has been intense interest in using IL-24 clinically, there are major gaps in knowledge about this protein, including how its production is regulated and the mechanisms by which it performs its biological functions. The goal of the work proposed in this IDEA Award application is to begin to fill in these knowledge gaps and determine whether further study into NKG2D-induced IL-24 production by anti-melanoma CD8+ T cells is warranted.

Impact: This proposal involves preclinical animal studies and human cell culture studies aimed at increasing therapy options for melanoma. These studies will provide the proof of principle needed to perform larger studies investigating the potential of targeting NKG2D in metastatic melanoma patients to make IL-24-

secreting CD8+ T cells that are better killers of melanoma cells. United States active duty and Veteran populations are at an increased risk of melanoma due to the nature of their duties. This makes novel ways of preventing and treating this disease a priority for the military.

Proposal Title:	Microsomal Glutathione Transferase as a New Target in Melanotic Melanoma
Log Number:	ME220116
Current PI Name:	Kenneth Tew
Award Number:	HT9425-23-1-0649
Current Contracting Organization:	Medical University of South Carolina
Current Performing Organization:	Medical University of South Carolina
Web Approval Date:	07-12-2023

One in five Americans will get skin cancer in their lifetimes. Skin cancers can affect your appearance as well as your health. Skin cancer is the most common form of cancer in the United States, with more than 3.5 million cases diagnosed yearly – more than all other cancers combined. Melanoma is the most deadly type of skin cancer. Despite some high-profile success stories using targeted and immunotherapy, it remains difficult to cure. Among cancers, melanoma is unique in its requirement for melanin, a pigment found in skin cells known as melanocytes. Melanin is a molecule that is created from amino acids through incompletely characterized pathways that produce both pheomelanin and eumelanin. Humans differ broadly in their capacities to generate melanin, and this creates a situation where certain individuals are more susceptible to melanoma initiation and progression. Our preliminary studies have shown that specific steps in melanin synthesis are catalyzed by enzymes that if knocked out, adversely alter melanin levels in melanoma cells and survival of melanoma-bearing mice. We hypothesize that one key step in melanin synthesis is altered in the absence of the enzyme (MGST1) and wish to examine this step, permitting a greater understanding of the missing link between this enzyme and melanoma. Moreover, humans are subject to genetic variation in GST enzymes, that is, some individuals do not have the types of enzymes that are integral to the melanin synthetic pathway. This may alter their susceptibility to ultraviolet-light induced conversion of melanocytes and other surrounding cells to a precancerous, and eventually cancer state. Our goal is to interrogate the melanin biosynthetic pathway, define the importance of an enzyme that we think influences melanin and pigmentation levels (also having antioxidant properties), relate this to alterations in immunity and determine whether new drugs that inhibit this enzyme can be developed as therapeutics. We have established evidence in preclinical models that depletion of the target enzyme significantly depletes pigmentation, delays cancer growth and metastases, and prolongs survival. Our short-term goals will include studies on how existing chemo-, targeted, and immunotherapies can be positively influenced by either genetic or pharmacologic inhibition of enzymatic catalysis. Results will prove the importance of MGST1 as a target and improve understanding of molecular pathways that influence metastatic spread, addressing fiscal year 2022 Melanoma Research Program Areas of Focus. If successful, a novel approach to the treatment of metastatic melanoma would improve survival of active-duty Service Members, Veterans, and the American public who suffer from this disease.

Proposal Title:	Role of Perilesional Reactive Astrocytosis in Melanoma Brain Metastases
Log Number:	ME220134
Current PI Name:	Joshua Jackson
Award Number:	HT9425-23-1-0633
Current Contracting Organization:	Drexel University
Current Performing Organization:	Drexel University
Web Approval Date:	10-03-2023

Melanoma is the deadliest form of skin cancer, and U.S. Military personnel are at an increased risk due to occupational exposures. Melanoma spreads to the brain with a high frequency, where it triggers neurologic symptoms including seizures. While melanoma restricted to the skin is often treatable, if the disease spreads to other organs - especially the brain - prognosis is poor. Once in the brain, melanoma interacts with resident cells including neurons, microglia, and astrocytes. We are particularly interested in interactions with the astrocytes, which normally provide pro-survival factors and regulate nutrient and local blood flow. These cells also regulate neuronal excitability by removing neurotransmitters preventing excessive activation and seizures. Following injury or infection, astrocytes also play an immune-like role that is characterized by changes in cell shape, protein expression and the functional support to neurons (called reactive astrocytosis). Reactive astrocytes have been found to surround melanoma brain metastasis at autopsy; however, little is known regarding how these cells influence the growth of melanoma in the brain and their resistance to treatment.

Our goal of the current proposal is to understand reactive changes in astrocytes in response to melanoma, how these responses might in turn influence melanoma growth in the brain, and whether treatments targeting reactive astrocytes could be harnessed for better outcomes. This project is a collaborative effort, bringing tools and expertise from the fields of neuroscience and basic cancer biology fields together to highlight the importance of interactions of melanoma brain metastases with cells that form the local brain microenvironment.

This work has no potential risks for patients and will directly benefit the men and women of the U.S. military, who have a higher incidence rate for melanoma. The current proposal addresses the “Understanding the Tumor Microenvironment” Focus Areas of the fiscal year 2022 Melanoma Research Program Idea Award.

Proposal Title:	Defining the Glyco-RNAome in Melanoma Progression
Log Number:	ME220138
Current PI Name:	Florian Karreth
Award Number:	HT9425-23-1-0513
Current Contracting Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Current Performing Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Web Approval Date:	07-11-2023

Cells attach sugar molecules to proteins to regulate their function and localization. The machinery that controls the attachment of sugars is often perturbed in cancers such as melanoma, rendering tumor cells more aggressive and metastatic and invisible to the immune system. A recent study reported that sugar molecules can also be attached to RNAs and that such “glyco-RNAs” decorate the outside of cells, likely to be presented to the cells’ surroundings. However, the molecular functions of glyco-RNAs and their effect on the interaction of cancer cells with their host are completely unknown. In this Idea Award application, we propose to identify glyco-RNAs that play important roles in the aggressive behavior of melanoma cells. Importantly, sugar modification of RNAs may be controlled by dietary intake of sugars, such as fucose, to reduce the risk of melanoma formation and metastasis. Thus, characterizing the roles of glyco-RNAs in melanoma aligns well with the fiscal year 2022 (FY22) Melanoma Research Program (MRP) Challenge Statement. Moreover, since glyco-RNAs are presented at the cell surface where they interact with the environment and immune cells, which could affect progression and metastasis, our study directly addresses two FY22 MRP Focus Areas: “Identify how the tumor microenvironment impacts tumor initiation, response to therapy, progression, recurrence, and/or dormancy” and “Delineate the molecular pathways that influence metastatic spread, recurrence, and/or dormancy.” Our studies will uncover a completely unrecognized new dimension of RNA and glycobiology, and there is tremendous potential for discoveries that will have a significant impact on these aspects of melanoma biology and thus offer new avenues for therapeutic intervention. For instance, by controlling the dietary consumption of certain sugars, the risk of melanoma patients to develop recurrent or metastatic disease could be lowered. This Idea Award will enable us to define and lead the burgeoning glyco-RNA field and to improve our understanding of how the interaction of melanoma cells with their environment provokes more aggressive disease. This knowledge, in turn, will be instrumental in developing new ideas to limit these interactions for melanoma prevention and therapeutic intervention. This will significantly benefit active-duty Service Members and Veterans who, due to their service at locations with high UV indexes, are at a higher risk of developing melanoma.

Proposal Title:	FIND-MEL: Developing an Application for Following Images of Nevi to Detect Melanoma
Log Number:	ME220193
Current PI Name:	Veronica Rotemberg
Award Number:	HT9425-23-1-0848
Current Contracting Organization:	Sloan Kettering Institute for Cancer Research
Current Performing Organization:	Sloan Kettering Institute for Cancer Research
Web Approval Date:	10-03-2023

Dr. Rotemberg's goals in melanoma research are to become an independently funded physician-scientist who focuses on automatic ways to find melanoma. Dr. Rotemberg has been an advocate for high quality studies and public data for public use, and her goal is to continue to develop new technologies that can benefit patients in the most collaborative way that inspires future research all over the world.

The research and career development plans in the attached proposal support Dr. Rotemberg's research development as a leader in melanoma research as well as her technical development in technology research. She has partnered with Dr. Susan Swetter as a career guide, a stellar melanoma researcher, leader, and mentor. Dr. Dy is also a co-investigator on the award, who will continue to support Dr. Rotemberg's development toward her own research laboratory.

Automatic methodologies, such as artificial intelligence (AI), for detection of skin cancer is already better than many dermatologists, but it has mostly been tested outside of real-world conditions. Most of the research in this area has focused on dermoscopic images, which uses a specialized device that magnifies a skin lesion and uses polarized light. This proposal addresses the Melanoma Research Program (MRP) Focus Area of: Develop new tools for the detection and diagnosis of melanoma, which includes easily accessible technology (beyond the dermoscope) for primary care physicians and dermatologists. Melanoma is the deadliest form of skin cancer, but it can be cured with surgery if caught at an early stage. We see AI to use patient photos to help find melanomas as soon as possible. We have designed a study that will use all different types of photographs to improve automatic detection of melanoma on photographs captured by any smartphone or digital camera.

The study addresses the fiscal year 2022 MRP Challenge Statement by focusing on both improved detection and AI-enabled lesion monitoring to detect melanoma as early as possible. This will target diagnosing melanoma prior to invasion and preventing the development of metastases.

This research is very applicable for improving melanoma detection in melanoma patients and those who are at high risk for developing melanoma. We know patients who have had melanoma and those with other risk factors are at higher risk for developing melanoma. Through this project, we will take advantage of photographs that have been collected in dermatology clinics to improve the ability of AI to find melanomas remotely through a cell phone or digital camera photograph. The potential benefits, especially for patients who don't have access to a dermatologist nearby (including Veterans and deployed military personnel) are especially valuable, because most spots on a patient's body will end up being benign. Having an accurate way to rule out melanoma from a cellular phone without going to a dermatologist in person would be very valuable. However, it means these technologies must be very accurate before we use them directly. We hope that the techniques developed in this proposal will be used for rigorous clinical trials in the future so they can be comprehensively studied and deployed.

In the short term, our goals are to develop and validate a method for monitoring photographs of skin lesions without in-person dermatologist examination. We will then use this data to support development in the community by making the algorithm open source as well as use this work as preliminary data for a prospective clinical trial via additional funding. Our long-term goals are that this type of approach to monitor lesions over time reduces unnecessary biopsies and improves our remote ability to find melanoma. We hope that this will especially help patients in remote and austere areas, especially our Veterans and active-duty personnel, by reducing their need to find a dermatologist when they are doing well, and making triage of dangerous lesions easier so that the necessary people and lesions are appropriately referred.

Proposal Title:	Role of Vascular Mimicry in the Outgrowth of Dormant Melanoma Micrometastases
Log Number:	ME220194
Current PI Name:	Lucia Jilaveanu
Award Number:	HT9425-23-1-0670
Current Contracting Organization:	Yale University
Current Performing Organization:	Yale University
Web Approval Date:	09-15-2023

Since I started my laboratory in 2014 my research has focused on understanding the mechanisms involved in melanoma brain metastasis, for which I have established a research pipeline using an integrated proteogenomics approach and novel brain metastasis models that I developed. My current lab-based efforts are related to furthering our understanding of how the micrometastatic tumor microenvironment controls the outgrowth of dormant lesions. I have been heavily involved in the development of a translational melanoma brain metastasis research program at Yale, with the goal of developing new drugs and delivering personalized therapy for melanoma patients with brain metastases. These studies are part of a broader endeavor ongoing at Yale aimed at changing treatment paradigms for brain metastasis patients.

One of the biggest mysteries of melanoma, but other cancers as well, is how disseminated cancer cells can be dormant in distant organs for years, only to emerge later on as clinically difficult to cure lesions. The factors regulating the transition from dormant to progressively growing metastases continue to be understudied and not understood. This proposal focuses on establishing the importance of a scarcely studied process called vascular mimicry, in which cancer cells contribute to the formation and lining of functional blood vessels in tumors, and their acquisition of increased blood supply during expansion. Our preliminary work using novel preclinical models we have developed led to the hypothesis that vascular mimicry could be linked to the switch from dormancy to proliferative state in organs poorly vascularized such as the brain; therefore, the focus of these studies on brain metastatic melanoma. If proven true, this could have broad implications ranging from predilection to develop early metastasis and accelerated disease to ineffectiveness of drugs currently used in clinic to treat melanoma. Our application is highly responsive to the Melanoma Research Program Challenge Statement through its scope and addresses two specific Focus Areas: (1) it is designed to uncover new molecular pathways that influence melanoma dormancy and recurrence, and (2) it seeks to improve our understanding of how the tumor microenvironment, specifically neovascularization influences the transition from dormant state to progressive melanoma. If successful, our work will establish vascular mimicry as a potential weakness of brain metastatic melanomas, in that they may be reliant on a particular type of vasculature in the early stage during metastatic awakening, and might provide new opportunities to develop strategies to prevent expansion or stabilize 'dormant' metastases. Our studies may provide a strong rationale for exploiting this process as a therapeutic target to prevent the proliferative outbreak of dormant micrometastases or perhaps to develop novel approaches to eradicate dormant tumor cells, thus preventing disease recurrence.

Once melanoma metastasizes, it is typically treated with systemic therapies. In recent years major progress has been made in treating metastatic melanoma. However, studies that led to these advances have excluded patients with brain metastases and little is therefore known about systemic therapy for melanoma brain lesions. Moreover, there is a paucity of clinically relevant animal models of melanoma brain metastases. Therefore, my group has been developing resources to address these deficiencies in recent years, as described in the preliminary data sections. These resources will enable me to reach the goal of developing new drugs to either prevent or treat brain metastases and delivering personalized therapy for melanoma patients. From a translational perspective, I hope to bring discoveries to the clinic to improve our

understanding and knowledge of metastatic disease and to improve patient care. If successful, these studies will decrease the morbidity and mortality from melanoma brain metastases, which disproportionately affects Service Members and Veterans, and therefore decrease the secondary psychosocial distress among Family Members as well.

Proposal Title:	Beyond Tumor Staging: Measuring Immune Status at Initial Diagnosis to Detect and Prevent Melanoma Recurrence
Log Number:	ME220197
Current PI Name:	Georgia Beasley
Award Number:	HT9425-23-1-0746
Current Contracting Organization:	Duke University
Current Performing Organization:	Duke University
Web Approval Date:	09-15-2023

For the majority of patients diagnosed with melanoma, surgery is the only therapy ever needed for cure. However, for reasons poorly understood, in about 1 of every 6 cases, the original melanoma can still spread through the body and be discovered in other organs months to years later. Once metastases are discovered, they can cause unwanted symptoms and increase chance of death from melanoma. Currently, predicting which cases will spread is not very precise which leads in some cases to patients being given too much treatment, while in other cases patients do not receive enough treatment. There is also currently no way to detect melanoma recurrence, and usually the metastases are only discovered once a patient is experiencing symptoms (e.g., bleeding from intestine lesions). The current treatment for preventing melanoma recurrence /metastasis after surgery involves adjuvant therapy with anti-programmed cell death protein 1 (anti-PD-1) inhibitors, which are prescribed to patients with American Joint Committee on Cancer (AJCC) stage IIB or higher. However, it is known that the majority of metastatic melanoma tumors (over 60%) are resistant to anti-PD-1 therapy. Moreover, treatment with anti-PD-1 therapy will elicit no benefit for the majority of patients who have excellent prognosis after surgery alone. Overall, this under- or overtreatment of melanoma patients is due to two primary deficiencies in the field: (1) our inability to accurately assess metastatic potential/risk of recurrence; and (2) our inability accurately detect recurrence.

The current method to determine who is at risk for melanoma recurrence relies on factors about the tumor (e. g., tumor thickness or Breslow depth) while detection of recurrence is limited to physical exam. Melanoma has long been known to interact with the immune system. Indeed, a strong immune reaction is thought to be necessary to control melanoma. However, immune response to tumor is not currently measured in the clinic nor used to determine which melanoma are at risk for spreading. As a result, tumor staging too often underestimates the recurrence rate for the majority of patients in the U.S. who die of metastatic melanoma. Finally, there is currently no way to detect recurrence until symptoms (bleeding, pain) arise and only then is treatment given. There is a critical need for reliable methods of identifying patients at highest risk for melanoma recurrence and methods for detection of recurrence to improve treatment and survival rates in this patient population.

This proposal addresses three FY22 MRP Focus Areas: (1) Develop prediction and surveillance tools for distinguishing patient populations at risk for additional primary melanomas, recurrence, and/or metastasis. (2) Identify how the tumor microenvironment (e.g., stromal, immune, microbiome) impacts tumor initiation, response to therapy, progression, recurrence, and/or dormancy; and (3) Delineate the molecular pathways that influence metastatic spread, recurrence, and/or dormancy. Preliminary work from our group shows that measuring a melanoma patient’s immune status at initial diagnosis can serve as an accurate and reliable method for assessing metastatic potential. Specifically, our group has preliminary data showing that: (1) B cell orientation and activation in tumor draining lymph nodes (TDLN) can distinguish patients at high risk for recurrence; and (2) immune function measured in blood and TDLN associates with host antitumor responses and tumor control.

Furthermore, we have preliminary evidence showing that blood-based markers, including a novel test we are developing, can improve early detection of recurrence and monitor effectiveness of adjuvant therapy in patients with stage IIB-IIIC melanoma. Based on our preliminary data, we plan to test immune function in patients at initial melanoma diagnosis by studying B cells in TDLN, testing immune function in blood and TDLN, and collecting blood at multiple time points after initial diagnosis to detect recurrence in blood.

This proposal addresses the fiscal year 2022 Melanoma Research Program Challenge Statement by shifting the paradigm of melanoma progression prevention treatment toward accurately identifying those patients who are at the highest risk of recurrence and metastasis. Our preliminary data indicate that assessment of immune function at initial diagnosis can fill this knowledge gap, allowing identification of patients for whom effective adjuvant therapy will induce durable antitumor responses, setting them on the road to recovery. Conversely, immune function assessment will allow for the accurate identification of patients most likely to have excellent prognosis through surgery alone and for whom adjuvant therapy would prove unnecessary and not beneficial. Finally, detecting recurrence in blood before symptoms appear will dramatically improve effectiveness of therapy. The short-term impacts of this work will not only be the development of a reliable and accurate method of assessing patients' risk of recurrence, but also a significant reduction in the under- and overtreatment of melanoma patients after initial surgery. The long-term impact of this research will be a significant reduction in the morbidity and mortality of melanoma patients, with the ultimate goal of them never having to hear "your cancer is back."

Proposal Title:	Beyond Tumor Staging: Measuring Immune Status at Initial Diagnosis to Detect and Prevent Melanoma Recurrence
Log Number:	ME220197P1
Current PI Name:	Smita Nair
Award Number:	HT9425-23-1-0747
Current Contracting Organization:	Duke University
Current Performing Organization:	Duke University
Web Approval Date:	09-15-2023

For the majority of patients diagnosed with melanoma, surgery is the only therapy ever needed for cure. However, for reasons poorly understood, in about 1 of every 6 cases, the original melanoma can still spread through the body and be discovered in other organs months to years later. Once metastases are discovered, they can cause unwanted symptoms and increase chance of death from melanoma. Currently, predicting which cases will spread is not very precise which leads in some cases to patients being given too much treatment, while in other cases patients do not receive enough treatment. There is also currently no way to detect melanoma recurrence, and usually the metastases are only discovered once a patient is experiencing symptoms (e.g., bleeding from intestine lesions). The current treatment for preventing melanoma recurrence /metastasis after surgery involves adjuvant therapy with anti-programmed cell death protein 1 (anti-PD-1) inhibitors, which are prescribed to patients with American Joint Committee on Cancer (AJCC) stage IIB or higher. However, it is known that the majority of metastatic melanoma tumors (over 60%) are resistant to anti-PD-1 therapy. Moreover, treatment with anti-PD-1 therapy will elicit no benefit for the majority of patients who have excellent prognosis after surgery alone. Overall, this under- or overtreatment of melanoma patients is due to two primary deficiencies in the field: (1) our inability to accurately assess metastatic potential/risk of recurrence; and (2) our inability accurately detect recurrence.

The current method to determine who is at risk for melanoma recurrence relies on factors about the tumor (e. g., tumor thickness or Breslow depth) while detection of recurrence is limited to physical exam. Melanoma has long been known to interact with the immune system. Indeed, a strong immune reaction is thought to be necessary to control melanoma. However, immune response to tumor is not currently measured in the clinic nor used to determine which melanoma are at risk for spreading. As a result, tumor staging too often underestimates the recurrence rate for the majority of patients in the U.S. who die of metastatic melanoma. Finally, there is currently no way to detect recurrence until symptoms (bleeding, pain) arise and only then is treatment given. There is a critical need for reliable methods of identifying patients at highest risk for melanoma recurrence and methods for detection of recurrence to improve treatment and survival rates in this patient population.

This proposal addresses three FY22 MRP Focus Areas: (1) Develop prediction and surveillance tools for distinguishing patient populations at risk for additional primary melanomas, recurrence, and/or metastasis. (2) Identify how the tumor microenvironment (e.g., stromal, immune, microbiome) impacts tumor initiation, response to therapy, progression, recurrence, and/or dormancy; and (3) Delineate the molecular pathways that influence metastatic spread, recurrence, and/or dormancy. Preliminary work from our group shows that measuring a melanoma patient’s immune status at initial diagnosis can serve as an accurate and reliable method for assessing metastatic potential. Specifically, our group has preliminary data showing that: (1) B cell orientation and activation in tumor draining lymph nodes (TDLN) can distinguish patients at high risk for recurrence; and (2) immune function measured in blood and TDLN associates with host antitumor responses and tumor control.

Furthermore, we have preliminary evidence showing that blood-based markers, including a novel test we are developing, can improve early detection of recurrence and monitor effectiveness of adjuvant therapy in patients with stage IIB-IIIC melanoma. Based on our preliminary data, we plan to test immune function in patients at initial melanoma diagnosis by studying B cells in TDLN, testing immune function in blood and TDLN, and collecting blood at multiple time points after initial diagnosis to detect recurrence in blood.

This proposal addresses the fiscal year 2022 Melanoma Research Program Challenge Statement by shifting the paradigm of melanoma progression prevention treatment toward accurately identifying those patients who are at the highest risk of recurrence and metastasis. Our preliminary data indicate that assessment of immune function at initial diagnosis can fill this knowledge gap, allowing identification of patients for whom effective adjuvant therapy will induce durable antitumor responses, setting them on the road to recovery. Conversely, immune function assessment will allow for the accurate identification of patients most likely to have excellent prognosis through surgery alone and for whom adjuvant therapy would prove unnecessary and not beneficial. Finally, detecting recurrence in blood before symptoms appear will dramatically improve effectiveness of therapy. The short-term impacts of this work will not only be the development of a reliable and accurate method of assessing patients' risk of recurrence, but also a significant reduction in the under- and overtreatment of melanoma patients after initial surgery. The long-term impact of this research will be a significant reduction in the morbidity and mortality of melanoma patients, with the ultimate goal of them never having to hear "your cancer is back."

Proposal Title:	Targeting PRMT5 in MTAP-Loss Melanoma
Log Number:	ME220215
Current PI Name:	Taiping Chen
Award Number:	HT9425-23-1-0825
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	10-03-2023

Background: Cancer is driven by either the loss of a tumor suppressor or the gain of an oncogene. The CDKN2A/B locus is a tumor suppressor region that is deleted in roughly 20% of all cancer types and up to 30% in melanoma. Melanoma patients bearing deletion of this locus exhibit poor clinical outcome and resistance to currently available treatment, highlighting an unmet need to develop effective therapeutic approaches for these patients. Importantly, when the CDKN2A/B locus is deleted in cancer there is very often (80% of cases) also a deletion of closely linked metabolic enzyme called methylthioadenosine phosphorylase (MTAP), which is referred to as "collateral lethality." Given that MTAP is the enzyme responsible for producing a metabolite called methylthioadenosine (MTA), MTA levels are much higher in MTAP-null tumors than those in normal tissues. Recently, MTA has been shown to be a nature inhibitor for protein arginine methyltransferase 5 (PRMT5), an enzyme with critical roles in cancer development via regulating protein modification. Therefore, CDKN2A/B/MTA-null tumors display a unique vulnerability to PRMT5 inhibitors (PRMT5i). Since then, there have been numerous efforts by Pharma and Biotech to develop PRMT5i, and there are at least 20 different types of PRMT5i. The efficacy of the first-generation inhibitors for cancer treatment was not impressive, likely due to the difficulty in establishing a therapeutic window. To address this issue, several companies (Mirati, Tango, and Amgen) have developed second-generation inhibitors, which only suppress the enzymatic activity of PRMT5 in presence of high MTA levels. Preliminary data from our groups show that these new inhibitors are exquisitely selective for MTAP-null tumors. In this manner, PRMT5 is total inhibited in the cancer cells that harbor the CDKN2A/B/MTA-deletion, while the normal cells remain untouched by these inhibitors, thus generating a very large and safe therapeutic window. It is important to note that MTA can also be secreted by MTAP-null tumors, which has been linked to a "cold" tumor-immune phenotype. Thus, we are dealing with two independent biological functions for elevated MTA levels in these cancer cells and their microenvironment: (1) The unique vulnerability (to PRMT5 inhibition) and (2) resistance (to immunotherapy). We expect that second-generation PRMT5 inhibitors will reduce tumor size, and as a consequence of this atrophy, the PRMT5i-treated tumors will also reduce the amount of secreted MTA, which in turn will sensitize the tumors to immunotherapy.

Melanoma Research Program Focus Areas Addressed: (1) Identify how the tumor microenvironment impacts tumor initiation, response to therapy. (2) Delineate the molecular pathways that influence metastatic spread and recurrence.

Rationale, Objective, and Aims: We hypothesize that by selectively inhibiting PRMT5 in MTAP-loss cells with high MTA, tumor volume will be reduced, resulting in the tumor microenvironment harboring low MTA and consequently becoming responsive to immunotherapy. Our objective is to develop a comprehensive plan to definitively evaluate the therapeutic potential of PRMT5 inhibitors (PRMT5i) with different mechanisms of action, alone and in combination with immunotherapeutic for MTAP-null melanomas. These goals will be achieved by the following Aims.

Aim 1: Develop melanoma mouse and cell models of MTAP loss to understand its impact on melanoma development and to better evaluate treatment efficacy.

Aim 2: Determine antitumor activity of PRMT5i in combination with immunotherapy in MTAP-loss melanoma.

Aim 3: Identify therapeutic targets for MTAP-loss melanoma that have developed PRMT5i resistance.

The Following Melanoma Patients Will Be Helped by This Proposal: Second-generation PRMT5 inhibitors specifically target a cancer cell vulnerability that occurs because of MTAP loss. This MTAP-null genotype accounts for roughly 30% of melanomas. Thus, there is a clear biomarker (MTAP expression) which can be used to stratify melanoma patients for treatment. Importantly, MTAP loss in tumor biopsies is routinely stained for at MD Anderson, thus a patient's MTAP status will be known at the time of melanoma diagnosis.

Potential Clinical Application: The objective of this project is to develop safe and effective personalized therapies for MTAP-loss melanoma patients. Our studies are expected to fill in the significant knowledge gap in the targetability of MTAP-loss tumors and identify novel therapeutic approaches with the potential for fast bench-to-bedside translation. Importantly, MD Anderson Cancer Center has two (Amgen and Mirati) active phase 1 trials for these second-generation PRMT5 inhibitors. The immunotherapeutic approach proposed for combinations has been approved to treat melanoma patients. Upon a successful completion of proposed studies, we expect that our findings will be translated into clinic within 2 years.

Proposal Title:	Targeting PRMT5 in MTAP-Loss Melanoma
Log Number:	ME220215P1
Current PI Name:	Weiyi Peng
Award Number:	HT9425-23-1-0826
Current Contracting Organization:	Houston, University of
Current Performing Organization:	Houston, University of
Web Approval Date:	10-03-2023

Background: Cancer is driven by either the loss of a tumor suppressor or the gain of an oncogene. The CDKN2A/B locus is a tumor suppressor region that is deleted in roughly 20% of all cancer types and up to 30% in melanoma. Melanoma patients bearing deletion of this locus exhibit poor clinical outcome and resistance to currently available treatment, highlighting an unmet need to develop effective therapeutic approaches for these patients. Importantly, when the CDKN2A/B locus is deleted in cancer there is very often (80% of cases) also a deletion of closely linked metabolic enzyme called methylthioadenosine phosphorylase (MTAP), which is referred to as "collateral lethality." Given that MTAP is the enzyme responsible for producing a metabolite called methylthioadenosine (MTA), MTA levels are much higher in MTAP-null tumors than those in normal tissues. Recently, MTA has been shown to be a nature inhibitor for protein arginine methyltransferase 5 (PRMT5), an enzyme with critical roles in cancer development via regulating protein modification. Therefore, CDKN2A/B/MTA-null tumors display a unique vulnerability to PRMT5 inhibitors (PRMT5i). Since then, there have been numerous efforts by Pharma and Biotech to develop PRMT5i, and there are at least 20 different types of PRMT5i. The efficacy of the first-generation inhibitors for cancer treatment was not impressive, likely due to the difficulty in establishing a therapeutic window. To address this issue, several companies (Mirati, Tango, and Amgen) have developed second-generation inhibitors, which only suppress the enzymatic activity of PRMT5 in presence of high MTA levels. Preliminary data from our groups show that these new inhibitors are exquisitely selective for MTAP-null tumors. In this manner, PRMT5 is total inhibited in the cancer cells that harbor the CDKN2A/B/MTA-deletion, while the normal cells remain untouched by these inhibitors, thus generating a very large and safe therapeutic window. It is important to note that MTA can also be secreted by MTAP-null tumors, which has been linked to a "cold" tumor-immune phenotype. Thus, we are dealing with two independent biological functions for elevated MTA levels in these cancer cells and their microenvironment: (1) The unique vulnerability (to PRMT5 inhibition) and (2) resistance (to immunotherapy). We expect that second-generation PRMT5 inhibitors will reduce tumor size, and as a consequence of this atrophy, the PRMT5i-treated tumors will also reduce the amount of secreted MTA, which in turn will sensitize the tumors to immunotherapy.

Melanoma Research Program Focus Areas Addressed: (1) Identify how the tumor microenvironment impacts tumor initiation, response to therapy. (2) Delineate the molecular pathways that influence metastatic spread and recurrence.

Rationale, Objective, and Aims: We hypothesize that by selectively inhibiting PRMT5 in MTAP-loss cells with high MTA, tumor volume will be reduced, resulting in the tumor microenvironment harboring low MTA and consequently becoming responsive to immunotherapy. Our objective is to develop a comprehensive plan to definitively evaluate the therapeutic potential of PRMT5 inhibitors (PRMT5i) with different mechanisms of action, alone and in combination with immunotherapeutic for MTAP-null melanomas. These goals will be achieved by the following Aims.

Aim 1: Develop melanoma mouse and cell models of MTAP loss to understand its impact on melanoma development and to better evaluate treatment efficacy.

Aim 2: Determine antitumor activity of PRMT5i in combination with immunotherapy in MTAP-loss melanoma.

Aim 3: Identify therapeutic targets for MTAP-loss melanoma that have developed PRMT5i resistance.

The Following Melanoma Patients Will Be Helped by This Proposal: Second-generation PRMT5 inhibitors specifically target a cancer cell vulnerability that occurs because of MTAP loss. This MTAP-null genotype accounts for roughly 30% of melanomas. Thus, there is a clear biomarker (MTAP expression) which can be used to stratify melanoma patients for treatment. Importantly, MTAP loss in tumor biopsies is routinely stained for at MD Anderson, thus a patient's MTAP status will be known at the time of melanoma diagnosis.

Potential Clinical Application: The objective of this project is to develop safe and effective personalized therapies for MTAP-loss melanoma patients. Our studies are expected to fill in the significant knowledge gap in the targetability of MTAP-loss tumors and identify novel therapeutic approaches with the potential for fast bench-to-bedside translation. Importantly, MD Anderson Cancer Center has two (Amgen and Mirati) active phase 1 trials for these second-generation PRMT5 inhibitors. The immunotherapeutic approach proposed for combinations has been approved to treat melanoma patients. Upon a successful completion of proposed studies, we expect that our findings will be translated into clinic within 2 years.

Proposal Title:	Targeting PRMT5 in MTAP-Loss Melanoma
Log Number:	ME220215P2
Current PI Name:	Han Xu
Award Number:	HT9425-23-1-0827
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	10-03-2023

Background: Cancer is driven by either the loss of a tumor suppressor or the gain of an oncogene. The CDKN2A/B locus is a tumor suppressor region that is deleted in roughly 20% of all cancer types and up to 30% in melanoma. Melanoma patients bearing deletion of this locus exhibit poor clinical outcome and resistance to currently available treatment, highlighting an unmet need to develop effective therapeutic approaches for these patients. Importantly, when the CDKN2A/B locus is deleted in cancer there is very often (80% of cases) also a deletion of closely linked metabolic enzyme called methylthioadenosine phosphorylase (MTAP), which is referred to as "collateral lethality." Given that MTAP is the enzyme responsible for producing a metabolite called methylthioadenosine (MTA), MTA levels are much higher in MTAP-null tumors than those in normal tissues. Recently, MTA has been shown to be a nature inhibitor for protein arginine methyltransferase 5 (PRMT5), an enzyme with critical roles in cancer development via regulating protein modification. Therefore, CDKN2A/B/MTA-null tumors display a unique vulnerability to PRMT5 inhibitors (PRMT5i). Since then, there have been numerous efforts by Pharma and Biotech to develop PRMT5i, and there are at least 20 different types of PRMT5i. The efficacy of the first-generation inhibitors for cancer treatment was not impressive, likely due to the difficulty in establishing a therapeutic window. To address this issue, several companies (Mirati, Tango, and Amgen) have developed second-generation inhibitors, which only suppress the enzymatic activity of PRMT5 in presence of high MTA levels. Preliminary data from our groups show that these new inhibitors are exquisitely selective for MTAP-null tumors. In this manner, PRMT5 is total inhibited in the cancer cells that harbor the CDKN2A/B/MTA-deletion, while the normal cells remain untouched by these inhibitors, thus generating a very large and safe therapeutic window. It is important to note that MTA can also be secreted by MTAP-null tumors, which has been linked to a "cold" tumor-immune phenotype. Thus, we are dealing with two independent biological functions for elevated MTA levels in these cancer cells and their microenvironment: (1) The unique vulnerability (to PRMT5 inhibition) and (2) resistance (to immunotherapy). We expect that second-generation PRMT5 inhibitors will reduce tumor size, and as a consequence of this atrophy, the PRMT5i-treated tumors will also reduce the amount of secreted MTA, which in turn will sensitize the tumors to immunotherapy.

Melanoma Research Program Focus Areas Addressed: (1) Identify how the tumor microenvironment impacts tumor initiation, response to therapy. (2) Delineate the molecular pathways that influence metastatic spread and recurrence.

Rationale, Objective, and Aims: We hypothesize that by selectively inhibiting PRMT5 in MTAP-loss cells with high MTA, tumor volume will be reduced, resulting in the tumor microenvironment harboring low MTA and consequently becoming responsive to immunotherapy. Our objective is to develop a comprehensive plan to definitively evaluate the therapeutic potential of PRMT5 inhibitors (PRMT5i) with different mechanisms of action, alone and in combination with immunotherapeutic for MTAP-null melanomas. These goals will be achieved by the following Aims.

Aim 1: Develop melanoma mouse and cell models of MTAP loss to understand its impact on melanoma development and to better evaluate treatment efficacy.

Aim 2: Determine antitumor activity of PRMT5i in combination with immunotherapy in MTAP-loss melanoma.

Aim 3: Identify therapeutic targets for MTAP-loss melanoma that have developed PRMT5i resistance.

The Following Melanoma Patients Will Be Helped by This Proposal: Second-generation PRMT5 inhibitors specifically target a cancer cell vulnerability that occurs because of MTAP loss. This MTAP-null genotype accounts for roughly 30% of melanomas. Thus, there is a clear biomarker (MTAP expression) which can be used to stratify melanoma patients for treatment. Importantly, MTAP loss in tumor biopsies is routinely stained for at MD Anderson, thus a patient's MTAP status will be known at the time of melanoma diagnosis.

Potential Clinical Application: The objective of this project is to develop safe and effective personalized therapies for MTAP-loss melanoma patients. Our studies are expected to fill in the significant knowledge gap in the targetability of MTAP-loss tumors and identify novel therapeutic approaches with the potential for fast bench-to-bedside translation. Importantly, MD Anderson Cancer Center has two (Amgen and Mirati) active phase 1 trials for these second-generation PRMT5 inhibitors. The immunotherapeutic approach proposed for combinations has been approved to treat melanoma patients. Upon a successful completion of proposed studies, we expect that our findings will be translated into clinic within 2 years.

Proposal Title:	Identification and Peripheral Tracking of Melanoma-Specific T Cells After Early-Stage Melanoma Diagnosis for Detection of Recurrence or Metastasis
Log Number:	ME220242
Current PI Name:	Noah Hornick
Award Number:	HT9425-23-1-0662
Current Contracting Organization:	Oregon Health and Science University - Portland
Current Performing Organization:	Oregon Health and Science University - Portland
Web Approval Date:	08-17-2023

My career plans are, and always have been, focused on the connections between things. I am most interested in studying the interaction between melanoma and the immune system. Because connecting basic science to clinical medicine is the central goal of my career, I pursue the study of that interaction through working, both as a dermatologist and as a scientist. Developing myself as a physician both gives me insight into what is actually needed for patient care and keeps me connected to the patients that inspire my research. That research, in return, provides me with the potential to push forward patient care both for my own patients and for all people with melanoma. My career development and sustainment plan are centered around developing the interaction between these two aspects of my professional life, and it will be guided by a like-minded and highly accomplished researcher in my Career Guide, Dr. Sancy Leachman. Through the support and collaboration that the Melanoma Academy can provide, I would be able to rapidly accelerate my research, it would also provide me with scientific connections and perspectives that will shape and direct my contributions to where they will be of most use to the melanoma research field, and thereby to melanoma patients.

The work outlined in this proposal is directed at the Focus Area challenge to develop prediction and surveillance tools for distinguishing patient populations at risk for second primary/recurrence and/or metastasis. I propose to meet this challenge by making use of immune cells that respond to melanoma, but are not able to eliminate it, in order to detect the presence of recurring or metastasizing melanoma before it would otherwise be recognized. This work will most directly benefit the many patients who are diagnosed with an early melanoma and need regular monitoring thereafter to detect possible recurrence or progression of their disease. The tools I plan to develop would provide additional means of monitoring and would enable melanomas that come back to be caught and treated earlier than is currently possible.

The human immune system has been refined continually throughout our evolutionary history and, as we now appreciate, plays an important role in protecting us from cancers as they begin to develop. It is only those cancers that are able to elude or escape from the immune system that eventually cause disease. Recent work, driven by the success of immune-stimulating medications in treating melanoma, has identified that, in the majority of disease-causing cancers, the immune system recognizes the malignant tissue but is unable to mount a successful response. Because the immune cells that respond to a tumor are defined by their ability to not only recognize a problem, but copy themselves so that the problem can be addressed again in the future, detecting the presence of cells that are copies of cells recognizing a melanoma is a way to assess whether there is additional melanoma present in a person. Because these cells circulate throughout the body to monitor for disease, they are detectable in blood, which presents an opportunity to monitor patients for progressive or recurrent melanoma after an initial melanoma diagnosis and surgery.

The difficulties in using these melanoma-targeted cells to monitor patients after a melanoma is found have, in large part, been due to the difficulty in identifying which cells are responding to melanoma. New technological advances have provided a set of tools that can be used to distinguish with more precision which cells in a person's original melanoma biopsy are the tumor-responsive cells that may be useful for monitoring. The work described in this proposal will leverage those tools to look for tumor-responsive immune cells in early melanoma biopsies, and then monitor the blood of patients over time. If our hypothesis is correct, levels of tumor-responsive cells will increase over time in the context of recurring or progressing melanoma, and will be difficult to find or entirely absent in people who have been cured of their disease.

These experiments will develop tools that can be quickly translated to the clinic, as they will be done directly on samples from patients with melanoma, while not requiring anything from those patients beyond a few additional blood draws. If our results show that this is a useful means of predicting the development of additional or worsening melanoma, it would mean the availability of a new tool with which we could assess the risk for a large number of people who have a relatively good prognosis after their melanomas are removed, but still require lifelong monitoring, potentially enabling earlier detection and earlier treatment for those patients whose disease is not cured after its initial recognition.

Earlier detection and earlier treatment of melanoma will mean better results for patients – fewer surgeries, fewer side effects, longer lives – and the ability to determine with better precision which patients will benefit from therapy will result in both fewer failed treatments and a better allocation of healthcare resources.

These results are what I hope to achieve with my future career in melanoma research, and the support of the Melanoma Academy would greatly accelerate my ability to do so.

Proposal Title:	Molecular Mechanisms and Therapeutic Potential of Heparan Sulfate Proteoglycans in Remyelination
Log Number:	MS220005
Current PI Name:	Fraser Sim
Award Number:	HT9425-23-1-0682
Current Contracting Organization:	New York, State University of, Buffalo
Current Performing Organization:	New York, State University of, Buffalo
Web Approval Date:	09-15-2023

The Focus Area of this application is "Central Nervous System Regenerative Potential in Demyelinating Conditions." The application utilizes a series of mechanistic studies to investigate the mechanisms by which the hostile inhibitory signaling environment in MS is generated and presents obstacles to repair in Multiple Sclerosis (MS). The hypothesis that heparan sulfate proteoglycans (HSPGs) are involved in these processes is novel and our approach is highly innovative as it utilizes a comprehensive set of new transgenic mice in carefully selected animal models.

The brain contains a population of progenitor cells, commonly referred to as oligodendrocyte progenitor cells (OPCs), that can give rise to specialized cells known as oligodendrocytes. Oligodendrocytes and the myelin that they produce are vital for normal neurological function. When oligodendrocytes are lost or damaged in demyelinating diseases such as MS this contributes to severe and progressive disability. Importantly, OPCs can generate new oligodendrocytes, restoring lost myelin and promoting functional regeneration known as remyelination. As such, OPCs represent a promising untapped source of stem /progenitors that when properly stimulated could lead to significant regeneration in MS and other diseases.

In MS, remyelination is thought to occur slowly over several months and is often insufficient. As a result, regions of permanent or chronic demyelination are commonly observed in patients with MS. As chronic demyelination is associated with neuronal death and the loss of neurological function, therapies aimed at enhancing remyelination are expected to restore lost function and prevent disease progression in MS.

We have begun deciphering a series of new mechanisms by which a class of proteins decorated with sulfated sugar sidechains known as heparan sulfate proteoglycans (HSPGs) can be modulated to improve remyelination. We recently identified sulfatase enzymes as part of a new mechanism that limits remyelination in animal models. Our initial studies found that genetic and pharmacological inhibition of sulfatases could accelerate remyelination in an animal model of toxin-induced demyelination. These enzymes play a relatively small part in a complex network of proteins along with enzymes that synthesize and modulate their heparan sulfate sugar sidechains. This network is collectively known as the heparanome and represents a new area for remyelination research. Unlike previous approaches that have typically focused on a single receptor-mediated pathway, the heparanome interacts with several pathways in a highly coordinated manner. As such, we anticipate that modulation of this novel component of the lesion environment will be fruitful in our search for effective strategies to improve remyelination. In the current proposal, we are working to define the precise make-up of inhibitory HSPGs, to define the potential contribution of HSPG inhibition in regions of grey matter injury, and ask whether small molecules can result in therapeutic improvement in the mouse EAE model of MS. The results from these studies will provide critical information on the HSPG-dependent cell-cell interactions that act during demyelination and further the identification of factors that promote myelin repair in MS.

Our goals are to (1) determine the specific contributions of synthetic enzymes known as 2-S and 6-S sulfotransferases on HSPG signaling following demyelination, (2) determine the role of neuron-expressed

sulfatase, an enzyme capable of editing HSPG sulfation, and ask if this mechanism contributes to the inhibitory environment present in cortical lesions in the MS brain, and (3) determine the therapeutic potential of heparan sulfate mimetics as agents to promote remyelination in EAE. The animal models we will employ provide insight into specific aspects of the regenerative process in MS and allow us to determine the potential for these strategies when translated into early clinical trial. All experiments will be performed using rigorous approaches with equal numbers of adult male and female mice, using appropriate statistical methods, and by blinding of the investigator to remove bias.

This project is significant as it will provide further insight into the potential for targeting HSPG and their sulfated sugar side chains in myelin repair and provide critical mechanistic information necessary for the development of strategies capable of clinical translation. The potential impact of a small molecule that alleviates the inhibitory microenvironment in chronic MS lesions is great. Not only would enhanced remyelination have the capacity to restore function but, importantly, myelin repair will act to protect these axons from further damage and degeneration. If our research is successful, we would anticipate clinical translation to occur within 10 years. We expect that a future therapeutic capable of mobilizing OPCs for repair may prevent or halt progression in MS and fill an urgent need for MS patients.

Proposal Title:	Investigating Lesion-Specific Mechanisms Driving Retrograde Transsynaptic Degeneration of the Visual Axis in Multiple Sclerosis
Log Number:	MS220041
Current PI Name:	Omar Al-Louzi
Award Number:	HT9425-23-1-0571
Current Contracting Organization:	Cedars-Sinai Medical Center
Current Performing Organization:	Cedars-Sinai Medical Center
Web Approval Date:	09-03-2023

Background: Multiple sclerosis (MS) results in the accelerated death of nerve cells in the brain, retina, and spinal cord. The exact mechanisms and types of lesions that promote this significant cell death are poorly understood. This accelerated nerve cell death in turn contributes to the long-term disability experienced by MS patients, even those who do not have evidence of active inflammation in the brain, spinal cord, or visual pathway. Most treatments available to treat MS target the inflammatory components of the disease, but there are no current interventions available to help slow down the nerve cell death that occurs with this condition.

Trans-synaptic degeneration refers to the spread of nerve cell death, from one nerve cell to adjacent cells, across the connections between these cells, which are called "synapses". In MS, trans-synaptic degeneration of the retina occurs in response to some but, importantly, not all lesions that affect the visual pathways in the brain. We know very little about which types of lesions promote this process and how someone's predisposition to develop trans-synaptic degeneration is associated with visual loss, clinical disability, or accelerated nerve cell death in the other areas of the brain or spinal cord. Recently, several types of lesions have been shown to be more specific for MS pathology, such as those that form around small veins in the brain (called central vein sign lesions), while other lesion types have been shown to reflect chronic active inflammation and higher risk of disability accumulation (called paramagnetic rim lesions). The relationship between different lesion types and the occurrence of trans-synaptic degeneration has not been investigated previously in MS and can help shed light on the specific lesion types that are related to higher cumulative nerve cell loss.

Objectives and rationale: This project will address the FY22 MSRP Focus Areas (1) Correlates of Disease Activity and Progression in MS, and (2) Biology and Measurement of MS Symptoms. We aim to use the visual pathway as a model to investigate innovative ways to quantify trans-synaptic degeneration in the retina, using a retinal imaging technique called optical coherence tomography. For our first aim, we will track the effect of two different MS lesion types (central vein sign positive and paramagnetic rim lesions) in the visual pathway in the brain and study how these lesions contribute to the spread of nerve cell degeneration to the retina. For our second aim, we will study how the process of trans-synaptic degeneration affects the visual symptoms experienced by MS patients, and how it relates to visual loss in specific parts of the visual field over time using a test called standard automated perimetry. For our third aim, we will use brain magnetic resonance imaging (MRI) scans and obtain regular disability assessments on study participants to examine whether the presence of trans-synaptic degeneration in the visual pathway can be used as a biomarker for widespread nerve cell loss in the brain and/or spinal cord, as well as the risk of patients experiencing progressively worsening disability over a 2-year follow-up period. These results will help validate the role of trans-synaptic degeneration in predicting long-term disability risks in MS.

What types of patients could it potentially help and how?

This project is expected to help all patients with MS, including both relapsing-remitting and progressive forms, by studying factors contributing to the chain death of nerve cells in the visual pathway. Additionally,

this work will also help MS patients who experience visual symptoms by measuring visual symptom severity and the visual field findings that are specifically caused by trans-synaptic degeneration in the visual pathway of MS patients.

What are the potential clinical applications, benefits, and risks?

We anticipate that in the short term (within approximately 5 years of completion of this study), the research findings from this project will introduce new ways to facilitate the identification of MS patients who are at risk of developing trans-synaptic degeneration in the visual pathway, the specific symptoms that herald this process, and the types of MS lesions that are more likely to cause it. These findings can also help identify MS patients at risk of widespread neurodegeneration and cumulative nerve cell loss from MS lesions and, therefore, who might be at higher risk for progressive clinical disability. In the long term, we think that the findings from this research will help validate accurate biomarkers to track trans-synaptic degeneration in the visual pathway, help detect and monitor it clinically, and potentially be used in proof-of-concept clinical trials of neuroprotection to prevent progressive nerve cell loss in MS.

Proposal Title:	Analyzing EBV Genomes and Epigenomes and EBV-Dependent B Cell Proteomes to Identify Fundamental Viral Triggers of MS
Log Number:	MS220049
Current PI Name:	Tobias Lanz
Award Number:	HT9425-23-1-0595
Current Contracting Organization:	Leland Stanford Junior University, The
Current Performing Organization:	Leland Stanford Junior University, The
Web Approval Date:	09-01-2023

Understanding how Epstein Barr Virus (EBV) Causes Multiple Sclerosis: The objective of this proposal is to learn about the genetics of EBV and how it contributes to the development of multiple sclerosis (MS). Approximately one million Americans suffer from MS. It is the most common cause of disability in young adults. MS is an autoimmune disease in which the patient's own immune cells attack components of the central nervous system (CNS, brain and spinal cord). Common symptoms include loss of vision, fatigue, numbness and tingling, muscle spasms and stiffness, bladder problems, and weakness. Prior research has shown that 100% of MS patients have been infected with EBV before their initial symptoms. EBV is therefore considered to be a pre-requisite to developing MS. However, the large majority of the general population is also infected with EBV, and it is unclear why some individuals develop MS and others do not. The overarching objective of this proposal is to identify fundamental molecular mechanisms by which EBV alters the patient's immune system to promote development of MS. Over the last decade our laboratories have developed several unique technologies to study the role of EBV in MS, which include methods to characterize the immune response to viral components and ways to sequence the viral DNA itself. This award will enable us to use our molecular technologies to investigate how EBV contributes to the development of MS. In particular, we will answer two important questions: Do cells from MS patients that become infected with EBV react differently to the virus than the same cells in healthy individuals? And are there distinct sub-strains of EBV that cause MS?

This proposal will address the Focus Area "Factors Contributing to or Associated with MS Etiology, Prodrome, Onset, and Disease Course." By addressing the etiology of MS, our research will address fundamental questions in MS research: What is the cause of the disease? What are the main molecular mechanisms that explain the association between the major risk factor EBV and the development of MS?

MS is a complex and very heterogeneous disease that requires individualized therapeutic strategies. As our research tackles a fundamental question of MS, we anticipate that our results will be relevant to all MS patients. In addition, it could be possible that we identify several distinct kinds of immune cell responses and certain EBV sub-strains, which distinguish some groups of patients from others, and which could be further developed for use as biomarkers to guide therapeutic decisions. Understanding the molecular mechanisms that cause MS will also enable us to design novel fundamental therapies for the treatment of MS. These include antiviral therapies, therapies that influence the immune response to EBV, or vaccines against EBV.

While currently antiviral therapeutics against EBV show limited efficacy, there are several antiviral treatments for other related herpesviruses. Their development have equipped the scientific community with an extensive amount of experience in this field that can be utilized to develop improved therapies against EBV. Several development campaigns are ongoing at the moment and their timelines will be relatively short. Further, multiple vaccine trials against EBV are currently ongoing, including two mRNA vaccine trials by Moderna.

In addition to antiviral therapeutics, there are multiple therapeutic compounds that interfere with activating pathways in immune cells. Upon discovery of the relevant pathways, we can "repurpose" these existing therapeutic compounds to treat EBV and its effect on immune cells in MS patients. Thus, success of the proposed studies in demonstrating how EBV promotes MS could be rapidly translated into novel therapies for MS patients. These therapies would likely be effective and more specific than current therapies, which for the most part have promiscuously large effects on the immune response.

Our proposed study is not a treatment study but an observational experimental study, carried out on patients' blood and autopsy tissue. The only potential risk to the participating subjects is loss of confidentiality. No identifying information will be associated with the sample during analysis and clinical data utilized for research data analyses will be anonymized. Therefore, the risk to release confidential health information to the public is minimized. Risks to participating subjects who will donate blood are limited to transient hematoma, a very low risk of infection, and minor pain, which is self-limiting.

Proposal Title:	Probing Oligodendrocyte Lineage Cell Autocrine Signals to Treat Multiple Sclerosis
Log Number:	MS220064
Current PI Name:	Ye Zhang
Award Number:	HT9425-23-1-0387
Current Contracting Organization:	California, University of, Los Angeles
Current Performing Organization:	California, University of, Los Angeles
Web Approval Date:	09-29-2023

Objectives and Rationale: Neurons in the brain form neural circuits by connecting with one another with long and thin axons similar to electric wires. Oligodendrocyte-lineage cells (OLCs) form myelin that wraps around axons similar to insulating plastic covers of electric wires. Myelin is essential for the function of axons and the electrical communications within neural circuits.

In multiple sclerosis (MS), the immune system attacks myelin, leading to myelin and axon damage, and loss of the abilities to walk, talk, see, and remember. Currently available MS treatments can reduce immune attacks of myelin, thus slowing down the deterioration of symptoms. However, these treatments cannot repair damaged myelin or axons and most patients on these treatments eventually deteriorate as the disease progresses. To develop new treatments aimed at repairing damaged myelin and curing MS, scientists need to know what signals stimulate OLCs to form new myelin. In the past several decades, scientists have made great progress in understanding signals that work inside individual OLCs, However, whether OLCs communicate with one another and whether their communication stimulates the formation of new myelin are unclear.

Progress in studying OLC communication has been slow in the past several decades because signal molecules from OLCs and other types of cells in the brain are mixed and thus hard to decipher. In pilot studies, we developed a new technology to block communication only between OLCs. This technology is similar to creating the "mute" button in a Zoom meeting, allowing us to "mute" one participant in a Zoom meeting to observe what happens when this participant is not allowed to communicate. This selective "mute" technology thus allows us to understand the contribution of this participant to the accomplishment of the group. When we applied our new method to "mute" OLC communications in mice, we found that OLCs form less myelin and the mice suffer from movement problems. This interesting discovery suggests that OLCs produce signals that encourage themselves and other OLCs to make new myelin, similar to people in a community that encourage one another to work for a common cause. We reasoned that we could use this encouragement signal to stimulate new myelin formation in MS patients.

In this proposed study, we will exploit the OLC-to-OLC encouragement signal to promote brain repair. In Specific Aim 1, we will apply our new method to "mute" OLC communication in a mouse model of MS and identify the involvement of OLC communication in the formation of new myelin after myelin loss and the recovery of motor function. In Specific Aim 2, we will generate a catalog of the communication signals used by OLCs. We will next identify which communication signal molecule(s) can promote OLC growth and myelin repair.

Focus Area: "Central nervous system regenerative potential in demyelinating conditions." We will identify cell-cell interactions and factors that promote remyelination and functional recovery.

The Applicability of the Research to Advance MS Patient Care: Recent data from clinical trials suggest that it is possible to improve the neurological function and the quality of life of MS patients by stimulating new

myelin formation even after myelin has been lost for a long time. However, no large-scale clinical trial that stimulates new myelin formation has been successful so far, highlighting the need to identify novel ways to stimulate new myelin formation. In contrast to previous studies that target myelin-promoting signals working inside individual OLCs, our study will target OLC-to-OLC communication and discover myelin-promoting signals that work outside of OLCs. Our approach holds promise for discovering a novel group of myelin-promoting molecules that have not been previously tested and may lead to a new category of MS therapies. Results from this study can be immediately translated into clinical trials. If successful, this study may lead to new treatments that ameliorate disabilities and disease progression in all types of MS patients in 7 to 10 years.

Proposal Title:	Early, Intermediate, and Late Retinal Biomarkers for Assessing Neuroprotection Following Acute Optic Neuritis: Insight and Exploration of RENEW, Opicinumab in AON Phase 2 Clinical Trial
Log Number:	MS220071
Current PI Name:	Shiv Saidha
Award Number:	HT9425-23-1-0551
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	09-03-2023

Acute optic neuritis (AON, or inflammation of the optic nerve, the nerve that transmits visual signals from the retina of the eye to the brain), causes impairment of vision and typically results in injury to the insulation sheaths (myelin) surrounding the fibers or cables comprising the nerves. The fibers or cables of the optic nerves originate from the top layer of the retina (retinal nerve fiber layer; RNFL), and the cell bodies or nerve cells (ganglion cells) from which these fibers are derived are located in a layer under the RNFL called the ganglion cell layer (GCL). AON is a frequent manifestation of multiple sclerosis (MS), that also commonly occurs during the course of MS. Because the eye is the most external part of the central nervous system, it is amenable to structural and functional interrogation in ways that other parts of the brain cannot be investigated. For example, we now have highly sophisticated retinal imaging techniques (like optical coherence tomography; OCT – explained below) that allow us to image the retina with up to 20 times the resolution of standard MRI, and accurately quantify layers like the RNFL and GCL. Similarly, using physiology-based techniques like visual evoked potentials, the speed at which signals move from the eyes to the back of the brain can be measured. This provides information regarding the integrity of myelin, as a function of fiber insulation. In addition, we can clinically test the visual system by assessing ability to read letters at different levels of illumination/contrast. Because of the ability to test structure and function of the retina so easily and accurately, AON is now frequently used as an event of interest in the design of clinical trials of new medications that may act to protect nerves from injury (neuroprotection), or even promote new myelin to be put down (remyelination). The identification and development of therapies for neuroprotection and remyelination represent a key focus in MS research. However, clinical trials of such agents have been hampered because of a lack of validated techniques for testing their effectiveness, highlighting again the role of the visual system, and interest in studying AON for the testing of such drugs. The RENEW trial was a 32-week placebo-controlled study of opicinumab in AON. This drug to thought to turn on myelin producing cells and has been shown to have remyelinating and neuroprotective effects in animal models of MS, and has the potential to encourage remyelination and/or neuroprotection after attacks or relapses in MS (such as AON).

In the current proposal we will re-analyze the entirety of the retinal OCT scans acquired in the RENEW trial. OCT, the optical analogue of ultrasound, allows extremely high-resolution retinal imaging and quantification of the integrity of the discrete retinal layers affected by AON. We will apply state-of-the-art validated techniques, including the highest level of quality control of the OCT images, and what is widely regarded as the best OCT image processing procedures to ensure the scans provide the most accurate quantification of the retinal layers of interest. In doing so, we will address the FY22 MSRP Investigator-Initiated Research Award Focus Area on Correlates of Disease Activity and Progression in MS, by validating the role of OCT

in trials of potentially neuroprotective and/or remyelinating therapies, as well as providing novel insights regarding mechanisms of tissue injury following inflammatory attacks in AON and MS in general. Although the RENEW trial was deemed "negative" on account of not meeting the pre-specified primary outcome at a particular timepoint, based on our assessment, numerous relevant and important signals in the trial are actually highly suggestive of a treatment effect of opicinumab. We hypothesize that opicinumab treatment in AON was associated with significantly reduced amounts of GCL thinning (an early marker of nerve injury in the retina after AON). Furthermore, analysis of the deeper retinal layers (the inner layer [INL] and outer nuclear [ONL] layers), was not included in the RENEW trial, although based on our extensive work, changes in these layers are known to occur during and after AON, and may reveal different important biologic processes to GCL thinning. We hypothesize that in our analyses of the RENEW dataset we will observe significantly lower increases in INL and ONL thicknesses during the initial months of AON, and significantly less residual INL thinning following AON in the opicinumab group.

Our proposal will validate that specific and distinct retinal layer changes occur at different time points and identify novel chronologic (early, intermediate, and late) retinal biomarkers of AON-induced injury in MS, that can be employed as outcome measures in clinical trials of remyelination and/or neuroprotection. Additionally, the completion of the proposed study will provide immediate data to help determine whether opicinumab therapy may have neuroprotective benefits following AON, and might therefore warrant further investigation in AON, and MS in general. If our hypotheses are correct, but this study is not performed and the findings are not confirmed, it could represent a missed opportunity to propel further study of what has been undoubtedly the most promising remyelinating therapy (opicinumab) to reach human testing to date.

Proposal Title:	Boosting Myelination Using Visual Flicker in the Acute Cuprizone Intoxication Model
Log Number:	MS220087
Current PI Name:	Bart Krekelberg
Award Number:	HT9425-23-1-0568
Current Contracting Organization:	Rutgers, New Jersey, State University of
Current Performing Organization:	Rutgers, New Jersey, State University of
Web Approval Date:	09-03-2023

Myelin is an insulating substance that wraps around healthy axons -- the wires that connect neurons -- and allows them to communicate rapidly, reliably, and over long distances.

Demyelinating diseases such as multiple sclerosis (MS) damage myelin and result in visual, motor, and cognitive deficits. Because the search for drugs that restore myelin has been unsuccessful, more than one million people in the United States alone suffer from the progressively worsening symptoms of MS.

About 8 years ago, researchers discovered that increasing neural activity (in mice) boosts myelin growth. So far, no one has tested whether this also works in humans, presumably because the tools used to increase neuronal activity in mice are not suitable for use in humans. Recently, however, we found that a particular kind of flickering light ("Flicker" for short) results in a widespread increase in neuronal activity throughout the brain. This finding led us to the new idea underlying this proposal: could increasing neural activity using Flicker stimulate the regrowth of myelin?

This project takes the first step from this idea toward a novel, non-pharmaceutical treatment for MS. Specifically, we want to show that Flicker boosts myelination in mice. Following a common approach in the field, we will feed mice a diet with the toxin cuprizone mixed in; over a few weeks, this results in almost complete demyelination and strong impairments in visual and motor behavior. After returning the animals to a regular diet, they recover, at least partially. We will measure their impairments using sensitive, noninvasive measures of myelination (visual evoked potentials, optomotor responses, and wheel running). Animals will receive either a daily dose of Flicker or no treatment. We predict that mice exposed to Flicker will show fewer impairments and a faster or more complete recovery.

We aim to use the brain's innate potential for (re)-myelination. Therefore, this project falls within the MSRP Focus Area that seeks to understand and use the Central Nervous System's Regenerative Potential in Demyelinating Conditions. Our immediate goal (less than 2 years) is to provide evidence that Flicker boosts myelination in mice, but our long-term goal (2-5 years) is to develop this into a novel treatment approach in humans. We designed the animal experiments with this in mind; the Flicker treatment and our experimental measures of impairments all have analogous methods appropriate for use in humans. This project's outcomes will therefore help design a randomized clinical trial to test our idea in MS. If we are successful, this could lead to a novel, non-pharmaceutical approach to remyelination that may stop or even revert the progression of MS.

Proposal Title:	Angiogenesis-Hypoxia Biomarkers and Disease Improvement in Progressive MS
Log Number:	MS220093
Current PI Name:	Carlos Camara-Lemarroy
Award Number:	HT9425-23-1-0878
Current Contracting Organization:	Calgary, University of
Current Performing Organization:	Calgary, University of
Web Approval Date:	09-29-2023

We don't know why people with multiple sclerosis (MS) experience progression (worsening of function). We also don't have accurate means of predicting who will progress. Currently, we use neurologic exams and magnetic resonance imaging to try and estimate progression. We also don't have good medications to stop progression; medications are good at stopping inflammation (like in relapses) but do not slow degeneration as seen in progressive MS. Although progressive MS tends to worsen over time, there is a small group of people who actually improve spontaneously. We want to study why this is, so that we can identify new ways of predicting how people will do over time but also to develop new treatments.

Researchers have found that there is hypoxia (lack of oxygen) in the brain of people with MS. Hypoxia could be one way the brain suffers degeneration in MS. One of the responses to hypoxia is trying to get more blood flow into the brain. Creating new blood vessels, or angiogenesis, is one of these responses. There are natural molecules that make blood vessel cells grow, produced by our own bodies. However, these molecules also make other cells grow, including brain cells, and help myelin develop too. We believe that a normal response to hypoxia in MS is the release of these "angioneurin" molecules, that they may also help stop neurodegeneration and increase repair in the brain. Some of the molecules we have explored include hepatocyte growth factor and angiopoietin-2.

An abnormal response (not enough of these molecules) could be a reason why some people show worsening, and an adequate response (enough of these molecules) a reason why some people improve. We have measured some of these molecules and found that high levels of them in blood actually can predict neurologic improvement in people with progressive MS. However, we need to study more samples to confirm these findings. We also want to test if more of these molecules are found in the brain in places where there has been recovery of lost myelin. Finally, we want to test if cells from people with progressive MS are unable to produce these molecules in adequate amounts after they experience hypoxia. We believe that an adequate hypoxia-angioneurin connection explains improvement in MS, and that we can evaluate if this happens analyzing blood samples of people with MS. We have access to existing blood samples to test our idea. This would be the first time these molecules are tested with this purpose, and we have experience in investigating novel biomarkers in MS.

This project could also tell us if the molecules we are investigating could be used to develop new treatments to promote improvement. For example, one of these molecules (hepatocyte growth factor) is being tested as a therapy for Alzheimer's disease, another neurodegenerative disease. If hypoxia and angiogenesis are indeed important for improvement, we could explore treatments that modify them, or that target these molecules. Our project therefore could have both short- and long-term benefits. We could find easily obtained (in blood), inexpensive and simple-to-analyze biomarkers, but also develop future treatments.

Proposal Title:	Harnessing Endogenous Repair in Multiple Sclerosis
Log Number:	MS220094
Current PI Name:	Carlos Camara-Lemarroy
Award Number:	HT9425-23-1-0812
Current Contracting Organization:	Calgary, University of
Current Performing Organization:	Calgary, University of
Web Approval Date:	09-26-2023

In Multiple Sclerosis (MS), new brain lesions appear because of inflammation and loss of myelin (the structure covering nerves, allowing them to work properly). Treatments used for MS stop inflammation, but cannot repair the damage already done. Repairing myelin might be an effective treatment in progressive MS, but there are no medications available that do this. Our own bodies have ways of repairing myelin. However, these are not working properly in MS. We want to study a new way to improve myelin repair (remyelination).

Remote ischemic preconditioning (or REIP) is not a medication. It consists of blocking the blood flow of a limb (an arm or leg) for short periods of time (using a blood pressure cuff). These short periods of time without blood flow are not harmful, but they do trick the body into thinking that harm may be coming. The body then produces substances that could be helpful in the case of real harm. These substances can travel all the way to the brain and could help with remyelination by changing how brain cells work.

REIP can be applied in mice by inflating blood pressure cuffs in the hind limbs. We have performed REIP in mice and have results that are promising, but we need additional information. We want to treat mice with REIP after we cause demyelination in their central nervous system by injecting a chemical. This is done under anesthesia. The area of demyelination caused by this injection goes on to remyelinate in predictable ways. We will test if REIP can accelerate remyelination and change how nerve cells work. We will also test if REIP can improve remyelination when there is inflammation, using another mouse model of MS. We will study in detail the changes associated with REIP in brain cells responsible for remyelination and repair.

REIP can protect the central nervous system of mice against stroke and trauma. Although REIP has been tested in people with stroke before, to our knowledge no one has looked at people with MS or in models of remyelination. This is a novel and exciting way to study remyelination that does not involve drugs or medications. There is an unmet need for strategies that can improve remyelination. This study could provide the foundation for a new way to do this, using REIP. Our results could pave the way for more studies aimed at better understanding REIPs effects in the brain. Since REIP is safe and easy to do, even in humans, this study could encourage the development of studies to determine if REIP could be helpful for patients with all types of MS in the short term. Emphasis would be placed on progressive forms of the disease, where there are few treatment options focused on repair/recovery of function.

Proposal Title:	Examining the Link Between Hippocampal Pathology and Meningeal Inflammation in Progressive MS
Log Number:	MS220097
Current PI Name:	Jennifer Gommerman
Award Number:	HT9425-23-1-0933
Current Contracting Organization:	Toronto, University of
Current Performing Organization:	Toronto, University of
Web Approval Date:	10-01-2023

Focus Area: This fiscal year 2022 Multiple Sclerosis Research Program Investigator-Initiated Research Award project addresses the Focus Area "Correlates of Disease Activity and Progression in Multiple Sclerosis." In this project we will use brain specimens acquired from the Netherlands Brain Bank to look for molecules and cells that are specifically associated with progressive MS (PMS) compared to relapsing-remitting MS (RRMS) and other non-MS conditions. We will focus our efforts on the compartment that ensheaths the brain -- the leptomeninges -- and assess how molecules and clusters of cells in the leptomeninges impacts the hippocampus. These tissues, along with a unique animal model that recapitulates aspects of PMS disease, will support our research activities.

Proposal Rationale: Patients living with MS experience major cognitive disabilities including memory impairment. There is substantial evidence that the hippocampus, a specialized area of the temporal cortex, is critical for the consolidation and recollection of episodic memories, social memory, and the temporal organization of events. Studies on MS brain tissue collected at autopsy have shown that the PMS hippocampus can be profoundly damaged. Thus, hippocampal injury may explain some of the clinical deficits experienced by PMS patients including impaired memory and learning as well as depressive symptoms. The hippocampus is lined with meninges -- the envelope that ensheaths the brain.

Hypothesis: The leptomeninges of progressive MS patients harbours an assortment of immune cells that contribute to hippocampal pathology in the progressive stage of MS.

Proposal Objectives: To test this hypothesis, we will (1) provide a complete inventory of the amount and types of immune cells as well as their by-products in the area of the leptomeninges that is adjacent to the hippocampus; (2) use all of the pathology and clinical metadata that we have for each subject to determine if what we detect in the hippocampus is relevant to the overall disease state of the PMS patient; (3) use a new animal model that mimics the same kind of hippocampus pathology and shows evidence of a PMS disease course (See Zuo et al, JCI Insight 2022) to test the relevance of what we find in human patients to the overall disease process, i.e., ascertain causality. With these tissues (both human and mouse) we will apply an innovative technique that we have already validated in the MS brain (see Ramaglia et al, ELife, 2019) and has been recently used in the COVID-19 brain (see Schwabenland et al, Immunity 2021). This technique allows us to measure ~50 separate markers (proteins) on a single slice of brain tissue. Combined with other molecular approaches, our proposed project represents an unprecedented opportunity to gain a deeper understanding of how the hippocampus is injured in PMS and to use our animal model to test these findings and develop therapies.

Applicability to MS Patient Care: The mechanisms that explain how the MS hippocampus is damaged are not fully understood. Because the hippocampus is involved in memory, understanding what goes wrong in this tissue could improve the lives of people living with MS.

Risk versus Benefits: The drugs we have available to treat MS have largely failed to impact cognitive decline in PMS. Thus, a better understanding of PMS is urgently needed, and identifying drug targets that reduce the burden of PMS disease is an obvious benefit. The risk in this project is that we may fail to identify druggable targets that are specific to MS hippocampal injury in Aim 1. However, we have already identified some leading candidates based on both our recently published data (See Zuo et al, JCI Insight 2022) and additional preliminary data, that will be prioritized for further inquiry in the proposed project. Moreover, our animal model has the advantage of allowing us to monitor and measure the signs of disease progression over time, and also provides the ability to harvest tissues and subject them to powerful techniques such as RNA sequencing for a global assessment of the gene changes associated with hippocampal pathology. This animal model serves as an additional means for identifying druggable targets that can be verified in patient samples (Aim 1).

Timing to Achieve Patient Outcomes: In the short term (within the timeframe of this project, i.e., 3 years), the work in this proposal has the potential to link candidate (druggable) molecules of the immune system to hippocampal pathology and disease severity in PMS, validating these drug targets in a relevant animal model. In the medium term, (immediately following the project) we will confirm the relevance of these candidate molecules in a large group of PMS patients to ascertain if what we observe holds up upon secondary testing (see letter of support from UCSF). In the long term, appropriate drugs will be developed and tested in carefully selected groups of patients that are experiencing PMS-associated deficits in cognition.

Proposal Title:	Investigation and Therapeutic Targeting of Metabolic Pathways in Microglia for Efficient Remyelination in MS
Log Number:	MS220110
Current PI Name:	Deepak Kaushik
Award Number:	HT9425-23-1-0691
Current Contracting Organization:	Memorial University of Newfoundland
Current Performing Organization:	Memorial University of Newfoundland
Web Approval Date:	09-15-2023

My lab is interested in finding the cause of multiple sclerosis (MS) and is dedicated to finding a cure for its treatment. In MS, immune cells such as macrophages (immune cells that eat damaged cells and cause inflammation), T and B cells (other types of immune cells) reach the brain and damage the myelin. Myelin is a fatty material that wraps around the brain cells called neurons. The loss of myelin causes neurons to become less effective, causing MS symptoms such as difficulty in walking, blurred vision, etc. Microglia are the macrophages inside the brain that eat damaged myelin to remove them from the brain tissue. This is important because it allows the new myelin to be wrapped around neurons by oligodendrocytes, the myelin-making cells in the brain. Microglia use special energy (metabolism) that helps these cells to remove damaged myelin. But microglia become less effective in myelin removal during the long-term MS, and we believe it is due to the changes within microglia metabolism, details of which are not yet known. My lab is interested in finding this information for microglia so we can use drugs to help microglia recover their normal functions. In this grant, we propose to find and stop the disease-related metabolic changes in microglia. We believe that this strategy will help repair the lost myelin and reduce disease progression in MS brains.

Basis of our hypothesis: Our earlier studies found that macrophages that travel from blood to the brain during MS depend on glucose metabolism (glycolysis). We also found that if we blocked glycolysis using dietary factors such as cinnamic acids, it reduced the disease in the mouse models of MS. Because microglia are the brain macrophages and promote MS progression (steady decrease in brain health), drugs that directly target microglia metabolism can be effective in treating the progressive forms of MS.

Working on the ideas of "Central Nervous System Regenerative Potential in Demyelinating Condition" we have set the following short-term and long-term goals to achieve our objectives:

1. Short term goals (1-2 years):

a. Using different mouse models of MS and human MS brain samples, we will study and recognize the energy needs of microglia in MS.

b. We will test drugs that block disease-causing metabolic pathways within microglia to reduce inflammation and increase myelin removal by these cells.

2. Long-term goals (2-5 years):

a. We will study changes within microglia in old mice to understand age-related changes in microglia functions.

b. We will study how microglial metabolism directly affect oligodendrocytes for increasing their myelin-wrapping around neurons.

c. We will build collaborations with basic and clinical researchers (local and international) to promote novel treatments such as replenishing compromised energy in non-functional microglia for patient use. We will test if dietary factors such as cinnamic acid will help restore the lost energy in microglia

Patients benefitting from this approach: Because microglia are involved in early and progressive MS, we believe that our findings will benefit relapse-remitting MS patients as well as progressive MS patients.

Risks and benefits: Existing MS drugs are known to target metabolism in different kinds of immune cells and have shown no side effects, so we are certain that targeting metabolism of immune cells will pose no risks. Further, our approach of using dietary factors will be easily tolerated and safe for patient use. We believe that our methodology will increase myelin generation, heal chronic lesions, and treat MS progression.

Proposal Title:	Elucidating the Biological Mechanisms Underlying the Association Between Epstein-Barr Virus and Multiple Sclerosis
Log Number:	MS220122
Current PI Name:	Alberto Ascherio
Award Number:	HT9425-23-1-0871
Current Contracting Organization:	Harvard T. H. Chan School of Public Health President and Fellows of Harvard College
Current Performing Organization:	Harvard T. H. Chan School of Public Health President and Fellows of Harvard College
Web Approval Date:	10-01-2023

Infection with the Epstein-Barr virus (EBV) is likely the leading cause of multiple sclerosis (MS). In a recently published study, infection with EBV was associated with a 32-fold increased risk of MS, and among the 801 patients included in the study, only one was not infected with the virus before developing MS. While these findings are intriguing, it is not yet known how EBV is causing the disease. Some studies have found that EBV peptides, which are parts of proteins, resemble peptides in the brain and other parts of the central nervous system, which could cause the immune system to accidentally attack important structures in the brain, such as healthy nerve cells, when trying to eliminate the virus, a theory called molecular mimicry. Still, it remains unclear whether this can fully explain why EBV is causing MS. In addition to causing the disease, EBV could also affect disease activity and symptoms in MS patients. Recently, a study proposed that EBV may play a similar role in MS as gluten plays in celiac disease and that by targeting EBV, safer and more effective treatments than those currently available could be developed. Thus, more knowledge about the mechanisms by which EBV is causing MS could have major implications both for the prevention and treatment of MS. Therefore, we propose to conduct a comprehensive study that will characterize the immune response to EBV in detail and evaluate whether molecular mimicry plays an important role for the development of the disease. Further, we aim to determine whether we can identify individuals who are at high risk of developing MS based on their immune response to EBV shortly after being infected with the virus, which could have implications for early detection and diagnosis of MS.

The aims of this project are to (1) investigate whether an infection with EBV affects the immune response to other viruses and bacteria, (2) to evaluate whether human peptides resembling EBV peptides in previous studies increase the risk of MS, and (3) to conduct analyses of several thousand human peptides and examine whether there is an immune response to other, currently unknown, peptides that could help to explain how EBV is causing MS, and whether the immune response to these peptides can be used to identify those at high risk of later developing MS.

The first aim will evaluate whether EBV, a virus that co-evolved with humans for thousands of years, in addition to causing diseases such as MS and specific types of cancer, also could play an advantageous role for the immune system, for example by strengthening immunity against other viruses and bacteria. This would be important to determine for the implementation of an EBV vaccine in the general population. The second aim will validate whether serum levels of human peptides previously suggested to cross-react (by molecular mimicry) with EBV are associated with the risk of MS when measured years before the first symptoms of the disease. In the third aim, we will explore whether other, currently unknown, human peptides cross-react with EBV and are associated with MS risk. Results from the two last aims have implications for the development of more targeted MS treatment, identification of biomarkers for interventions targeting EBV, and for early detection and diagnosis of MS patients.

The proposed study will be conducted using clinical data and serum samples from active-duty military personnel. While being on active duty, serum specimens are collected from Service Members on average every 2 years, and these samples are stored in the Department of Defense Serum Repository. Currently, this

repository contains over 60 million archived serum specimens from over 11 million individuals. For the past 20 years, we have identified individuals who developed MS while being on active duty. The proposed study will include samples that have already been collected during our previous work on MS in this population. In these serum samples, we will use three assays: (1) VirScan, which measures the immune response to all viruses known to infect humans and selected bacteria, (2) Human peptidome (HP), which measures peptides covering all human proteins, and (3) Nucleic Acid Programmable Protein Array (NAPPA), which measures all known human full-length proteins, including those previously suggested to be targeted by the immune system because of molecular mimicry.

While new treatments have improved the quality of life and prognosis of MS patients, the disease continues to be the most common cause of disability in young adults and the incidence is increasing worldwide, mostly in women and, in the U.S., in Blacks. As EBV is likely the leading cause of MS, more research on the underlying mechanisms by which the virus is causing the disease is critically needed. The proposed project stands to produce important results that will help to elucidate these mechanisms.

Proposal Title:	Real-World Clinical Trial of an Online Cognitive Behavioral Therapy for Managing Fatigue in Multiple Sclerosis
Log Number:	MS220136
Current PI Name:	Robert McBurney
Award Number:	HT9425-23-1-0482
Current Contracting Organization:	Accelerated Cure Project, Inc.
Current Performing Organization:	Accelerated Cure Project, Inc.
Web Approval Date:	09-26-2023

Up to 70% of people with multiple sclerosis (MS) report fatigue as their worst and most troubling symptom. To date, studies that use drugs to treat MS fatigue have yielded inconclusive and/or negative results on their effectiveness. Real World Clinical Trial of an Online Cognitive Behavioral Therapy for Managing Fatigue in MS is a study designed to test an intervention that does not rely on drugs. The fatigue treatment being studied is Elevida, which has been rigorously studied and is approved and reimbursed for use in Germany.

Elevida is an MS-specific fatigue management program based on cognitive behavioral therapy (CBT) delivered via the internet. CBT is a psychology tool that helps a person connect their physical symptoms with their emotional reactions and cognitive thinking and has been proven effective for treating a wide range of conditions including depression, pain, and anxiety. Internet-based interventions such as Elevida could provide treatment for people with MS who might not have access to providers due to their location, transportation, or mobility problems or who lack the financial resources for personalized therapy sessions.

This is a real-world large-scale clinical trial of 2,000 people that will look at the results in key subgroups of women, men, racial, and ethnic subgroups. The participants will be randomly assigned to one of three groups. All study participants will continue with their treatment as usual, and no one will be asked to change their usual MS treatments. The first group will continue treatment with no additional intervention offered. The second group will continue their usual treatment with the addition of internet-based resources about MS and fatigue. The third group will continue with treatment as usual combined with the Elevida program. This study will be conducted over a period of 3 months with all participants being asked to complete online assessment tools at regular intervals to demonstrate the effectiveness of each treatment.

Positive results from this study would provide compelling evidence for support and reimbursement of Elevida in the U.S. as an effective MS fatigue treatment that can reach areas where therapists are unavailable or provide care in settings with limited resources. Study participants will come from the Veterans Administration MS Centers of Excellence and the iConquerMS network, a division of the Accelerated Cure Project for MS.

Proposal Title:	Mechanism Governing Association Between Viral Respiratory Infection Relapse Generation and Disease Progression in Persons with MS
Log Number:	MS220138
Current PI Name:	Andrew Steelman
Award Number:	HT9425-23-1-0692
Current Contracting Organization:	Illinois, University of, Champaign/Urbana
Current Performing Organization:	Illinois, University of, Champaign/Urbana
Web Approval Date:	09-01-2023

Multiple sclerosis (MS) is a disease of the brain and spinal cord in which the patient's own immune system damages tissue, causing disability. It has been estimated that 85% of persons with multiple sclerosis are initially diagnosed with a relapsing-remitting form of disease. This type of MS is characterized by periods of increased disease activity (relapses) followed by periods of partial recovery (remission). Since 1965, data have repeatedly shown that disease symptoms worsen in an estimated 27%-41% of relapsing-remitting MS patients within 5 weeks of contracting a respiratory viral infection. More recent research has indicated that many different types of viruses including those that cause the "common cold," influenza virus, and SARS-CoV-2 (the virus that causes COVID-19) are linked to this phenomenon. However, how these common viruses act to increase disease activity in the brains of persons with MS is not yet known. Since 85% of persons with MS are initially diagnosed with having the relapsing-remitting subtype of disease, a very large proportion of patients remain at risk for increased disease activity once they get a respiratory infection. Until we understand exactly how viral infections worsen disease, this patient population will continue to be at risk for disease exacerbation when exposed to prevalent viruses such as SARS-CoV-2.

We previously performed experiments in mice to address the role of influenza virus infection on the disease course of an animal model of MS. As occurs in the human disease, our data show that mice developed relapses shortly after exposure to virus and developed worse disease than control mice. Notably, a subset of immune cells, termed T cells, were found to be affected by infection in a manner that made them able to worsen disease. The ability for T cells to cause disease in the animal model is dependent on signals from a separate type of cell, termed dendritic cells, which also act to detect viral infections. Therefore, we developed the hypothesis that viral infections affect T cell function indirectly by targeting dendritic cells. To test this hypothesis we will determine if proteins from different types of viruses (i.e., influenza and SARS-CoV-2) are capable of affecting the interactions between dendritic cells and T cells in a manner that causes T cells to stimulate disease activity. We will also determine how T cells from virus-infected mice affect the cells that make up the brain (neurons, microglia, astrocytes, endothelial cells, and oligodendrocytes). By understanding these interactions we hope to determine targets for future drugs that can inhibit cell death that occurs within the brains of persons with MS. Finally, we have identified a specific pathway, activated by infection, that we hypothesize promotes the generation of disease-causing T cells. We will test whether inhibiting this pathway can decrease the likelihood of infection-induced relapse. Since the immune system is needed to protect against viral infections we will also test whether the drug is safe to use during an active viral infection.

While this research relies heavily on animal models of disease, we expect to identify a novel, but unifying, mechanism whereby viruses influence relapse generation and disease progression that is applicable to human MS. The results from our proposed experiments should reveal molecular targets that may prove to be clinically beneficial in the prevention of infection-induced disease exacerbations and may slow disease progression in general. Finally, identifying specific interactions between T cells and cells within the brain should shed light on mechanisms that control cell death. Our hope is that these data could be capitalized upon to reduce disease or promote repair.

Proposal Title:	Sex Differences in Gray Matter Atrophy and White Matter Disruption Progression in Early Multiple Sclerosis
Log Number:	MS220157
Current PI Name:	Allan MacKenzie-Graham
Award Number:	HT9425-23-1-0868
Current Contracting Organization:	California, University of, Los Angeles
Current Performing Organization:	California, University of, Los Angeles
Web Approval Date:	09-29-2023

Sex differences exist in multiple sclerosis (MS). Although women are more susceptible to MS than men by a ratio of approximately 3:1, studies have shown that men are at higher risk for worse disability, have worse cognitive decline, and demonstrated a shorter time to conversion from relapsing-remitting MS (RRMS) to secondary progressive MS (SPMS). Together, these studies suggest the possibility that men with MS may have a worse prognosis than women with MS. However, sex differences in whole brain size, structure volumes, and other measures have been shown during health and these differences in healthy brains are a confound in the study of sex differences in MS. For rigor in the study of sex as a biologic variable, this confound must be addressed. In a cross-sectional study, we compared female MS patients to female healthy controls and male MS patients to male healthy controls to remove the confound of known sex differences in healthy controls. We demonstrated more extensive gray matter (GM) loss in male MS patients than in female MS patients. In this proposal, we will determine sex differences over time in the progression of GM loss and white matter (WM) damage in early MS. We propose that male MS patients will exhibit more GM loss earlier than female MS patients, each compared to their respective healthy controls. We also expect that male MS patients will show WM damage earlier than female MS patients, again compared to their respective healthy controls.

Disability differences exist in MS. MS is multifocal, characterized by distinct disabilities affecting cognition, vision, and fine motor control, to name a few. Individual MS patients exhibit a variable set of disabilities, with different rates of change in each disability over time. We have previously demonstrated distinct regions of GM loss that corresponded with specific disabilities in a cross-sectional study of RRMS patients. Here, we will create maps of GM loss and WM damage for cognition, vision, and fine motor control disabilities, and evaluate their change over time in female and male MS patients. We propose that distinct patterns of GM loss will be associated with worsening in specific disabilities and that these patterns will differ between female and male MS patients. Further, we propose that distinct patterns of WM damage will also be associated with worsening in specific disabilities, and that these patterns will be different between female and male MS patients.

We will perform these experiments by leveraging data from an existing longitudinal dataset of early MS patients and healthy controls comprising 280 subjects (from our collaborators at Charité Universitätsmedizin Berlin) and using state-of-the-art image processing technologies.

We believe that sex differences in disease progression may alter future clinical trial design and may inform clinician's decisions on treatment for their patients. Further, disability-specific maps will permit a more accurate measure of disability progression and may be a more sensitive biomarker for use during evaluation of future treatments targeting remyelination and neural repair.

Proposal Title:	Cardiometabolic Comorbidity and Outcomes in Multiple Sclerosis: A Polygenic Risk Score Approach
Log Number:	MS220164
Current PI Name:	Kathryn Fitzgerald
Award Number:	HT9425-23-1-0680
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	09-15-2023

What is the problem and how are we trying to solve it? Cardiometabolic health is a term that refers to the health of a person's heart, blood, and blood vessels. It also is related to how a person's body can turn food components like sugar and fat into energy. Health conditions like heart disease, diabetes, obesity, or high blood pressure are called cardiometabolic diseases. People with multiple sclerosis (MS) are at a higher risk for developing these conditions compared to people without MS. A person with MS who has one of these conditions may be at higher risk of their MS worsening. For example, a person with MS and high blood pressure might have more MS disability than a person with MS who does not have high blood pressure. However, the results from studies so far have not been the same. Sometimes in studies where participants are not randomized (like the flip of a coin) to a study treatment, there can be bias. The bias could have played a role in the different conclusions. We want to reduce these biases by studying genetic factors that are related to a person's risk for diabetes or high blood pressure. Genetic factors do not change over a person's lifetime. There are many of these genetic factors that play a role in a person's risk for diabetes or high blood pressure. Together, they are called "diabetes genetic scores" or "high blood pressure genetic scores." The goal of this study is to see if these genetic scores can predict MS progression. There are also genetic scores that can affect how well diabetes medicines or high blood pressure medicines work. Sometimes the genetic factors can make the medicines work better. We also want to see if the genetic factors making the medicines work better can predict less MS disability. We will use genetic factors for many kinds of diabetes or high blood pressure medicines. We expect to find that there are genetic factors for one type of medicine that can predict less disability. If so, then future studies could test if taking the medicine itself can prevent disability progression in MS. Since all these medicines are already available and can be prescribed by doctors today, the results could open the doors for many new potential medicines to treat MS progression.

What types of patients could it potentially help and how? Diabetes and high blood pressure are very common conditions. They affect many people with MS. Our study focuses on the genetic scores and not the actual conditions themselves. So, the results of this study can be important for all types of people with MS. The results may suggest there are treatments for high blood pressure or diabetes that might also slow MS disability.

What are the potential clinical applications, benefits, and risks? Our study can help us understand how conditions like diabetes or high blood pressure are linked with changes in MS disease. It can also help us to know if medicines for these conditions can slow down MS disability. We do not think there will be any risks with this study. We have collected all the data for this study already. We removed any information that could identify an individual person. We will also take important steps to make sure it is analyzed in a safe way.

What is the projected time it may take to achieve a patient-related outcome? We will see how diabetes or high blood pressure can affect MS disability. Our primary goal focuses on identifying predictors of disability progression, which is related to a person's quality of life. As a result, a patient-related outcome might be achieved in the short-term or 1 to 2 years. In the long term (more than 2 years), the results of this study may suggest that medicines for diabetes or high blood pressure might help prevent MS disability.

How are we addressing fiscal year 2022 MSRP Focus Areas for (1) Factors Contributing to or Associated with MS Etiology, Prodrome, Onset, and Disease Course and (2) Correlates of Disease Activity and Progression in MS? We want to find out if having disorders like diabetes or high blood pressure are related to more MS disability. Our approach will use "diabetes genetic scores" or "high blood pressure genetic scores," which may help to reduce some common study biases. To meet our goals, we plan to use existing data and samples from four studies of people with MS who are from Canada and the U.S. Our study will include over 2,000 people with MS. All the research we want to perform will use samples and information that already exist.

Proposal Title:	Treatment of Cognitive Deficits in Multiple Sclerosis with High-Definition Transcranial Direct Current Stimulation
Log Number:	MS220175
Current PI Name:	John Hart
Award Number:	HT9425-23-1-0618
Current Contracting Organization:	Texas, University of, at Dallas
Current Performing Organization:	Texas, University of, at Dallas
Web Approval Date:	09-15-2023

A debilitating and common cognitive symptom in those with multiple sclerosis is the inability to retrieve words when needed. This inability to retrieve words affects one's conversations, ability to fluidly exchange information verbally, and retrieve information from memory. Our group has studied the ability to retrieve words using functional neuroimaging (e.g., fMRI) and electroencephalography (EEG), and we have discovered patterns of brain activity that are important for word retrieval. We have also found abnormal patterns of brain activity in patients who have problems retrieving words as a consequence of multiple conditions, including multiple sclerosis.

We have used our observations of patterns of brain activity to design a noninvasive, non-pharmacological treatment for those with word retrieval deficits that uses High Definition transcranial Direct Current Stimulation (HD tDCS). We use HD tDCS to deliver low levels of painless and safe electrical stimulation to specific regions of the head to stimulate the brain regions underlying that electrode location. This stimulation is delivered from electrodes in a cap that is placed on the subject's head, which safely and painlessly delivers the stimulation.

In this study, we plan to investigate the delivery of HD tDCS over specific brain regions of patients with multiple sclerosis and who have word retrieval deficits to determine if this will improve performance in verbal retrieval tasks. We will administer pre-treatment tests of verbal retrieval measures (assessing finding words, memory of the names of items in a list) as well as recording brain electrical activity from the scalp using EEG. This EEG will record both resting brain electrical activity and activity associated with performing tasks involving verbal retrieval.

We will then administer 10 sessions of HD tDCS in approximately 60 multiple sclerosis patients, randomly assigned to receive active or sham stimulation, and to evaluate placebo and longitudinal effects. As there is nothing a person feels when the electrical stimulation is on, these patients will not know that they are receiving the stimulation or not. We will then compare performance between the "active" group receiving the HD tDCS and the sham group by performing the same tests of verbal retrieval that we performed prior to the treatment, immediately after the last treatment and 8 weeks after the last treatment session. This will allow for assessing whether the HD tDCS treatment had a significant effect on performing verbal retrieval tasks and to determine if there are changes in patterns of brain electrical activity to account for how this treatment worked.

At the conclusion of this study, the effectiveness of HD tDCS for improving verbal retrieval deficits will be established, and we will determine if this is a useful treatment modality that significantly and functionally changes not only the performance on neuropsychological tests but also affects a person's day-to-day functions and quality of life. We predict that the treatment will improve a patient's ability to produce their words fluently in a conversation and remember the words that they want to say, leading to improved skills in both social and work situations. This treatment has the added advantage of having few, if any, known side effects, such as an individual might experience when taking medicine. The system used to deliver these

electrical pulses is relatively inexpensive and easy to use. This treatment can be made widely available for Warrior Transition Units, military clinics, and Department of Veterans Affairs/Department of Defense medical centers, and even in civilian medical centers.

Proposal Title:	Isolation and Engineering of Potent Anti-EBV Neutralizing Antibodies for the Treatment of MS
Log Number:	MS220176
Current PI Name:	Theodore Jardetzky
Award Number:	HT9425-23-1-0565
Current Contracting Organization:	Leland Stanford Junior University, The
Current Performing Organization:	Leland Stanford Junior University, The
Web Approval Date:	09-01-2023

Objectives and rationale for the proposed research project: Recent studies have provided compelling evidence that Epstein-Barr virus (EBV) infection predisposes people to developing MS, with EBV infection increasing the likelihood of developing MS by 32-fold. Although EBV infection is not the sole cause of MS, preventing EBV infection could substantially reduce the emergence of new MS cases. In addition, MS patients who have received anti-EBV T cell therapy have shown clinical improvement in clinical trials, indicating that anti-EBV therapeutic approaches may improve outcomes for current MS patients. In this proposal, we will develop a multi-pronged approach to targeting EBV for the treatment and prevention of MS. Our overall goal in this proposal is to develop novel neutralizing antibodies to two key viral protein targets, gHgL and gB, to block EBV infections and to suppress EBV reactivation in infected individuals. Both of these proteins are critically important for EBV infections and primary targets of the immune response, but highly potent antibodies are likely necessary to fully suppress EBV infection. The gB protein exists in an unstable form on the surface of the virus, making it challenging to develop as a vaccine or to use for the identification of neutralizing antibodies. However, we have engineered a stabilized version of the EBV gB protein, which is a key innovation that enables the proposed studies. The isolation of new anti-EBV antibodies and further stabilization of the EBV gB protein will also inform ongoing EBV vaccine design efforts.

FY22 MSRP Focus Area(s): This research project will address the FY22 MSRP Focus Area "Factors Contributing to or Associated with MS Etiology, Prodrome, Onset, and Disease Course" by developing novel approaches to blocking primary and re-activated EBV infections.

Applicability of the research to advance MS patient care: What types of patients could it potentially help and how? The promising results that have been observed with anti-EBV T cell therapy strongly indicate that targeting EBV in existing MS patients could have therapeutic benefit and potentially slow the course of the disease. Based on these and other preclinical studies, we believe that the anti-EBV neutralizing antibodies that we will develop in this proposal could similarly provide an important approach to the treatment of MS. Furthermore, our studies will provide an important foundation for the development of EBV vaccines. An EBV vaccine has the potential to prevent new MS cases by blocking primary EBV infections, although it remains to be established what percentage of MS cases would be prevented by this approach.

What are the potential clinical applications, benefits, and risks? As indicated above, we believe that anti-EBV neutralizing antibodies could provide clinical benefit to existing MS patients. EBV establishes life-long latency in infected individuals and undergoes periodic reactivation and replication, which may lead to cross-reactive immune responses to normal tissues and the associated progression of MS disease. By suppressing EBV replication in infected MS patients, it may be possible to reduce the severity of disease. The benefits to this approach, if effective, would be to mitigate the role of EBV in triggering MS and the associated risks are likely to be low and similar to other antiviral antibody therapies used clinically.

What is the projected time it may take to achieve a patient-related outcome? Anti-EBV antibody therapeutics, once validated in preclinical studies, would have to go through a development process prior to entering clinical trials. Once candidate antibodies have been identified and validated in the laboratory setting, these could potentially be moved into clinical trials within 2 to 3 years given sufficient financial resources. Although vaccines for SARS-CoV-2 were developed and approved within a year, the time frame for development of an EBV vaccine is likely to take longer, on the order of 5 to 10 years prior to reaching U. S. Food and Drug Administration approval. EBV vaccines are currently in clinical trials, although these do not incorporate stabilized forms of the gB protein that is being developed in this proposal. The efficacy of current EBV vaccine candidates remains to be determined.

Proposal Title:	Identification of Protein Pathways and Novel Biomarkers in Pre- and Early Clinical MS Individuals with Distinct Patterns of Autoimmunity
Log Number:	MS220185
Current PI Name:	Ahmed Abdelhak
Award Number:	HT9425-23-1-0499
Current Contracting Organization:	California, University of, San Francisco
Current Performing Organization:	California, University of, San Francisco
Web Approval Date:	09-03-2023

Multiple sclerosis (MS) is the most common cause of disability in young adults, following traumatic causes. Despite intensive research over the last decades, the events preceding the onset of brain and spinal cord injury remain largely unknown. This damage is caused by different mechanisms, including the activation of different arms of the immune system and aberrant activity of the supporting (i.e., glial) cells in the brain, like the so-called astrocytes and microglial cells. The combined effect of those mechanisms will ultimately lead to the loss of the insulating myelin sheath covering nerve fibers (i.e., axons) in the central nervous system (CNS). Early and precise detection of the different pathways leading to neuronal death provides a unique window of opportunity to understand the MS etiology, initiate the appropriate preventive mechanisms, and protect the nerve cells from irreversible damage. Unfortunately, however, our ability to untangle the complexity of those mechanisms and to measure the effect of each of them separately using standard approaches and clinical cohorts is limited.

Therefore, we hypothesize that advanced, sensitive tests using blood samples can lead to a better understanding of what causes MS development, help determine the initial events, and reveal the timing and pace of different mechanisms leading to damage to underlying nerve fibers. In a preliminary work conducted in our lab, such tools allowed for the discovery of a subset of People with MS (PwMS) with unique antibody clusters that can target the brain and spinal cord.

In this project, we will use state-of-the-art protein measurement and blood biomarker discovery tools to determine the protein signature that characterizes PwMS before the first clinical symptoms. Through those highly accurate analytical tools, we can compare the abundance of thousands of proteins between presymptomatic samples with evidence of a specific antibody cluster compared to PwMS without any specific immune cluster and healthy controls.

Our project will use de-identified, pre-collected samples from unique samples from the Department of Defense Serum Repository (DoDSR) Presymptomatic MS cohort, established in 2019, to assess environmental and genetic risk factors for MS before onset symptoms and diagnosis. MS cases in this cohort (n=250) were selected from the population-based Gulf War Era MS (GWEMS) cohort (n=2,691), which consists of incident MS cases within the U.S. military population with active-duty service between 1990-2007. In collaboration with the Armed Forces Health Surveillance Branch, the DoDSR was used to identify the earliest serum sample (mean 5.0 years) before the first symptom onset of MS and a second sample shortly (mean 1.2 years) after the first symptom onset for 250 MS cases. Another source of samples is the unique ORIGINS cohort at University of California, San Francisco, which includes hundreds of samples from PwMS collected in the earliest clinical stage.

All the samples and clinical data will be made available under the study-specific ID according to the Health Insurance Portability and Accountability Act of 1996. No access to the 18 protected health identifiers will be granted to the project personnel, and stringent data protection rules will be imposed.

Our work can help reveal the mechanisms leading to neuronal damage and disability accumulation at the earliest stages of the disease, even before the clinical symptoms appear. That can explain the contribution of early events to long-term disability. In addition, detecting early cellular elements contributing to nerve fiber degeneration in the preclinical stage can guide the development of novel targeted preventive strategies, especially for high-risk individuals.

Proposal Title:	In-Depth Analysis of Compartmentalized Inflammation in Progressive MS: IMPROVE MS
Log Number:	MS220186
Current PI Name:	Tradite Neziraj
Award Number:	HT9425-23-1-1007
Current Contracting Organization:	Universitat Basel
Current Performing Organization:	Universitat Basel
Web Approval Date:	10-02-2023

Multiple sclerosis (MS) is the most prevalent chronic inflammatory disease of the central nervous system (CNS) affecting over 2.5 million people worldwide. It is thought to be caused by dysregulated immune cells that mistakenly attack the patient's own brain and spinal cord (CNS). The CNS is responsible for basic neurological processes including movement, sensibility, vision, and cognition. The disease course of patients with MS is heterogeneous. About 85% of MS patients develop a relapsing remitting MS. Within 10-20 years, up to 50% of the relapsing remitting patients suffer from increasing neurological deficiencies characterized by paralysis and cognitive impairment (secondary progressive MS). About 15% of MS patients show a constant worsening of symptoms from the onset of the disease (primary progressive MS patients).

While enormous progress has been made in treating the relapsing form of MS, progressive MS remains one of the biggest clinical challenges as therapeutic options are still sparse. Progressive MS is characterized by inflammatory processes of the CNS that partly consist of aggregates of B cells. B cells are white blood cells, which are mostly known for their function of producing antibodies to fight infections. In MS, harmful B cells can enter the CNS, where they contribute significantly to disease progression by attacking the myelin sheath, the isolating layer that protects our nerve fibers, and by affecting other immune cells to enhance inflammation. However, some B cells are known to have a beneficial function by suppressing immune responses. Beneficial, regulatory B cells that produce the antibody IgA were found to travel from the gut to the CNS. The gut microbiota, which represents all microorganisms including bacteria that live in a person's digestive tract, is suggested to be a major contributor to disease progression and the activation of B cells. Yet, little is known about the interaction between the gut microbiota and beneficial B cells in progressive MS.

IMPROVE MS aims to understand diverse B cell functions and the influence of gut bacteria on beneficial B cell responses in progressive MS with consequences on the development of novel therapeutic strategies and thus patient care. Specifically, the following Focus Areas and aims will be addressed:

Factors contributing to or associated with MS Etiology, Prodrome, Onset, and Disease Course: We will analyze the influence of changes in the gut bacterial composition of different MS patients on beneficial, regulatory B cell responses in MS.

1. Our first aim is to characterize harmful and beneficial B cells in the cerebrospinal fluid (a fluid that surrounds the brain and spinal cord), blood, and gut biopsies from patients with progressive MS versus patients with new diagnosed relapsing remitting MS and healthy controls.

2. Our second aim is to identify the gut bacteria in MS patients that can activate beneficial B cell responses in different MS patients. Understanding the role of gut bacteria in MS and their effect on B cells will pave the way for the development of therapeutic approaches that modulate the composition of bacteria in our gut and induce a beneficial, regulatory B cell response in progressive MS patients.

3. Correlates of Disease Activity and Progression in MS: Our third aim is to assess the correlation of the antibody IgA, which is produced by beneficial B cells, with disease activity and progression in an existing large cohort of well-characterized MS patients. We aim to identify a correlation between IgA and disease activity and progression and by this develop a novel, personalized biomarker to predict disease prognosis and assess treatment response.

IMPROVE MS will provide new insights about the role of gut bacteria and beneficial B cell responses in progressive MS. It has the potential (i) to fundamentally advance our knowledge of the development of progressive MS by characterizing diverse B cells of different tissues, (ii) to assess new biomarker candidates, and (iii) to pave the way for new tailored therapies that induce regulatory B cell responses in the CNS with the objective to ultimately improve the lives of our patients with progressive MS.

Proposal Title:	Determining the Mechanisms Underlying Remyelination by Surviving Oligodendrocytes
Log Number:	MS220187
Current PI Name:	Lindsay Osso
Award Number:	HT9425-23-1-0561
Current Contracting Organization:	Colorado, University of, at Denver
Current Performing Organization:	Colorado, University of, at Denver
Web Approval Date:	09-03-2023

Rationale and Objective: Myelin, the electrical insulation around neuronal axons, enables rapid information transmission across the nervous system. In multiple sclerosis, the immune system destroys myelin, causing demyelination of axons and leading to motor, sensory, and cognitive impairment. Creating new myelin, also known as remyelination, is the primary means for restoring function. However, remyelination that occurs naturally in patients is often limited, leading to disease progression in many people with multiple sclerosis. New strategies to increase remyelination are necessary to improve functional recovery in patients. Prior approaches to enhance remyelination have focused exclusively on increasing the formation of new oligodendrocytes, the cells that make myelin. However, recent findings from our laboratory and others have demonstrated that remyelination also occurs through other means. These studies found that oligodendrocytes that survive demyelination can also participate in remyelination, making new myelin sheaths to remyelinate axons. Enhancing the capacity of surviving oligodendrocytes to remyelinate axons could be a successful strategy to increase remyelination and functional recovery, especially considering that new oligodendrocyte formation is often inhibited in multiple sclerosis. Critically, we do not yet understand the molecular mechanisms regulating the capacity of surviving oligodendrocytes to remyelinate. Thus, I propose to investigate these mechanisms. I hypothesize that surviving oligodendrocytes increase the expression of genes that are typically involved in myelin formation by new oligodendrocytes. Additionally, I hypothesize that the gene Fyn, which is known to promote myelin formation by new oligodendrocytes, enables surviving oligodendrocytes to form new myelin.

FY22 MSRP Focus Area: This project directly addresses the "Central Nervous System Regenerative Potential in Demyelinating Conditions" FY22 MSRP Focus Area. This Focus Area outlines the need for scientific studies that identify new factors that promote myelin repair. By elucidating the molecular mechanisms regulating the capacity of surviving oligodendrocytes to remyelinate, this study will identify new factors involved in myelin repair.

Advancing Multiple Sclerosis Patient Care: Understanding the molecular mechanisms mediating surviving oligodendrocyte remyelination is the critical first step toward enhancing this myelin repair pathway in patients. Future work will test drugs known to target this molecular mechanism for their ability to increase the remyelinating capacity of surviving oligodendrocytes. This work would commence immediately following the identification the molecular mechanisms mediating surviving oligodendrocyte remyelination and could result in the identification of new drugs to increase remyelination efficiency and restore function in multiple sclerosis patients. Recent evidence from multiple sclerosis patients indicates that surviving oligodendrocytes are present in regions of previous demyelination known as shadow plaques. A drug that enhances remyelination by surviving oligodendrocytes could be administered to patients at all stages of progression to improve remyelination of shadow plaques or of future demyelinating lesions.

Proposal Title:	The Role of Microglial Lipid Phagocytosis and Recycling in Remyelination
Log Number:	MS220189
Current PI Name:	Lisa Golden
Award Number:	HT9425-23-1-0901
Current Contracting Organization:	Colorado, University of, at Denver
Current Performing Organization:	Colorado, University of, at Denver
Web Approval Date:	09-29-2023

Objectives and Rational: Multiple sclerosis (MS) patients develop lesions in the central nervous system (CNS) in which myelin is damaged, known as demyelination. Myelin is a membrane made of lipids, or fats, produced by oligodendrocytes that surrounds and protects axons. After a neurological injury, oligodendrocytes can regenerate myelin to repair the damage, known as remyelination. However, remyelination of MS lesions is often incomplete, leading to permanent damage. It is unknown why remyelination of MS lesions is incomplete. Thus, this proposal aims to understand the remyelination failure in MS.

The immune system plays a large role in MS disease; B cells produce antibodies that bind to myelin and target it for attack by immune cells. Notably, no current animal models reflect this specific mode of demyelination, making it difficult to study MS-specific mechanisms of remyelination failure. Therefore, we developed a new model of demyelination and remyelination using these MS-specific antibodies. When the antibodies are applied to mouse brain tissue, they induce demyelination. Upon removal, the tissue remyelinates. However, if the antibody remains (simulating an MS lesion environment) there is inefficient remyelination.

Microglia, the immune cells in the CNS, are important for "eating" myelin debris after demyelination and recycling the lipids to be reused. Of importance, oligodendrocytes use lipids to create new myelin. However, early studies suggest that MS antibodies impair microglial functions. Highlighting the importance of these cells in remyelination, if microglia are absent from a recovering tissue slice, lesion repair is also inefficient. Therefore, this proposal tests the hypothesis that myelin removal and recycling by microglia is crucial for helping in MS lesion remyelination.

In our model system, inefficient remyelination is defined by the presence of abnormal myelin structures not localized to axons, and a lack of proper myelin on the axons. Understanding why these two phenomena occur is crucial for understanding the remyelination failure in MS lesions. The aims of this project are (1) to investigate how excess lipid accumulation in microglia after eating myelin affects their function during remyelination, and (2) to study the importance of recycling the lipids from the myelin that microglia removed back to oligodendrocytes to produce new myelin for lesion repair.

MSRP Focus Area: This proposal addresses the "Central Nervous System Regenerative Potential in Demyelinating Conditions" Focus Area of the Multiple Sclerosis Research Program. We have developed an innovative, disease-specific model system of demyelination and repair that utilizes myelin-binding antibodies from MS patients. This model will be used to study microglia-oligodendrocyte interactions in order to understand why remyelination fails in MS patient lesions, and identify new biological mechanisms that can be targeted to improve remyelination.

Applicability to MS Patient Care: The results from this study will provide important new information about the role of microglial lipid recycling during remyelination. We will identify new druggable targets for the

recycling mechanisms that may overcome the remyelination limitations in MS lesions. Such a treatment could be available within 10 years and would greatly enhance the lives of MS patients as it would stall, if not, reverse permanent damage to the brain, ideally alleviating cognitive deficits.

Proposal Title:	Identifying and Testing Molecular Therapies for Schwannoma
Log Number:	NF220007
Current PI Name:	Lu Le
Award Number:	HT9425-23-1-0321
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	07-31-2023

Background: Neurofibromatosis Type 2 (NF2) is an autosomal dominant disorder that results in growth of multiple tumors in the nervous system. These tumors, called “schwannomas” because they arise from Schwann cells, can cause numbness, weakness, and chronic pain and can create pressure on vital organs, impairing their function. A hallmark tumor of NF2 is bilateral vestibular schwannoma (VS): these tumors develop on the 8th cranial nerve and lead to balance issues and deafness. Currently, there is no effective FDA-approved drug to treat schwannomas, and surgery is the main treatment. The NF2 gene encodes a tumor suppressor protein called Merlin. In 2015, the Clapp lab reported a mouse model of schwannoma in which the Merlin gene is deleted in Schwann cells and the mice develop tumors that resemble human schwannoma (Gelhausen et al., Hum Mol Gen, 2015). In 2020, the Le lab generated a different mouse schwannoma model based on deregulation of HIPPO signaling in Schwann cells (Chen et al., JCI Insight, 2020). These two unique animal models targeting different parts of the HIPPO pathway, as well as work from Dr. Read, who has an extensive biobank of human schwannoma, have revealed molecular pathways critical for schwannoma formation. In this application, we propose to use both of these preclinical schwannoma models to test new drug combination treatment strategies targeting these key pathways in schwannoma development. We will also use these models to investigate the “crosstalk” between key pathways involved in schwannoma development, which should lead to identification of new therapeutic targets. Finally, we will validate any new targets we find in our mouse models in human schwannoma samples. The critical resources and expertise of the collaborating PIs and the synergy of these approaches will contribute to the identification of important molecular targets with potential for clinical use in schwannoma treatment.

Scientific Rationale and Objective: Certain signaling pathways have been shown in animal models and human tumor samples to be involved in schwannoma development, but whether and how these pathways interact with each other is not known. The HIPPO and the MAPK signaling pathways have been implicated in schwannoma development in NF2 mouse models; however, the interaction of these pathways downstream of NF2/Merlin has not been elucidated. Our preliminary data also show that another protein, called AXL, would be a good candidate for drug targeting. We hypothesize that combined targeting of these pathways will have a synergistic effect to block tumor growth, and that a detailed investigation of the crosstalk between these pathways could inform new treatment strategies. To this end, we propose the following aims. In Aim 1, we will use our two schwannoma mouse models to test the effectiveness of a novel treatment regimen that combines TEAD inhibitor (to block dysregulated HIPPO signaling) plus MEK inhibitor (to block MAPK signaling) for blocking schwannoma growth. Response of the tumors to drug treatment will be measured, and then treated tumors will be comprehensively analyzed to identify potential new targets. In Aim 2, we will extend our experiments to test AXL inhibitors, both alone and in combination with inhibitors of MAPK and other key pathways in schwannoma formation. In Aim 3, we will use human schwannoma samples from NF2 patients to confirm the expression of any newly identified genes arising from Aims 1 and 2. Likewise, sequencing data from these human schwannoma samples will be mined for potentially relevant genes and molecular pathways critical for schwannoma development, which will then be tested in our mouse NF2 models.

Types of Patients/Clinical Benefits and Timeline: The studies proposed in this application will benefit NF2 patients as well as other patients with schwannoma. The clinical benefits of these studies will not be realized

for some time, as preclinical studies must be followed up by clinical studies in human patients and must then undergo FDA review before drug approval. However, preclinical studies are a necessary first step to identifying effective drugs/ drug combinations that will work to slow/prevent schwannoma growth or cause regression.

Likely Contributions of Proposed Research to Advancing NF Research/Patient Care: Our two unique mouse models of schwannoma provide powerful platforms to study important questions in schwannoma. The synergistic studies proposed in this application will leverage these two mouse models of NF2 to (1) test new combination treatment regimens for schwannoma, (2) explore the mechanistic underpinnings of key signaling pathways involved in schwannoma development, and (3) genetically and pharmacologically test potential targets identified in our studies. Finally, the availability of extensive human schwannoma samples from the Read lab offers the opportunity to validate findings from our mouse models, as well as for mining for new targets, thus making this work very translational. A major innovation in our proposal is leveraging the two existing NF2 genetic mouse models that develop schwannoma with 100% penetrance for pre-clinical drug testing along with synergistic feedback and validation from human schwannoma samples. The identification of key signaling events in schwannoma development is critical for designing effective targeted therapies, which are currently lacking for schwannoma treatment and are desperately needed to improve the quality of life of NF2 patients.

Proposal Title:	Identifying and Testing Molecular Therapies for Schwannoma
Log Number:	NF220007P1
Current PI Name:	David Clapp
Award Number:	HT9425-23-1-0322
Current Contracting Organization:	Indiana University
Current Performing Organization:	Indiana University
Web Approval Date:	07-31-2023

Background: Neurofibromatosis Type 2 (NF2) is an autosomal dominant disorder that results in growth of multiple tumors in the nervous system. These tumors, called “schwannomas” because they arise from Schwann cells, can cause numbness, weakness, and chronic pain and can create pressure on vital organs, impairing their function. A hallmark tumor of NF2 is bilateral vestibular schwannoma (VS): these tumors develop on the 8th cranial nerve and lead to balance issues and deafness. Currently, there is no effective FDA-approved drug to treat schwannomas, and surgery is the main treatment. The NF2 gene encodes a tumor suppressor protein called Merlin. In 2015, the Clapp lab reported a mouse model of schwannoma in which the Merlin gene is deleted in Schwann cells and the mice develop tumors that resemble human schwannoma (Gelhausen et al., Hum Mol Gen, 2015). In 2020, the Le lab generated a different mouse schwannoma model based on deregulation of HIPPO signaling in Schwann cells (Chen et al., JCI Insight, 2020). These two unique animal models targeting different parts of the HIPPO pathway, as well as work from Dr. Read, who has an extensive biobank of human schwannoma, have revealed molecular pathways critical for schwannoma formation. In this application, we propose to use both of these preclinical schwannoma models to test new drug combination treatment strategies targeting these key pathways in schwannoma development. We will also use these models to investigate the “crosstalk” between key pathways involved in schwannoma development, which should lead to identification of new therapeutic targets. Finally, we will validate any new targets we find in our mouse models in human schwannoma samples. The critical resources and expertise of the collaborating PIs and the synergy of these approaches will contribute to the identification of important molecular targets with potential for clinical use in schwannoma treatment.

Scientific Rationale and Objective: Certain signaling pathways have been shown in animal models and human tumor samples to be involved in schwannoma development, but whether and how these pathways interact with each other is not known. The HIPPO and the MAPK signaling pathways have been implicated in schwannoma development in NF2 mouse models; however, the interaction of these pathways downstream of NF2/Merlin has not been elucidated. Our preliminary data also show that another protein, called AXL, would be a good candidate for drug targeting. We hypothesize that combined targeting of these pathways will have a synergistic effect to block tumor growth, and that a detailed investigation of the crosstalk between these pathways could inform new treatment strategies. To this end, we propose the following aims. In Aim 1, we will use our two schwannoma mouse models to test the effectiveness of a novel treatment regimen that combines TEAD inhibitor (to block dysregulated HIPPO signaling) plus MEK inhibitor (to block MAPK signaling) for blocking schwannoma growth. Response of the tumors to drug treatment will be measured, and then treated tumors will be comprehensively analyzed to identify potential new targets. In Aim 2, we will extend our experiments to test AXL inhibitors, both alone and in combination with inhibitors of MAPK and other key pathways in schwannoma formation. In Aim 3, we will use human schwannoma samples from NF2 patients to confirm the expression of any newly identified genes arising from Aims 1 and 2. Likewise, sequencing data from these human schwannoma samples will be mined for potentially relevant genes and molecular pathways critical for schwannoma development, which will then be tested in our mouse NF2 models.

Types of Patients/Clinical Benefits and Timeline: The studies proposed in this application will benefit NF2 patients as well as other patients with schwannoma. The clinical benefits of these studies will not be realized

for some time, as preclinical studies must be followed up by clinical studies in human patients and must then undergo FDA review before drug approval. However, preclinical studies are a necessary first step to identifying effective drugs/ drug combinations that will work to slow/prevent schwannoma growth or cause regression.

Likely Contributions of Proposed Research to Advancing NF Research/Patient Care: Our two unique mouse models of schwannoma provide powerful platforms to study important questions in schwannoma. The synergistic studies proposed in this application will leverage these two mouse models of NF2 to (1) test new combination treatment regimens for schwannoma, (2) explore the mechanistic underpinnings of key signaling pathways involved in schwannoma development, and (3) genetically and pharmacologically test potential targets identified in our studies. Finally, the availability of extensive human schwannoma samples from the Read lab offers the opportunity to validate findings from our mouse models, as well as for mining for new targets, thus making this work very translational. A major innovation in our proposal is leveraging the two existing NF2 genetic mouse models that develop schwannoma with 100% penetrance for pre-clinical drug testing along with synergistic feedback and validation from human schwannoma samples. The identification of key signaling events in schwannoma development is critical for designing effective targeted therapies, which are currently lacking for schwannoma treatment and are desperately needed to improve the quality of life of NF2 patients.

Proposal Title:	Identifying and Testing Molecular Therapies for Schwannoma
Log Number:	NF220007P2
Current PI Name:	Renee Read
Award Number:	HT9425-23-1-0323
Current Contracting Organization:	Emory University
Current Performing Organization:	Emory University
Web Approval Date:	07-31-2023

Background: Neurofibromatosis Type 2 (NF2) is an autosomal dominant disorder that results in growth of multiple tumors in the nervous system. These tumors, called “schwannomas” because they arise from Schwann cells, can cause numbness, weakness, and chronic pain and can create pressure on vital organs, impairing their function. A hallmark tumor of NF2 is bilateral vestibular schwannoma (VS): these tumors develop on the 8th cranial nerve and lead to balance issues and deafness. Currently, there is no effective FDA-approved drug to treat schwannomas, and surgery is the main treatment. The NF2 gene encodes a tumor suppressor protein called Merlin. In 2015, the Clapp lab reported a mouse model of schwannoma in which the Merlin gene is deleted in Schwann cells and the mice develop tumors that resemble human schwannoma (Gelhausen et al., Hum Mol Gen, 2015). In 2020, the Le lab generated a different mouse schwannoma model based on deregulation of HIPPO signaling in Schwann cells (Chen et al., JCI Insight, 2020). These two unique animal models targeting different parts of the HIPPO pathway, as well as work from Dr. Read, who has an extensive biobank of human schwannoma, have revealed molecular pathways critical for schwannoma formation. In this application, we propose to use both of these preclinical schwannoma models to test new drug combination treatment strategies targeting these key pathways in schwannoma development. We will also use these models to investigate the “crosstalk” between key pathways involved in schwannoma development, which should lead to identification of new therapeutic targets. Finally, we will validate any new targets we find in our mouse models in human schwannoma samples. The critical resources and expertise of the collaborating PIs and the synergy of these approaches will contribute to the identification of important molecular targets with potential for clinical use in schwannoma treatment.

Scientific Rationale and Objective: Certain signaling pathways have been shown in animal models and human tumor samples to be involved in schwannoma development, but whether and how these pathways interact with each other is not known. The HIPPO and the MAPK signaling pathways have been implicated in schwannoma development in NF2 mouse models; however, the interaction of these pathways downstream of NF2/Merlin has not been elucidated. Our preliminary data also show that another protein, called AXL, would be a good candidate for drug targeting. We hypothesize that combined targeting of these pathways will have a synergistic effect to block tumor growth, and that a detailed investigation of the crosstalk between these pathways could inform new treatment strategies. To this end, we propose the following aims. In Aim 1, we will use our two schwannoma mouse models to test the effectiveness of a novel treatment regimen that combines TEAD inhibitor (to block dysregulated HIPPO signaling) plus MEK inhibitor (to block MAPK signaling) for blocking schwannoma growth. Response of the tumors to drug treatment will be measured, and then treated tumors will be comprehensively analyzed to identify potential new targets. In Aim 2, we will extend our experiments to test AXL inhibitors, both alone and in combination with inhibitors of MAPK and other key pathways in schwannoma formation. In Aim 3, we will use human schwannoma samples from NF2 patients to confirm the expression of any newly identified genes arising from Aims 1 and 2. Likewise, sequencing data from these human schwannoma samples will be mined for potentially relevant genes and molecular pathways critical for schwannoma development, which will then be tested in our mouse NF2 models.

Types of Patients/Clinical Benefits and Timeline: The studies proposed in this application will benefit NF2 patients as well as other patients with schwannoma. The clinical benefits of these studies will not be realized

for some time, as preclinical studies must be followed up by clinical studies in human patients and must then undergo FDA review before drug approval. However, preclinical studies are a necessary first step to identifying effective drugs/ drug combinations that will work to slow/prevent schwannoma growth or cause regression.

Likely Contributions of Proposed Research to Advancing NF Research/Patient Care: Our two unique mouse models of schwannoma provide powerful platforms to study important questions in schwannoma. The synergistic studies proposed in this application will leverage these two mouse models of NF2 to (1) test new combination treatment regimens for schwannoma, (2) explore the mechanistic underpinnings of key signaling pathways involved in schwannoma development, and (3) genetically and pharmacologically test potential targets identified in our studies. Finally, the availability of extensive human schwannoma samples from the Read lab offers the opportunity to validate findings from our mouse models, as well as for mining for new targets, thus making this work very translational. A major innovation in our proposal is leveraging the two existing NF2 genetic mouse models that develop schwannoma with 100% penetrance for pre-clinical drug testing along with synergistic feedback and validation from human schwannoma samples. The identification of key signaling events in schwannoma development is critical for designing effective targeted therapies, which are currently lacking for schwannoma treatment and are desperately needed to improve the quality of life of NF2 patients.

Proposal Title:	Targeting SOX11-FAK-Mediated Immune Exhaustion in NF1-Associated Malignant Peripheral Nerve Sheath Tumor (MPNST)
Log Number:	NF220008
Current PI Name:	Steven Rhodes
Award Number:	HT9425-23-1-0143
Current Contracting Organization:	Indiana University
Current Performing Organization:	Indiana University
Web Approval Date:	05-01-2023

Malignant peripheral nerve sheath tumor (MPNST) is a highly aggressive form of cancer that is the leading cause of early death in persons with neurofibromatosis type 1 (NF1). These tumors are highly resistant to treatment due to their ability to escape destruction by the immune system. We have discovered a protein, SOX11, that becomes highly expressed in MPNST and that we believe is critical for allowing tumor cells to hide from immune attack. In this proposal, we will determine the impact of SOX11 on key factors required by the immune system to recognize and destroy human MPNST cells. Additionally, we will establish whether genetic deletion of Sox11 in mice that develop MPNST can prevent tumor growth by promoting anti-tumor immune responses. In other forms of human cancer, SOX11 has been shown to act through a protein called focal adhesion kinase (FAK) to shut down anti-tumor immunity. Defactinib is a drug that blocks the activity of FAK and is already being used in clinical trials to treat patients with other types of cancer. We will determine whether defactinib can reduce the growth of MPNST in mice by enhancing the ability of the immune system to attack and destroy the tumor cells. These studies will enhance our understanding of immune escape pathways in MPNST and, furthermore, have strong potential to justify clinical trials of drugs such as defactinib that block FAK activity in MPNST where no effective treatments currently exist.

Proposal Title:	Genetics and the Environment: Evaluating How Maternal Dietary Exposure Affects Neurodevelopment and Cognition in NF1
Log Number:	NF220009
Current PI Name:	Yuan Pan
Award Number:	HT9425-23-1-0239
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	07-10-2023

Rationale: Neurofibromatosis type 1 (NF1) is a genetic disease caused by mutations in the NF1 gene that affects 1/2,500 people worldwide. Up to 80% of children with NF1 suffer from cognitive manifestations, such as problems with attention, executive function, learning, and motor learning. The treatment options for NF1-associated cognitive deficits are very limited, and the underlying mechanisms remain incompletely understood. In addition, cognitive issues in NF1 display significant clinical variance, and the confounding factors (genetic and environmental) are poorly understood.

Oligodendrocytes are cells that provide insulation (the myelin sheath) to neurons to facilitate fast neuronal communication and are required for healthy brain function. Oligodendrocytes are constantly being made from oligodendrocyte precursor cells (OPCs). Our preliminary data show that the Nf1 mutation results in abnormal patches with hyperdense OPCs that are impaired in making oligodendrocytes, leading to disrupted cognitive function. In addition, we showed that maternal obesity (a nutritional/environmental modifier) and its associated interleukin-6 (IL6) elevation causes increased OPC abundance early in development, suggesting that modifiers such as maternal obesity could interact with the Nf1 mutation to induce or exacerbate neurological issues in NF1.

Objective: In this study, we propose a series of experiments designed to determine the interaction between Nf1- mutation and maternal dietary exposure (high-fat-high-sugar diet). We propose to determine whether maternal obesity exacerbates Nf1-related anomalies in OPCs (Aim 1), oligodendrocyte production (Aims 1-2), and cognition (Aim 2). We will also determine the role of IL6 by inhibiting the function of its receptor (Aims 1-2). Our proposal addresses two of the areas of focus in the 2022 DOD NFRP IIRA: “Non-tumor manifestations (cognitive manifestations)” and “Nutritional, environmental, and other modifiers of NF1.”

What Types of Patients Will It Help and How Will It Help Them? The experiments proposed herein will help patients with NF1 by evaluating how two genetic and environmental factors (NF1 mutation and maternal obesity) affect cognitive symptoms and determine whether there is an interaction between these factors. This study is relevant to the clinical heterogeneity of cognitive phenotypes in NF1 and provides the initial proof-of-concept studies that environmental exposure may modify NF1-associated phenotypes to worsen cognitive deficits.

What Are the Potential Clinical Applications, Benefits, and Risks? The experiments proposed will determine whether maternal obesity-induced IL6 elevation worsens NF1-associated cognitive deficits. This will help lay the groundwork for interventional studies to determine whether IL6 receptor (IL6R) inhibition (e.g., tocilizumab, FDA-approved) improves cognitive manifestations in individuals with NF1. Potential risks of treating pregnant women with IL6R inhibitors would need to be carefully studied in the future prior to implementation.

What Is the Projected Time It May Take to Achieve a Patient-Related Outcome? This study will provide a potential rationale for further human investigation. These investigations could involve the correlation of

maternal and/or childhood serum IL6 levels with imaging abnormalities or cognitive deficits in NF1. Such a study would provide additional rationale for trialing IL6R inhibitors in patients with NF1. The projected time of this project together with the follow-up human investigations may take 5-10 years.

What Are the Likely Contributions of the Proposed Research Project to Advancing the Field of NF Research and/or Patient Care? Successful completion of the proposed studies will advance the field by deepening our understanding of how Nf1 mutation interacts with environmental/nutritional factors to induce cognitive impairments. Our studies will provide a rationale for further identifying treatments to ameliorate OPC /oligodendrocyte dysfunction and the associated cognitive manifestations in NF1 patients. They may also provide preclinical evidence to support the use of IL6R inhibitors to prevent or improve certain cognitive manifestations. In addition, our developmental studies will provide valuable evidence about the timeline of OPC/oligodendrocyte deficits in NF1 relevant to cognition, which might be helpful in determining the optimum time for intervention in individuals with NF1.

Proposal Title:	SMaRT-Based Repair of NF1 Pre-mRNA
Log Number:	NF220013
Current PI Name:	Andre Leier
Award Number:	HT9425-23-1-0185
Current Contracting Organization:	Alabama, University of, at Birmingham
Current Performing Organization:	Alabama, University of, at Birmingham
Web Approval Date:	05-03-2023

The proposed research project investigates the applicability and suitability of a method called “spliceosome-mediated pre-mRNA trans-splicing” (SMaRT) for correcting faulty pre-mature NF1 messenger RNA (pre-mRNAs) in cells. If successful, this method could be used to transiently correct a patient’s NF1 mutation – not in the NF1 gene (DNA) but in NF1 transcripts (RNA) before these are translated into NF1 protein (neurofibromin). The technique utilizes the cell’s own splicing machinery, the spliceosome, which by default removes non-coding regions, so called introns, from premature messenger RNAs and combines the coding sequences, so-called exons, into mature mRNAs. SMaRT requires the delivery of an engineered RNA molecule, called the pre-trans-splicing molecule (PTM), to the cell’s nucleus. The PTM contains the replacement sequence for one or more NF1 exons in the pre-mRNA that harbor a pathogenic mutation and will be designed to specifically bind to intronic NF1 pre-mRNA. Interaction between spliceosome, PTM, and mutant NF1 pre-mRNA will then result in the insertion of the PTM’s replacement exon(s) into the NF1 pre-mRNA instead of the corresponding disease-causing exon(s), as well as generation of a correct transcript, allowing translation into functional neurofibromin.

SMaRT has been studied for over two decades and applied to several target pre-mRNAs and disease contexts with varying degrees of successful repair. In recent years, several improvements in the PTM design have increased the efficiency and specificity of trans-splicing. For example, trans-splicing efficiencies of more than 40% have been achieved in human cells following optimization of the PTM targeting pre-mRNA from a gene, called rho-dopsin. The level of NF1 repair that is ultimately achieved depends on many factors, including the sequence of the targeted NF1 intron, the position and length of the intronic NF1 pre-mRNA binding domain, and other PTM design elements. Recently published research by us and collaborators suggests that less than 50% NF1 repair is necessary to observe at least partial reconstitution of normal cellular behavior. Moreover, a genetically engineered mouse that produces only approximately 50% of functional neurofibromin in its cells does not show any Neurofibromatosis Type I (NF1) phenotype. Together, this suggests that, thanks to improved PTM designs, SMaRT-based repair efficiencies of mutant transcripts are now reaching levels that could potentially normalize NF1 downstream signaling and be therapeutic for NF1 patients. This is the underlying hypothesis of our proposal.

Of note, our approach does not correct mutant DNA, which would be permanent, but instead it corrects faulty RNA. Consequently, PTMs (RNA) would likely have to be repeatedly delivered to the patient’s cells to be therapeutic. On the other hand, safety issues of gene replacement and gene editing therapies such as insertional mutagenesis, overexpression of the target gene, or off-target genome editing are of no concern with SMaRT. If successful, our approach could help patients with pathogenic NF1 variants, where the mutation is in an exon that can be efficiently replaced using a designed PTM. We envision the use of therapeutic PTMs that systemically correct a patient’s NF1 germline mutation (first hit) in the transcript to reduce the overall risk of NF1-associated tumors. In addition, PTMs correcting NF1 germline and/or somatic exon mutations may also be therapeutic against NF1-related tumors. While off-target effects such as binding of the PTM to pre-mRNA other than NF1 are, in principle, possible, they are likely not harmful if the product of such an event is non-functional or readily degraded. Moreover, it has been shown that such specificity issues can be reduced by improving the PTM design.

The likeliest major contribution of this project will be a proof of concept showing that SMaRT-based correction of mutant NF1 transcripts is possible in human cells. More specifically, as part of this proposal, we will screen the 57 NF1 introns for their suitability as target introns using SMaRT. The screening will rank introns based on their natural splice sites' strength. For the most promising introns – those with weak splice site strength are preferred, as they are direct competitors to a PTM's splice site – we will design PTMs that are able to replace exons that are in the (pre-)mRNA sequence either 5' (i.e., before) or 3' (i.e., after) of the respective intron. Several PTMs will be pre-screened in a fluorescence assay in human cells, whereby higher fluorescence intensity indicates more efficient repair. Subsequently, we will test the best-performing PTMs directly on mutant NF1 and quantify levels of repaired neurofibromin and downstream cell signals, which will inform about the impact that the repair has on the reconstitution of normal cell behavior. We may further improve the design of PTMs to increase levels of repair. Our work will also inform about faulty exons (and with that patient mutations) that may be corrected with our approach. Together, the collected data will allow us to assess whether the development of the proposed NF1 therapy can move on to the next phase, namely testing of SMaRT in mouse models with patient mutation and disease phenotype.

Proposal Title:	Modeling Risk of Radiation-Associated Malignant Progression in Familial NF2 with Machine Learning
Log Number:	NF220014
Current PI Name:	Chay Paterson
Award Number:	HT9425-23-1-0434
Current Contracting Organization:	Manchester, University of
Current Performing Organization:	Manchester, University of
Web Approval Date:	09-15-2023

NF2 is a rare hereditary disorder that predisposes patients to benign tumors on the acoustic nerve called vestibular schwannomas. Radiotherapy is a non-invasive treatment that is sometimes recommended for patients who have developed problematic tumors, but it has proven very difficult to evaluate the risk that radiation promotes malignancy, and much more serious brain tumors.

A large, long-term study on the after-effects of radiation therapy on patients with NF2 was recently carried out by a team based at the University of Manchester. Using data from this long-running study, we will develop a machine learning-based model that computes an individualized assessment of the risk of post-radiation malignancy. The clinical applications will be more precise guidance about treatment-related risk, allowing patients and clinicians to make personalized decisions to lower the chance of complications.

This model will be packaged in an open-source plugin compatible with widely used medical imaging software, and the modelling approach will be communicated to medical physicists and neurosurgeons in peer-reviewed publications. If there is confirmed to be an increased risk of malignancy, and risk factors identified, these will be communicated to the scientific and neurosurgical communities directly. The timescale to delivering outcomes relevant to patients should be short, with clear recommendations and freely available software tools by the end of the project.

This research will advance the care of patients with NF2 by ensuring that they receive the correct treatment and advice, and thus enjoy a lower risk of malignancy overall. The bioinformatic methods developed may also help to identify new genomic risk factors in future research.

Proposal Title:	Deciphering the Role of LZTR1 in Schwannomatosis
Log Number:	NF220018
Current PI Name:	Stephanie Mo
Award Number:	HT9425-23-1-0248
Current Contracting Organization:	New York University School of Medicine
Current Performing Organization:	New York University School of Medicine
Web Approval Date:	07-07-2023

Schwannomatosis is a rare genetic disorder that affects around 1 in 40,000 people. Schwann cells are responsible for protecting the nerves; however, in schwannomatosis, abnormal growth of these cells occurs around the spinal and peripheral nerves. As a result, they form multiple noncancerous tumors called schwannomas. Individuals with schwannomatosis often experience chronic pain in early adulthood as well as other symptoms such as numbness, weakness, and vision changes. These symptoms can vary between individuals depending on a number of factors like the size, number, and location of the tumors. To date, treatments for schwannomatosis include the surgical removal of schwannomas and pain management. However, there is no cure or medication for schwannomatosis.

The cause of schwannomatosis is unclear; however, in a number of cases, it has been found to be linked to a gene called LZTR1, which provides the instructions to make the LZTR1 protein. It has been hypothesized that LZTR1 regulates the growth of cells. However, in schwannomatosis, the LZTR1 gene is modified. As a result, the protein no longer functions like normal, and cells instead grow in an uncontrolled manner, which ultimately leads to the development of tumors. Currently, there are no studies that explain how the modified LZTR1 gene leads to the development of schwannomatosis. Therefore, my research proposal aims to identify how this modified LZTR1 gene leads to the development of tumors in schwannomatosis. From this research, I hope to identify therapeutic targets that will form the foundation of a drug discovery pipeline. Ultimately, my research proposal will aim to improve our understanding of this understudied genetic disorder and aid the development of a potential therapy for schwannomatosis.

Proposal Title:	Cellular Origin of Bone Manifestations in Neurofibromatosis Type 1
Log Number:	NF220019
Current PI Name:	Celine Colnot
Award Number:	HT9425-23-1-0359
Current Contracting Organization:	Institut National de la Sante et de la Recherche Medicale (INSERM), Montpellier
Current Performing Organization:	Institut National de la Sante et de la Recherche Medicale (INSERM), Montpellier
Web Approval Date:	09-15-2023

Neurofibromatosis type 1 (NF1) is an autosomal dominant disease due to mutations in the NF1 tumor suppressor gene. NF1 patients can be affected by a variety of symptoms with a wide range of severity, including cutaneous, neurological, orthopedic and malignant manifestations. Bone manifestations in NF1 affect approximately 50% of patients and comprise osteoporosis, bone dysplasia, pseudarthrosis, cranial anomalies and spine deformities. Scoliosis is the most common osseous defect associated with NF1 as 20% of NF1 patients have a disorder of the spine. Non-dystrophic deformity can be managed similarly to idiopathic scoliosis with expert examination and bracing. However, dystrophic scoliosis associated with vertebral and rib anomalies, often requires surgical fusion with instrumentation and bone grafting. Long bone dysplasia affects 5% of NF1 patients but can lead to congenital pseudarthrosis of the tibia (CPT) a very severe pathology. Treating CPT is very challenging for orthopaedic surgeons and requires complex surgical procedures including fixation, bone grafting, periosteum grafting and induced membrane techniques. The efficacy of these various treatments is still not demonstrated and risk of amputation remains high.

To improve the clinical care of NF1 patients a better understanding of underlying genetic bases and mechanisms of NF1 pathophysiology is needed. NF1 patients carry a heterozygote inactivating mutation in the NF1 gene. The NF1 gene has been described as bearing one of the highest mutation rates in the human genome. Research on the genetic bases of neurofibromas (NFBs) and café-au-lait macules (CALMs) revealed that somatic mutations in specific cell types, i.e., Schwann cells and melanocytes respectively, occur in these localized NF1 lesions causing the loss of the second NF1 allele. However, for bone lesions, the cell types carrying a potential mutation in the second NF1 allele have not been determined.

The goal of this project is to characterize which cell types are affected in bone manifestations in NF1 in human and in a clinically relevant mouse model. We will study the role of a specific cell population derived from the neural crest during embryonic development that give rise to Schwann cells and melanocytes in the skin. Inactivation of both NF1 alleles in these cells causes cutaneous and plexiform NFBs, as well as CALMs and other NF1 symptoms in mice. Our preliminary evaluations also show bone anomalies reminiscent of NF1 symptoms and indicates that the same neural crest derivatives producing Schwann cells and skeletal stem/progenitor cells in bone may be the cell of origin of bone manifestations. This animal model is unique as it is the first model to recapitulate several aspects of the NF1 disease and may serve as a valuable pre-clinical model.

We therefore propose to perform an advance characterization of the cell types responsible for the bone anomalies in this mouse model and how NF1 gene inactivation impacts their functions. In parallel, we will analyze bone samples from NF1 patients affected by congenital pseudarthrosis of the tibia and dystrophic scoliosis. This patient population requires complex surgical procedures to correct the bone anomalies. Bone samples collected during these surgical procedures will be analyzed to identify the cell types carrying inactivation of NF1 gene.

The strength and novelty of this project is to combine the analyses of a clinically relevant animal model and patient samples to elucidate the mechanisms of NF1 bone manifestations and identify potential common

mechanisms between tumor and non-tumor NF1 manifestations. In the long term, deciphering the cell type of origin of bone lesions and the molecular pathways affected by NF1 mutation will help design new pharmacological and cellular approaches to treat bone manifestations in NF1.

Proposal Title:	Tailoring an Online Platform to Promote Evidence-Based Care in Children and Adults with Neurofibromatosis 1 to Address Medical and Social Barriers to Care
Log Number:	NF220020
Current PI Name:	Vanessa Merker
Award Number:	HT9425-23-1-0457
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	09-15-2023

Neurofibromatosis type 1 (NF1) is a complex multisystem disorder that predisposes people to multiple types of benign and malignant tumors, as well as cognitive, behavioral, bone, vascular, and other medical issues.

Regular medical evaluations are critical to identify NF1-related problems as early as possible and offer appropriate treatment. Professional medical societies in the United States (U.S.) have issued guidelines about what routine medical evaluations are needed by NF1 patients, and we previously showed these guidelines are strongly supported by NF specialists. But, in the U.S., a majority of NF1 patients don't go to specialty NF clinics for their care. Our preliminary research shows that these patients who don't go to specialty NF clinics are significantly less likely to get recommended NF1-related medical evaluations than patients who do go to these clinics. We hypothesize that if we could help primary care providers (PCPs) perform routine NF1-related medical evaluations, we could significantly improve the quality of care for patients who don't go to specialty NF clinics, and ultimately, improve their overall health and wellbeing.

To achieve this goal, we propose to adapt an innovative online platform that has already successfully improved care for people with Down Syndrome (DS). The "Down Syndrome Clinic to You" platform consists of a website where parents/caregivers fill out questions about their loved one's health. Based on the answers to those questions, the website automatically generates a list of personalized medical recommendations for the person with DS, such as whether the person is due for an eye exam, should be tested for sleep apnea, or needs another medical evaluation. These recommendations get automatically formatted into two letters – one written for parents/caregivers and one written for PCPs. Parents can then share the PCP letter with their loved one's doctor at their next visit, so that the PCP knows what evaluations the person with DS needs and why. Our proposed study will adapt this online platform to suit the unique needs of people with NF1. We will test whether our new "NF Guidelines to You" platform can increase evidenced-based healthcare for people with NF1 by providing personalized care recommendations directly to NF1 patients/parents and their PCPs across the U.S.

This project will have three stages. In the first stage, we will ask adults with NF1, parents of children with NF1, and PCPs who care for individuals with NF1 about their primary care visits and the problems they have seeking out or delivering NF1-related medical evaluations during these visits. In the second stage, we will create the "NF Guidelines to You" website based on recommendations from individuals in stage 1 and with the help of an advisory board of NF1 patients/parents, PCPs, and scientific/medical experts. We will also create tailored educational resources as a means of empowering individuals with NF1 to advocate for their medical care needs. These resources will be tested to be as widely accessible as possible, including for people who speak Spanish, have learning disabilities, or are young adults managing their care for the first time. In the third stage, we will test whether using "NF Guidelines to You" is helpful for adults with NF1, parents of children with NF1, and PCPs across the U.S. We will focus on whether the website is easy to use

and if people find the letters and educational resources useful so that we can make any final improvements as needed. We will also preliminarily look at whether patients who use the platform receive more guideline-recommended NF1 care and are more satisfied with their care. At the end of this process, we will have optimized the “NF Guidelines to You” platform and will be ready to test if it is better than the current standard of care in a large clinical trial.

This platform could help the roughly 63,000 people with NF1 who do not attend specialty NF clinics receive better care by delivering personalized, actionable healthcare recommendations to NF1 patients and their PCPs, and by empowering individuals and families with NF to advocate for the care they need. As a free platform available in both English and Spanish on all devices (e.g., PCs, Macs, iPhones, Android, etc.), it will have a wide reach. It can be easily updated in the future to include additional languages, new or updated NF1 guidelines, and other NF1-related resources. It could also be expanded to target other kinds of physicians (like local neurologists) and provide guidelines for neurofibromatosis 2 and schwannomatosis. Ultimately, we hope to develop resources that ensure all NF patients, no matter where they live or what doctors they see, can get the high-quality care they need to monitor and treat their NF.

Proposal Title:	Quantitative Sodium Magnetic Resonance Imaging and Chemical Exchange Saturation Transfer (CEST) of Low-Grade Gliomas in Children with Neurofibromatosis Type 1
Log Number:	NF220021
Current PI Name:	Aashim Bhatia
Award Number:	HT9425-23-1-0345
Current Contracting Organization:	Children's Hospital, Philadelphia
Current Performing Organization:	Children's Hospital, Philadelphia
Web Approval Date:	07-12-2023

Currently, the standard approach of monitoring brain tumors includes radiology imaging, such as MRI. These MRIs provide important information for the clinical doctors but have many limitations in knowing whether there is a brain tumor and whether a cancer treatment is working. Children with neurofibromatosis type 1(NF1) have an increased risk over their lifetime of developing brain tumors; however, they also develop benign lesions in the brain that we cannot distinguish from tumors on MRI, due to their similar imaging appearance on MRI.

We are researching an MRI approach to looking at more advanced imaging techniques, such as sodium levels in the brain and brain tumors, since we know brain tumors have increased sodium and protein levels based on our preliminary data and literature. However, much more data are needed, and there are no research studies in children. Low-grade gliomas are common brain tumors in children with NF1 that can be treated with chemotherapy. After treatment, the goal is for the tumor to shrink in size or stop growing, but treatment changes can make the tumor look as it is still progressing with more enhancement on MRI, and we currently do not have an easy way to determine this with clinical MRIs. Biopsy cannot be performed at multiple timepoints during treatment due to the risk of complications. Biopsy is not a simple procedure for patients; there is sedation, time needed in the hospital, and risk of bleeding and infections. We believe measuring the sodium values and protein levels in tumors with these advanced imaging techniques (sodium MRI and chemical exchange saturation transfer) proposed in this grant will help know earlier than current imaging being performed in hospitals on when children with NF1 develop brain tumors. This research study will also help radiologists, such as myself, know whether the signal abnormality in the brain is a tumor or rather a benign abnormality in the brain that looks like tumor commonly seen in NF1 patients. These advanced imaging techniques have the potential to help understand whether a child needs chemotherapy and if cancer treatment is working.

This study overall will help us understand the behavior in brain tumors that cannot be obtained with the current clinical MRIs. We will use this data from this study to help support funding for larger studies in NF1 patients, including adults through the collaboration at the University of Penn/Penn Hospital. These advanced MRI techniques will make an accurate diagnosis of tumors, and determine whether they are responding or not responding to treatments, allowing doctors to make treatment decisions quickly to improve outcomes of children with NF1 and brain tumors.

Proposal Title:	Leveraging a Precision Medicine Platform to Predict Novel Therapies for Malignant Peripheral Nerve Sheath Tumors
Log Number:	NF220025
Current PI Name:	David Largaespada
Award Number:	HT9425-23-1-0251
Current Contracting Organization:	Minnesota, University of, Twin Cities
Current Performing Organization:	Minnesota, University of, Twin Cities
Web Approval Date:	06-22-2023

One of the most common cancers affecting adults with neurofibromatosis type l (NFl) is the malignant peripheral nerve sheath tumor (MPNST), a highly aggressive tumor. Sadly, for those individuals with NFl who develop these cancers, there are limited treatment options, and the vast majority of people with these malignancies will die within 5 years of diagnosis. Over the past decade, research has revealed that NFl-MPNST represents a group of diverse cancer types, characterized by different genetic changes. In this respect, there are likely different subtypes of MPNST that each harbor distinct sets of molecular changes and thus have different responses to rationally-chosen treatments. Unfortunately, current small-animal models of NFl-MPNST used to discover and evaluate new therapies are largely engineered with one set of genetic changes, limiting their ability to fully represent the human condition. For this reason, we believe that the failure to discover effective treatments is partly due to the inability of these preclinical models to accurately model the genetic landscape of the human tumors as well as the ability of the tumors to adapt to single drugs. To address this problem, we have started to develop and characterize a set of patient-derived MPNST mouse models (called PDX) obtained directly from actual human tumors.

In this proposal, we plan to continue to generate new models for use in the NFl research community and extend these models to 3D cultures that enable rapid testing of preclinical drugs. This effort is essential for two reasons. First, PDX models can only be used for a limited period of time before they develop other genetic changes that cause them to differ from the human tumor from which they came and thus no longer mirror that tumor. As such, it is essential to continue to generate new models in order to maintain the resource for the NFl community. Second, we want to ensure that we can identify all of the relevant subtypes of this rare tumor. By growing the models in 3D cultures, we will be able to rapidly screen multiple drugs and drug combinations and determine which subtypes respond better to each given therapy. Additionally, we will analyze the changes brought about by the drugs by measuring changes in RNA and proteins. This will help us learn how the tumors "adapt" to drugs and identify other drugs that can prevent this adaptation if administered in combination.

This work will give us the body of data needed to rationally design clinical trials in a personalized manner using information from patients' tumors to choose which therapy the patient should receive. Currently, there are no effective therapies for metastatic NFl-MPNST and survival is dismal. Our hope is that, by the end of this funding period, we will be applying for funding for a clinical trial and thus, in the next few years, we will have the ability to explore this treatment paradigm in patients with NFl-MPNST-a patient population that desperately needs better therapies.

Proposal Title:	Leveraging a Precision Medicine Platform to Predict Novel Therapies for Malignant Peripheral Nerve Sheath Tumors
Log Number:	NF220025P1
Current PI Name:	Christine Pratilas
Award Number:	HT9425-23-1-0252
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	07-31-2023

One of the most common cancers affecting adults with neurofibromatosis type l (NFl) is the malignant peripheral nerve sheath tumor (MPNST), a highly aggressive tumor. Sadly, for those individuals with NFl who develop these cancers, there are limited treatment options, and the vast majority of people with these malignancies will die within 5 years of diagnosis. Over the past decade, research has revealed that NFl-MPNST represents a group of diverse cancer types, characterized by different genetic changes. In this respect, there are likely different subtypes of MPNST that each harbor distinct sets of molecular changes and thus have different responses to rationally-chosen treatments. Unfortunately, current small-animal models of NFl-MPNST used to discover and evaluate new therapies are largely engineered with one set of genetic changes, limiting their ability to fully represent the human condition. For this reason, we believe that the failure to discover effective treatments is partly due to the inability of these preclinical models to accurately model the genetic landscape of the human tumors as well as the ability of the tumors to adapt to single drugs. To address this problem, we have started to develop and characterize a set of patient-derived MPNST mouse models (called PDX) obtained directly from actual human tumors.

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Proposal Title:	Leveraging a Precision Medicine Platform to Predict Novel Therapies for Malignant Peripheral Nerve Sheath Tumors
Log Number:	NF220025P2
Current PI Name:	Sara Gosline
Award Number:	CDMRPL-0-NF220025P2
Current Contracting Organization:	Battelle Memorial Institute
Current Performing Organization:	Battelle Memorial Institute
Web Approval Date:	07-31-2023

One of the most common cancers affecting adults with neurofibromatosis type l (NFl) is the malignant peripheral nerve sheath tumor (MPNST), a highly aggressive tumor. Sadly, for those individuals with NFl who develop these cancers, there are limited treatment options, and the vast majority of people with these malignancies will die within 5 years of diagnosis. Over the past decade, research has revealed that NFl-MPNST represents a group of diverse cancer types, characterized by different genetic changes. In this respect, there are likely different subtypes of MPNST that each harbor distinct sets of molecular changes and thus have different responses to rationally-chosen treatments. Unfortunately, current small-animal models of NFl-MPNST used to discover and evaluate new therapies are largely engineered with one set of genetic changes, limiting their ability to fully represent the human condition. For this reason, we believe that the failure to discover effective treatments is partly due to the inability of these preclinical models to accurately model the genetic landscape of the human tumors as well as the ability of the tumors to adapt to single drugs. To address this problem, we have started to develop and characterize a set of patient-derived MPNST mouse models (called PDX) obtained directly from actual human tumors.

In this proposal, we plan to continue to generate new models for use in the NFl research community and extend these models to 3D cultures that enable rapid testing of preclinical drugs. This effort is essential for two reasons. First, PDX models can only be used for a limited period of time before they develop other genetic changes that cause them to differ from the human tumor from which they came and thus no longer mirror that tumor. As such, it is essential to continue to generate new models in order to maintain the resource for the NFl community. Second, we want to ensure that we can identify all of the relevant subtypes of this rare tumor. By growing the models in 3D cultures, we will be able to rapidly screen multiple drugs and drug combinations and determine which subtypes respond better to each given therapy. Additionally, we will analyze the changes brought about by the drugs by measuring changes in RNA and proteins. This will help us learn how the tumors "adapt" to drugs and identify other drugs that can prevent this adaptation if administered in combination.

This work will give us the body of data needed to rationally design clinical trials in a personalized manner using information from patients' tumors to choose which therapy the patient should receive. Currently, there are no effective therapies for metastatic NFl-MPNST and survival is dismal. Our hope is that, by the end of this funding period, we will be applying for funding for a clinical trial and thus, in the next few years, we will have the ability to explore this treatment paradigm in patients with NFl-MPNST-a patient population that desperately needs better therapies.

Proposal Title:	Leveraging a Precision Medicine Platform to Predict Novel Therapies for Malignant Peripheral Nerve Sheath Tumors
Log Number:	NF220025P3
Current PI Name:	Angela Hirbe
Award Number:	HT9425-23-1-0253
Current Contracting Organization:	Washington University in St Louis
Current Performing Organization:	Washington University in St Louis
Web Approval Date:	06-22-2023

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Proposal Title:	Leveraging a Precision Medicine Platform to Predict Novel Therapies for Malignant Peripheral Nerve Sheath Tumors
Log Number:	NF220025P4
Current PI Name:	David Wood
Award Number:	HT9425-23-1-0254
Current Contracting Organization:	Minnesota, University of, Twin Cities
Current Performing Organization:	Minnesota, University of, Twin Cities
Web Approval Date:	06-22-2023

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Proposal Title:	NF2/Merlin in Vascular Smooth Muscle Cells
Log Number:	NF220030
Current PI Name:	Brian Stansfield
Award Number:	HT9425-23-1-0240
Current Contracting Organization:	Augusta University Research Institute, Inc.
Current Performing Organization:	Augusta University Research Institute, Inc.
Web Approval Date:	06-22-2023

Therapies tailored for persons with neurofibromatosis type 2 (NF2) are limited, and few clinical trials are actively recruiting persons with NF2 for participation. In seeking to identify new therapeutic targets for NF2, we have focused on recent reports demonstrating that hypertension is exceedingly common in persons with NF2 and that some manifestations may be particularly sensitive to therapies that are traditionally used to control blood pressure. In fact, use of a particular blood pressure medication (losartan) was recently shown to enhance radiation therapy in an animal model NF2-associated schwannoma with outcomes that closely mirrored higher-dose radiation.

Based on these promising outcomes in animal models and clinical evidence from cohorts of patients with NF2, we have developed animal models carrying mutations in Nf2 in vascular smooth muscle cells alone. These animals develop arterial stiffness and poor blood pressure control that accurately resemble clinical reports from persons with NF2. Both smooth muscle cells and whole arteries from these animals display increased contractility (a feature of hypertension), and we have identified a novel pathway for study that has not previously been explored in the context of NF2.

Loss of merlin, the protein encoded by the NF2 gene, in smooth muscle cells results in very low expression of the serotonin transporter. This transporter is primarily responsible for returning serotonin to the intracellular compartment to be degraded, and FDA-approved compounds targeting the serotonin transporter (Selective Serotonin Reuptake Inhibitors or SSRIs) interfere with serotonin metabolism and promote increased binding of serotonin to its receptor. Serotonin helps to control blood pressure but is also a critical mediator of pain signaling pathways. We provide clear evidence that loss of merlin sensitizes smooth muscle cells to angiotensin, a small protein that controls blood pressure and is targeted by losartan. In turn, activation of angiotensin signaling and loss of the serotonin transporter promote the effects of serotonin on the vascular system.

The long-term goal of our laboratory is to develop new insights and therapies for NF. Our goal in this Investigator-Initiated Award application is to identify a novel relationship between merlin, angiotensin, and serotonin in the control of blood pressure and arterial stiffness with an eye towards how these mechanisms may be exploited for hypertension and other manifestations related to NF2. At the conclusion of the funding period, we will have (1) defined a clear mechanism linking these important targets to merlin/NF2, (2) developed efficacy data from multiple FDA-approved and experimental therapies that may be used to treat high blood pressure and/or other manifestations of NF2, and (3) generated multiple surveys of small RNA, proteins, and pathways that are dysregulated in merlin-deficient smooth muscle cells, which will significantly inform and impact the NF2 research community.

Proposal Title:	Evaluation of Procaspase-Activating Compound-1 in Combination with Chemotherapy, MEK Inhibition, and/or Radiotherapy for Malignant Peripheral Nerve Sheath Tumors
Log Number:	NF220032
Current PI Name:	Gregory Riggins
Award Number:	HT9425-23-1-0214
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	06-22-2023

Neurofibromatosis type 1 is a complex genetic disorder characterized by the development of benign tumors along with the nervous, called neurofibromas that can cause disability and progress into malignant tumors. About 1 in 10 patients will have a benign neurofibroma become cancerous. These malignant tumors, known as malignant peripheral nerve sheath tumors (MPNST), are refractory to conventional treatments. Consequently, there is an urgent need to develop more efficient therapy for NF 1 patients. These incurable peripheral nerve tumors result from loss of Neurofibromin 1 (NF1) tumor suppressor gene function, causing constitutive activation of MAPK signaling. For patients not qualified for surgery resection, cytotoxic therapy is often employed as treatment. However, acquired resistance is often observed in patients treated with cytotoxic chemotherapy, and additional drug combinations are required to induce tumor regression. The acquired drug resistance occurs due to the selective pressure in cancer cells and the lack of specificity of these drugs. Therefore, one fundamental question is how to optimize drug combinations that induce tumor regression and preferentially target NF1 mutant cancer cells. The present research plan addresses these questions using a combination of therapies. Cancer cell death induction is a clinically relevant strategy, and we helped launch phase 1 clinical trials with a novel drug for cancer therapy, procaspase activating compound-1 (PAC-1). This procaspase-3 activator induces apoptosis and extends survival in several cancer models, including rodents and dogs. Therefore, PAC-1 drug combinations will likely have a synergistic effect with the most promising therapy used for NF-1-associated MPNST. Our hypothesis states that PAC-1 combined with radio/chemotherapy will impair MPNST growth in mouse models. In this hypothesis-driven and exploratory project, we aim to optimize combinations of PAC-1 with chemo/radiotherapy that would be effective for treating MPNSTs harboring NF1 mutation in vivo. We believe that with successful results in this project, the innovative data could guide translational research from bench to bedside.

Proposal Title:	Targeting the NF-kB Pathway to Treat NF1-Deficient Tumors
Log Number:	NF220035
Current PI Name:	Stephanie Bouley
Award Number:	HT9425-23-1-0831
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	09-15-2023

Neurofibromatosis type 1 (NF1) is a tumor-predisposing genetic condition with an incidence rate of 1 in 3,000 individuals. While there are several symptoms that individuals with NF1 may experience, one of the most common is the formation of benign tumors known as plexiform neurofibromas (PNs). In around 12% of individuals with NF1, PNs have the potential to transform into malignant peripheral nerve sheath tumors (MPNSTs), which are often unresectable due to their location and resistant to most therapies available; as a result, MPNSTs are often fatal. Both PNs and MPNSTs are comprised of Schwann cells (SCs), the major glial cell in the peripheral nervous system. While there are no therapeutic options for MPNSTs, there is only one FDA-approved therapy for treating PNs, the MEK1/2 inhibitor Selumetinib. While Selumetinib has been successful in treating PNs in children and adolescents with NF1, there are many unknowns surrounding long-term treatment with MEK inhibitors, including concerns regarding side effects and resistance. For these reasons, there still exists an unmet clinical need for identifying new therapeutic strategies for treating individuals with NF1.

To address this need, our laboratory has chosen to focus on identifying differences in the SCs that make up a NF1 patient's normal tissue and PN tissue. To do this, we used a panel of pairs of cell lines derived from patients or generated in our laboratory using CRISPR technology that were either like a patient's normal tissue (missing one copy of the NF1 gene) or like a patient's PN tissue (missing two copies of the NF1 gene) to identify altered signaling networks. We did this by measuring changes in the global cellular landscape between each pair of cells, which allowed us to identify a plethora of potential targets involved in a variety of cellular processes. We performed comparison analyses between our two matched sets of patient-derived SC lines to identify overlapping hits, and in doing so we were able to refine our list of potential targets.

Our proposal focuses on investigating one signaling network identified through these analyses: the NF-kappaB pathway. Within this network, we have identified multiple targets that we will be exploring as potential therapeutic targets or biomarkers in treating NF1-deficient SCs like those found in PNs and MPNSTs. We have already demonstrated the therapeutic potential of targeting some of these targets with drugs, though this was performed on cell lines we used in our initial study. We will confirm that inhibition of these targets decreases the viability of NF1-deficient SCs in new pairs of NF1-deficient SC lines that we have generated using CRISPR-based gene editing strategies; we will also test drugs against other potential targets in these cell lines. We will measure cell viability as well as proteins involved in the cell death process known as apoptosis. We will also measure changes in proteins downstream of these targets to determine the impact of targeting NF-kappaB on other signaling networks. All the therapeutics tested that result in reduced viability of NF1-deficient SCs will also be tested in combination with the FDA-approved MEK inhibitor Selumetinib. We then want to test our successful inhibitors in MPNST-derived cell lines, as some studies have suggested that NF-kappaB is increased in MPNSTs prior to or after treatment with Selumetinib. We plan to test our inhibitors as both single agents and in combination with Selumetinib and measure proteins involved in cell death in addition to measuring viability. Finally, we will measure genes regulated by the NF-kappaB pathway, as transcription is anticipated to be altered due to target knockdown, and we will quantify gene expression, as this could reveal to us novel biomarkers for treating NF1. Any potential biomarkers will be measured in samples of PNs collected from patients to confirm their expression.

The work we are proposing has the potential to benefit many individuals with NF1 who are impacted by PNs. Our research seeks to identify novel therapeutic strategies to reduce PN burden by targeting the NF1-deficient SCs that comprise PNs. By testing the therapeutic potential of inhibiting multiple targets, we aim to identify several therapeutic options, as it has already been demonstrated that NF1 is a complex condition for which one therapeutic strategy will not work for everyone. In addition, our work aims to build upon the already successful outcomes observed with Selumetinib by examining the efficacy of combination therapy between any of the therapeutic strategies identified in our work and MEK inhibition. If we see success in our in vitro models, we will push forward with performing in vivo studies of our proposed therapeutic options, such as in a mouse or pig model of NF1. Our ultimate goal is to identify novel therapeutic strategies for treating individuals with NF1, to reduce their tumor burden, and by doing so improve their overall quality of life.

Proposal Title:	Cotargeting RAF and MEK kinases in NF1-Associated Malignant Peripheral Nerve Sheath Tumor
Log Number:	NF220037
Current PI Name:	Angelina Vaseva
Award Number:	HT9425-23-1-0217
Current Contracting Organization:	Texas, University of, Health Science Center at San Antonio
Current Performing Organization:	Texas, University of, Health Science Center at San Antonio
Web Approval Date:	07-12-2023

The most common malignancy and a leading cause of death in patients with neurofibromatosis type 1 (NF1) is peripheral nerve sheath tumor (NF1-MPNST). NF1-MPNST is a type of highly aggressive soft-tissue sarcoma with very limited therapy options due to resistance to conventional therapies as well as metastatic propensity. There is an urgent need for novel and more effective therapies. The most common molecular characteristic of NF1-MPNST is loss-of-function alterations in the NF1 gene. This leads to uncontrolled activation of RAS oncoproteins and RAS- effector pathways, among which the RAF-MEK-ERK signaling cascade has proven an attractive therapeutic target. However, responses to MEK inhibitors have been limited and current efforts are focused on development of combinatorial strategies. Recent preclinical and clinical evaluation of a novel dual RAF/MEK inhibitor has been encouraging in RAS-driven cancers. Evaluation of such inhibitors in NF1-associated cancers, including MPNST, is still lacking. Our preliminary data indicated that such inhibitor could circumvent RAF-mediated resistance to traditional MEK inhibitors in NF1-MPNST cells. Moreover, when NF1-MPNST tumor xenografts were treated with dual RAF/MEK inhibitor, this led to tumor regression (as opposed to progressive disease upon treatment with conventional MEK inhibitors). Therefore dual RAF/MEK inhibitor could have clinical applications in NF1-MPNST. In addition, we found that RAF/MEK inhibition modulated DNA damage response pathways and can possibly sensitize NF1-MPNST to chemotherapy agents. These results are the basis of our proposal to (1) preclinically evaluate the activity of this inhibitor in NF1-MPNST and (2) design novel combinatorial approaches and evaluate effect on chemotherapy response. We will employ evaluation of patient-derived xenograft models, in vivo pharmacodynamic assays with clinically relevant drug exposures, and genomic and pharmacologic synthetic lethal screens. Accomplishment of our project has the potential to lead to design of much needed novel therapies for patients with NF1-MPNST.

Proposal Title:	Interrogating Tumor-Immune-Neuron Cross Talk at Single-Cell Resolution in a Panel of Novel Somatic Transgenic NF1 Tumor Models
Log Number:	NF220040
Current PI Name:	Joshua Breunig
Award Number:	HT9425-23-1-0269
Current Contracting Organization:	Cedars-Sinai Medical Center
Current Performing Organization:	Cedars-Sinai Medical Center
Web Approval Date:	09-14-2023

We now know an incredible amount about the genetic mutations associated with NF1 glioma. However, there is an urgent need for representative genetically engineered preclinical disease models that reflect these combinations of mutations, due to a lack of human cell lines for NF1-associated glioma. In addition, mouse models provide the opportunity to interrogate the role of neuroimmune crosstalk in the pathogenesis of glioma. Inhibiting this crosstalk is one of the potential exciting therapeutic approaches for NF1 as preclinical studies have identified critical pathways involved in the neuroimmune regulation of Nf1 optic pathway glioma. Current mouse models of brain gliomas are a mix of mutations that are either not prevalent in NF1-associated cases, or do not reflect the most common mutation signature. Specifically, combined loss of the genes Cdkn2a, Trp53, and Atrx (or Fgf1 amplification in the case of low-grade tumors). In addition, none of these models incorporate the inherited Nf1 mutation. Our approach is to generate novel NF1 glioma (low-grade and high-grade) models using our new state-of-the-art genetic engineering approaches for tumor modeling in mice, by precisely targeting each of the above mutations and NF1 patient derived inherited Nf1 mutations simultaneously (Breunig and Gutmann laboratories). In addition, we will test whether the neuron-immune axis/molecules that drive disease progression in NF1 optic pathway gliomas is also required for the progression of NF1-gliomas in the brain (Breunig and Pan laboratories). Completion of the novel experiments outlined in this proposal will directly address the FY22 NFRP areas of emphasis: “Heterogeneity of NF-related tumors” and “Target identification, drug discovery.”

Benefits and Risks: Deriving new cancer models can be time-consuming and fraught with challenges. However, our published approach has demonstrated the ability to accurately model multiple tumor types—even when assessing cell-by-cell with human tumors. Further, we can mix and match additional mutations in the future to expand to the less common tumor signature mutations, allowing for precision patient disease avatars. We will thus use these mice as patient avatars for the ongoing clinical trial by employing the same therapeutics as are used in the trial. Using survival analysis, we will assess the predictive nature of these models for therapeutic testing. In addition, using single-cell approaches, we will directly compare the mouse and human tumors on a cell-by-cell basis to assess their fidelity in recapitulating human tumors.

By credentialing our models against current frontline and experimental therapies, we have designed our proposal to be of maximal benefit to patients with NF1. Specifically, if our models our predictive, they can be used to assess safety and efficacy of combinatorial approaches and novel therapeutic interventions (e.g., targeting the neuron-immune axis) that would take years to decades to similarly complete in the clinical trial setting—all while patients with NF1 glioma continue to succumb to the disease. Our proposed models can additionally be used to determine tumor-intrinsic and extrinsic mechanisms of gliomagenesis in NF1 and derive new targets for therapeutic intervention.

Last, our approach is relatively cheap, simple, and faster than the traditional engineered mouse models. We have previously made our tools available to the scientific community (e.g., through open access repositories

such as Addgene) and will continue to do so, multiplying the potential for impact in the fight against NF1. There are no clinical risks to patients, and risks to animals will be minimized by following federal and institutional regulations of rodent care.

Taken together, we hope that these preclinical studies will lead to a new generation of patient NF1 disease models and new targeted therapeutic interventions with minimal side effects for the family members of the military with NF1 and civilians afflicted with this devastating syndrome.

Proposal Title:	Interrogating Tumor-Immune-Neuron Cross Talk at Single-Cell Resolution in a Panel of Novel Somatic Transgenic NF1 Tumor Models
Log Number:	NF220040P1
Current PI Name:	Yuan Pan
Award Number:	HT9425-23-1-0270
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	09-14-2023

We now know an incredible amount about the genetic mutations associated with NF1 glioma. However, there is an urgent need for representative genetically engineered preclinical disease models that reflect these combinations of mutations, due to a lack of human cell lines for NF1-associated glioma. In addition, mouse models provide the opportunity to interrogate the role of neuroimmune crosstalk in the pathogenesis of glioma. Inhibiting this crosstalk is one of the potential exciting therapeutic approaches for NF1 as preclinical studies have identified critical pathways involved in the neuroimmune regulation of Nf1 optic pathway glioma. Current mouse models of brain gliomas are a mix of mutations that are either not prevalent in NF1-associated cases, or do not reflect the most common mutation signature. Specifically, combined loss of the genes Cdkn2a, Trp53, and Atrx (or Fgf1 amplification in the case of low-grade tumors). In addition, none of these models incorporate the inherited Nf1 mutation. Our approach is to generate novel NF1 glioma (low-grade and high-grade) models using our new state-of-the-art genetic engineering approaches for tumor modeling in mice, by precisely targeting each of the above mutations and NF1 patient derived inherited Nf1 mutations simultaneously (Breunig and Gutmann laboratories). In addition, we will test whether the neuron-immune axis/molecules that drive disease progression in NF1 optic pathway gliomas is also required for the progression of NF1-gliomas in the brain (Breunig and Pan laboratories). Completion of the novel experiments outlined in this proposal will directly address the FY22 NFRP areas of emphasis: “Heterogeneity of NF-related tumors” and “Target identification, drug discovery.”

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Proposal Title:	Therapeutic Potential of Ezrin Inhibition in NF2-Associated Schwannomas
Log Number:	NF220059
Current PI Name:	Jeremie Vitte
Award Number:	HT9425-23-1-0404
Current Contracting Organization:	California, University of, Los Angeles
Current Performing Organization:	California, University of, Los Angeles
Web Approval Date:	09-15-2023

This proposal takes advantage of the complementary expertise of Drs. Jeremie Vitte, Marco Giovannini, (UCLA) and collaborator Andrea McClatchey (MGH/Harvard). It addresses the areas of emphasis of the FY22 NFRP: “NF2-related areas” and “Target identification, drug discovery.”

The main characteristic of neurofibromatosis type 2 (NF2) is the development of schwannoma, tumors that form around the auditory and other cranial and spinal nerves. Depending on location and size, these tumors can cause a range of neurological symptoms including deafness, tinnitus, dizziness, pain, paralysis and even death due to brain compression. Current treatment of schwannomas is largely limited to surgical resection, which carries some risks such as cerebrospinal fluid leak, meningitis, bleeding, cerebral edema, and even death. This lack of therapeutic options reflects, at least in part, our limited knowledge of the biology explaining the development of schwannomas.

Because schwannomas grow slowly, they are difficult to treat. Standard chemotherapies targeting fast diving cells, that are used as cancer treatment, do not usually work for these tumors. NF2 schwannomas are characterized by loss of the merlin protein due to mutations in the NF2 gene in Schwann cells, cells that wrap around neurons in nerves. Optimally, a treatment for NF2 schwannoma would exploit features specific of NF2 schwannoma cells and eliminate the broad consequences of merlin loss. We discovered that blocking ezrin, a protein in the same family as merlin, fixed some of the issues caused by merlin loss in Schwann cells. Here, we propose testing the idea that blocking ezrin stops schwannoma development, and therefore could represent a new therapeutic target for NF2 tumors.

The goals of this 1-year proposal are to test this idea by evaluating the effect of combined merlin/ezrin loss in cells and in mice. A new mouse model will allow us to inactivate simultaneously merlin and ezrin, and study the consequences on tumor development by counting cells in the tumors. Schwann cells isolated from these mice will be used to evaluate the effect of merlin/ezrin loss on the NF2-specific features and proliferation. In the second part, we will use small molecules to block ezrin function in Schwann cells and mouse with merlin loss. In these multiple experiments, if cells grow slower or loose NF2 features and tumors are smaller after ezrin loss or blockage, our initial idea that ezrin can be a therapeutic target for NF2 tumors will be validated.

The successful completion of this work will provide the NF2 research and clinical community with outstanding new tools to explore blocking ezrin as a new possible treatment for NF2 schwannomas. We believe that these studies will also have a strong impact on the NF2 field in the long term with the potential to use this therapeutic approach for other NF2-related tumors or in conjunction with other treatments.

Proposal Title:	Neuron-Glial Interactions in Schwannoma Initiation and Heterogeneity
Log Number:	NF220061
Current PI Name:	Andrea McClatchey
Award Number:	HT9425-23-1-0380
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	09-15-2023

Schwannomas that form around the auditory and other cranial and spinal nerves are the hallmark of Neurofibromatosis type 2 (NF2) and related schwannomatoses. It is a mystery as to why they form around some nerves and not others when Schwann cells and their relatives surround all nerves of the peripheral nervous system. Moreover, mutation of the NF2 tumor suppressor gene underlies most if not all familial and sporadic schwannomas with few if any additional mutations, yet these tumors behave, look and respond to drugs in very different and inconsistent ways. Surgery is currently the primary treatment option and patients are often subject to multiple, high-risk surgeries as tumors inevitably recur. The few targeted therapies that have been developed have shown heterogeneous and limited efficacy. New ideas for developing therapies for schwannoma are desperately needed.

Studies of other types of malignant brain tumors have revealed that chemical signaling between neurons and the glial cells that they interact with is very important for tumor development and expansion. Our lab has recently begun to develop an “atlas” of schwannoma development in well-established and validated mouse models and human tumor tissue. These studies have taught us about how schwannomas initiate and develop and suggest that they form around specific neuronal subtypes, supporting the notion that chemical signals from nerves trigger schwannoma initiation and subsequent heterogeneous development. The studies proposed here will begin to test this completely new idea. Given the wealth of existing information about neuronal signaling and vast resources dedicated to building a pipeline of drugs that modify neuronal signals, these studies could rapidly open up completely new therapeutic opportunities for schwannoma patients. They could also help to explain and combat the chronic pain that is associated with many schwannomas. In the longer term, these studies can be expanded to include studies of other forms of familial schwannomatosis using available mouse models (Smarcb1-, Lztr- mutant) and human tissue.

Proposal Title:	Has the Tasmanian Devil Revealed a New Mechanism to Induce Regression of NF1 Tumors?
Log Number:	NF220063
Current PI Name:	Raymond Mattingly
Award Number:	HT9425-23-1-0958
Current Contracting Organization:	East Carolina University
Current Performing Organization:	East Carolina University
Web Approval Date:	09-15-2023

Spontaneous cancer regression is a very rare event that has intrigued physicians and patients for centuries. In the 20th century, medical and scientific communities often dismissed such cases as anecdotal and of little value to the design of patient care. More recently, this early work on cancer regression recognizes these studies as the beginnings of the very exciting fields of cancer immunology and immunotherapy. Despite this revaluation, spontaneous cancer regression is very difficult to study for several reasons. First, it is estimated to occur at only about 1 in 60,000 human cases. Second, spontaneous cancer regression is defined as that which occurs in the absence of any treatment that could plausibly contribute to a positive response. In cancers such as the malignant peripheral nerve sheath tumors (MPNSTs) that cause significant mortality in NF1 patients, current treatment options of surgery, chemotherapy and radiation produce some patient benefits even though they are not usually curative. Thus, it would not be ethical to design a study in which material is collected for study, no treatment is given, and then later it is hoped that regressed material would be available to define how any rare spontaneous regression occurred.

Tasmanian devils are the apex predator on the island of Tasmania, Australia. In 1996, a new and rapidly fatal disease was described in the wild population that caused a sharp decrease in numbers and forecasts that the species would be extinct within 50 years. The malady is characterized by large tumors that grow on the face of the animals (hence devil facial tumor disease, or DFTD) with local tissue invasion and, in the majority of cases, metastasis to distant organs. In addition to the spread within the animal itself, DFTD is transmitted through the population through biting behaviors that directly transfer cancer cells to a new host. Thus, a malignant cancer, which would normally be limited by the lifetime of the originating host, has escaped that restriction to become a plague upon the entire population.

Most animal diseases receive little investigation unless occurring in agriculturally important or companion species. DFTD is an exception due to its extremely unusual characteristics and potential for immense ecological damage through the predicted extinction event. Tour-de-force research by (1) trapping animals to sample their cancers and install tracking, (2) releasing back into the wild, and (3) later retrapping to record disease progression/stability/regression and resample their cancers has revealed that:

1. DFTD cancers are MPNSTs that arise from Schwann cells, i.e., DFTD cancers have a similar cell of origin as NF1 nerve sheath tumors.

2. Spontaneous regression occurred in fewer than 20 cases from 10,000 affected animals.

3. Spontaneous regression was driven by a single point mutation that turned on a gene called RASL11A.

4. DFTD cancers that lack RASL11A did not regress and continued to kill the animals

5. Expression of RASL11A in cells derived from non-regressed DFTD cancers was sufficient to reduce their proliferation in a cell culture model.

We recently completed a small pilot study of gene expression in human cutaneous neurofibroma (cNF) samples from NF1 patients in comparison to Schwann cells with normal neurofibromin expression. One striking result was the loss of RASL11A expression in all of the cNF samples tested. This is a novel finding for the NF1 field: there are no published reports linking RASL11A and NF1. Our long-term goal is to define targeted, effective, and safe therapeutic approaches for NF1. For this exploration-hypothesis development award, we hypothesize that expression of the RasL11A protein will block proliferation and induce death of human MPNST cells. Successful completion of this project will include animal work to test whether RASL11A is sufficient to induce MPNST regression. Positive results from this project could lead to future translation into the clinic. While genetic engineering to replace lost neurofibromin expression in NF1 patients remains an extremely complex and distant challenge, recent advances in gene-based therapies suggest that an approach to increase RASL11A expression might be attainable within 5-10 years.

Proposal Title:	Improving Hearing in NF2 Patients Who Use the Auditory Brainstem Implant (ABI)
Log Number:	NF220064
Current PI Name:	Daniel Lee
Award Number:	HT9425-23-1-0272
Current Contracting Organization:	Massachusetts Eye and Ear Infirmary
Current Performing Organization:	Massachusetts Eye and Ear Infirmary
Web Approval Date:	07-13-2023

Rationale: The overall goal of this 2022 NFRP research proposal is to enhance hearing in deaf patients from neurofibromatosis type 2 (NF2). The growth of bilateral vestibular schwannomas results in progressive and permanent hearing loss in almost all NF2 patients. The only option for providing hearing to deaf NF2 individuals when hearing aids or cochlear implants (CIs) are not helpful is the auditory brainstem implant (ABI). The FDA-approved the ABI in the U.S. almost two decades ago for patients with NF2. The ABI is inserted (usually during tumor surgery) through a craniotomy approach to provide hearing sensations. The surgeon must place the ABI blindly using surrounding brain landmarks and rely on live electrical recording feedback as the target of the ABI, called the “cochlear nucleus,” is not directly seen. Most NF2 ABI patients have sound awareness that improves lip-reading accuracy, but only rarely do they understand speech without any visual cues. In addition, NF2 ABI users often experience side effects, requiring the audiologist to turn off one or several channels on the device. This modest performance seen with the ABI can be explained by (a) tumor growth that damages the brainstem, (b) challenging nature of the surgery in which there are no direct ways to “see” that the ABI is in the correct location, (c) limitations in our ability to program ABIs, and (d) the rigid design of the clinical ABI that does not allow it to wrap around curved brain tissue.

Objectives/Aims: To solve these unmet needs, our work seeks to enhance NF2 ABI outcomes by:

Aim 1. Predicting the best auditory performance based on detailed ABI location seen on three-dimensional (3D) CT (a technique that our group developed), as well as on 3D MRI scans and routine X-ray views. We will apply this new and exciting composite imaging technique on NF2 patients from Massachusetts Eye and Ear as well as from several more clinics in the U.S. and abroad to improve our ability to see different trends in the data with a greater number of patients. In addition, we will be applying artificial intelligence techniques to these data to more effectively test our predictive model of NF2 ABI user performance.

Aim 2. Developing new ways to test NF2 ABI patients and improve programming techniques to enhance hearing. We will use existing and new approaches to assess hearing with the ABI and determine how these responses agree with the ideal positioning of the device as described in Aim 1.

Aim 3. Using a mouse model of NF2 to better understand how a new-generation soft ABI stimulates the cochlear nucleus and provides hearing compared to a mouse model that lacks brain tumors. There are a number of important questions about the ABI that we will answer in Aim 3, including (1) what nerves are activated with the ABI and where are they located and (2) what is the effect of tumor growth on ABI responses in a mouse with NF2 compared to a normal mouse that does not have brain tumors? Advancing our knowledge about how the ABI works will help inform how we program the device as defined in Aim 2, and will also influence future designs of flexible ABI technology for use in human.

Target population: NF2 patients who are candidates or users of the ABI

Clinical Application/Timeline: A major strength of our proposal is the immediate potential for clinical translation. The “ideal” position of the ABI defined in Aim 1 can readily be used during surgery to guide placement, especially given the availability of surgical CT navigation technology and portable X-rays in the operating room, including at our institution. Optimizing the testing and programming of the ABI seen in Aim 2 can also be translated to our existing NF2 ABI user population. In addition, though more long-term in perspective, the flexible electrode and novel biomaterials used in Aim 3 have already been extensively tested in animal models by our research team, and we will exploit the benefits of this soft design to answer basic questions about how the ABI provides hearing in NF2.

Overall Impact/Contribution: It is extremely disappointing to offer an NF2 patient who is deaf a hearing implant that provides modest benefits and little to no speech recognition, unlike the CI or a hearing aid. Despite these limitations, the ABI device and its surgical implantation and programming techniques have remained largely unchanged since its initial development 30 years ago. Our proposal brings together a multi-disciplinary, multi-institutional team to address this neglected area of NF2 research and improve hearing abilities and quality of life for deaf NF2 patients.

Proposal Title:	Development of a Gene Therapy Approach for the Treatment of Neurofibromatosis Type 2
Log Number:	NF220067
Current PI Name:	David Jung
Award Number:	HT9425-23-1-0284
Current Contracting Organization:	Massachusetts Eye and Ear Infirmary
Current Performing Organization:	Massachusetts Eye and Ear Infirmary
Web Approval Date:	07-12-2023

Neurofibromatosis type 2 (NF2) is a tumor predisposing disease caused by loss of the NF2 gene and is associated with multiple nervous system tumors, including vestibular schwannomas (VS) that arise from Schwann cells. These nervous system tumors lead to tinnitus, deafness, disequilibrium, and facial paralysis, along with presenile cataracts and peripheral nerve tumors. Brainstem compression leading to hydrocephalus, stroke and death are rare, but deafness and facial paralysis are common. Although NF2 is a rare disease (1:33,000), sporadic VS and meningiomas are also caused by pathogenic NF2 variants in 95% of sporadic VS and in 50% of sporadic meningiomas. Meningiomas are the most common intracranial tumors, with an incidence in the U.S. of 8.33 per 100,000 person-years. Sporadic VS are the sixth most common intracranial tumor at 1.09 per 100,000 patient years. Overall, the prevalence of these tumors resulted in 31,990 meningiomas and 4,100 VS in the U.S. in 2019. The quality of life of NF2 patients is similar to that of patients with cancer. Additionally, NF2 patients have higher levels of psychosocial stressors, disease-related anxiety, personal and financial stress, and lack of social support.

Current treatment strategies for NF2 involve surgery and stereotactic radiation (SR), both of which are associated with worsening functional performance. Furthermore, surgery and SR are aimed primarily at slowing disease progression, rather than halting or reversing the disease process. No FDA-approved chemotherapeutic agents exist for the treatment of NF2, although multiple agents are in clinical trials. Disease-associated changes of the NF2 gene are stable mutations that are suitable for correction with targeted gene therapy. Adeno-associated virus (AAV) viral vectors have helped usher in the age of gene therapy and are clinically approved for several indications, including a form of blindness and spinal muscular atrophy. Successful gene therapy would be liberating for NF2 patients, with prevention of schwannoma formation and preservation of critical hearing, balance, and cranial nerve function.

In this proposal, we plan to develop a novel AAV-based gene therapy to add the NF2 gene back to Schwann cells that have lost the NF2 gene and thereby treat the schwannomas and hearing loss that NF2 patients currently experience. We will test the idea that NF2 gene replacement, using our novel capsid-promoter combinations, will (1) cause regression of human schwannomas and (2) preserve hearing in mice lacking Nf2 in Schwann cells, in the context of an otherwise normal auditory and nervous system. Successful gene therapy would be liberating for NF2 patients, with prevention of schwannoma formation and preservation of critical hearing, balance, and cranial nerve function.

Proposal Title:	Gap Junction-Mediated Communication in Schwannoma Development
Log Number:	NF220069
Current PI Name:	Christine MacKenzie
Award Number:	HT9425-23-1-0541
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	09-15-2023

Schwannomas that form around the acoustic and other cranial and spinal nerves are the hallmark of neurofibromatosis type 2 (NF2) and related familial schwannomatosis. Both familial NF2 and sporadic schwannomas are caused by mutation of the NF2 tumor suppressor gene, with few cooperating mutations. Despite this genetic uniformity, tumor behavior and therapeutic response is remarkably heterogeneous.

Currently, surgical resection is the primary treatment option and patients are often subject to multiple, high-risk surgeries as tumors inevitably recur. As such, targeted pharmacological therapies for schwannomas are desperately needed, but few have been developed and most of those that have reached clinical trials have shown heterogeneous responses and limited efficacy.

Our recent studies of schwannoma development in a mouse model have revealed that the earliest lesions form adjacent to neuronal cell bodies in the ganglia of the peripheral nervous system and express markers of a type of Schwann cells that normally form a single-layered coat around these cell bodies. Our preliminary data showed that these lesions progressively express Cx43, a protein that forms gap junctions, a type of intercellular connection that enables the direct passage of small molecules between the cytoplasm of two cells. Gap junction mediated communication plays an important role in chronic pain in the peripheral nervous system and drives tumor growth and drug resistance in other types of tumors, particularly in the central nervous system. However, no one has investigated the role of gap junctions in schwannomas. The overarching goal is to test the hypothesis that cells in schwannomas are coupled by and functionally depend on gap junctions. We will test this hypothesis using cells and tissues from a well-established mouse model of schwannoma, and from human schwannomas, along with state-of-the-art imaging techniques.

The successful completion of these studies may uncover a completely new understanding of schwannoma biology and multiple new avenues of therapeutic possibility. The research proposed here has the potential to benefit schwannoma patients, especially those with NF2 or schwannomatosis, who are in desperate need of new therapeutic options. In particular, gap junction-mediated communication has been linked to chronic pain, an important non-tumor manifestation of schwannoma that could be targeted with gap junction blockers.

Substantial focus on developing targeted therapies for schwannoma based on studies of cells grown in 2D in non-physiological conditions has yielded disappointing results. We believe that focusing on the complex biology of schwannoma is a better approach and will reveal new therapeutic ideas. Gap junction proteins could represent important biomarkers for schwannomas or could be targeted using gap junction blockers, which are widely used in preclinical studies and are currently being tested in clinical trials. While the studies proposed here only scratch the surface of a potential role for gap junctions in schwannoma and more comprehensive studies to unravel the complex biological structure of gap junctions will be required, it is important to note that the gap junction blocker currently being clinically evaluated, MFA, is an FDA-approved drug that is licensed as a non-steroidal anti-inflammatory drug, potentially shortening the time to testing in patients.

Proposal Title:	CRISPRa-Based Strategies for NF1
Log Number:	NF220070
Current PI Name:	James Walker
Award Number:	HT9425-23-1-0490
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	09-15-2023

This project is proposed as a collaboration between the Walker Lab at the Center for Genomic Medicine, Massachusetts General Hospital, and Infixion Bioscience, a drug discovery startup focused on developing drugs to radically improve life for NF1 patients by correcting a known root cause of symptoms, namely the insufficient amount of normal (wild-type) NF1 protein.

NF1 Genetic Background: Like other human genes, the NF1 gene has both a paternal and maternal copy. For people who do not have an NF1 mutation, each NF1 gene copy (or allele) produces ~50% of the NF1 protein (neurofibromin) needed to be healthy. However, for people with NF1, one of these two NF1 alleles is abnormal, producing either defective or no protein. The result is much less wild-type neurofibromin protein in every cell of the body. NF1 is considered an “autosomal dominant” genetic disorder because this abnormality in just one gene allele is enough to cause NF1 symptoms. Biological theory suggests that neurofibromin from this mutant allele either directly causes NF1 symptoms (e.g., is pathogenic) or that the lack of wild-type protein is the root cause. The lack of a normal protein leading to disease is referred to as “haploinsufficiency.” The idea to keep in mind is that people with NF1 do produce wild-type NF1 protein, but their one normal gene simply doesn’t make enough.

Rationale for Approaching NF1 by Targeting Haploinsufficiency: Many scientific publications have provided solid evidence that most NF1 symptoms are either caused directly by NF1 protein haploinsufficiency (i.e., cognitive/social deficits), or that this haploinsufficient condition drives symptom progression (i.e., tumor progression). It’s also well accepted that NF1 haploinsufficiency leads to dysregulation of many biological pathways (i.e., Ras/Mapk, HCN1, CRMP2, etc.), further explaining the wide variety of symptoms experienced by people with NF1. By correcting the underlying NF1 protein deficiency, the possibility exists to improve a wide range of NF1 symptoms. Additional research into other autosomal dominant and haploinsufficient genetic disorders, such as Willams-Beuren Syndrome, Supravalvular Aortic Stenosis and Sim1 (Giordano, et al.; Matharu, et al.), has shown that upregulation of a single normal allele can lead to improving symptoms. More specifically, the ability to correct dysregulation by simply eliminating haploinsufficiency has been demonstrated at the cellular level for NF1 (Mellert, et al., Wallis, et al.). Finally, the mechanisms that regulate gene expression from the NF1 allele have been well characterized, with clear evidence that NF1 gene expression can be regulated by several biological agents and mechanisms, including cytokines, histone acetylation, DNA methylation, and peptide hormones. So the approach proposed here is absolutely feasible.

Research Goals: Upregulating expression of neurofibromin in a variety of tissues to correct the underlying root cause of NF1, rather than treating individual symptoms or downstream pathway, is predicted to impact patients possessing any of the over 4,000 unique NF1 mutations already identified. Given this background, this research proposes to develop and study a novel CRISPR-based reagent to increase neurofibromin expression from the wild-type NF1 allele, thus compensating for the lack of normal NF1 protein produced by the mutated allele, and then to test this new reagent in NF1 patient derived cells, and in a mouse model of NF1.

Project Research Aims: Using a cutting-edge technique (Gro-Seq), we will first study exactly where production of NF1 protein begins (aka. transcription start site). Using this information will use CRISPRa

/dCas9 (gene activating) technology, along with a proprietary reporter cell line from Infixion Bioscience, to identify the ideal locations in the NF1 gene promoter region that can be “activated” to increase NF1 gene expression and subsequent protein production. Finally, in Aim 3, we will validate the impact of these newly developed CRISPRa reagents on NF1 protein (and downstream pathways) by testing both in NF1 patient-derived induced pluripotent stem cells (iPSCs) that have been differentiated into neurons, and by injecting this CRISPRa reagent into the CNS (i.e. brains) of NF1 mice. Readouts from these experiments will include NF1 protein levels, as well as the impact increased NF1 protein levels have on several biological pathways that are known to be dysregulated by NF1 protein haploinsufficiency.

Future Goals: Once we can demonstrate the impact these CRISPRa synthetic transcription factors have at the cellular level, future studies would then begin to assess symptoms in NF1 heterozygous mice and/or mini-pigs, likely starting with their impact on the cognitive, learning and social deficits experienced by people with NF1.

Proposal Title:	Targeting the Novel TXNRD1-cGAS-SASP Axis to Eradicate Stemlike Cells in Epithelial Ovarian Cancer
Log Number:	OC220011
Current PI Name:	Xue Hao
Award Number:	HT9425-23-1-0729
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	09-28-2023

Areas of Emphasis Relevance: (1) Understand the basic biology of ovarian cancer progression and recurrence and (2) Develop novel therapeutic strategies for treatment and prevention.

Rationale and Objective: Epithelial ovarian cancer (EOC) is the most lethal gynecological cancer in the United States with only 30% of patients living for 5 years or more. The standard of care for EOC is platinum-based therapy. Although the majority of EOC patients respond to chemotherapy well initially, relapse ultimately occurs with resistance. Emerging evidence suggests that cancer stem-like cells (CSCs) contribute to chemotherapy resistance.

However, approaches to eliminate CSCs in EOC remain an unmet need. Platinum-based therapy is known to induce senescence in EOC. Senescence is tumor suppressive by halting the proliferation of cancer cells while secreting a number of proinflammatory and pro-growth factors known as the senescence-associated secretory phenotype (SASP). Increasing evidence support that senescent cells contribute to chemoresistance by inducing CSCs through the SASP. However, directly eliminating senescent cells is not ideal because senescent cells are important to normal tissue structure and function. Therefore, it would be an ideal strategy to overcome chemoresistance by selectively eliminating the detrimental SASP while maintaining the tumor-suppressive role of senescence-associated growth arrest. In the proposed studies, we will explore this possibility by focusing on a newly discovered pathway that centers on a protein called TXNRD1. Of note, small molecule inhibitors of TXNRD1 that can be applied to this approach are already available.

Our proposal consists of two aims. First, we will investigate the mechanism underlying the SASP regulation by TXNRD1 during platinum-induced senescence. Second, we will develop a novel approach to overcome platinum-induced resistance by eliminating stem-like cells through targeting TXNRD1. Taken together, the objective of the proposed studies is to develop a novel therapeutic strategy to eradicate the CSCs induced by platinum-based chemotherapy in EOC by inhibiting TXNRD1.

Impact and Ultimate Applicability: We anticipate that this work could benefit ovarian cancer patients by developing urgently needed strategies for therapy-induced relapse. The ideal outcome would be that a combination of platinum and inhibitors targeting the TXNRD1 could be utilized to eliminate the CSCs to overcome chemoresistance in EOC.

In the immediate short term, the proposed studies will provide fundamental mechanistic insights into the role of TXNRD1 in regulating platinum-associated therapy relapse in EOCs. This sets the stage to ultimately develop an effective approach to target this newly discovered pathway. In the long term, the completion of the proposed studies will provide a scientific rationale for developing novel therapeutic strategies for chemoresistance and relapse in EOC, which is essentially incurable at the moment. If successful, this research will have a transformative impact on the management of chemoresistant EOC, a major clinical challenge.

Proposal Title:	Piecing the Puzzle Together: Unraveling the Genomic Landscape of Low-Grade Serous Ovarian Carcinoma (LGSOC) to Guide Novel Therapeutic Strategies
Log Number:	OC220022
Current PI Name:	Kathleen Pishas
Award Number:	HT9425-23-1-0994
Current Contracting Organization:	Melbourne, University of
Current Performing Organization:	Melbourne, University of
Web Approval Date:	09-28-2023

Ovarian cancer is the most lethal gynecological cancer, with 14,070 women dying from disease in the USA alone in 2021. Our laboratory focuses on a rare type of ovarian cancer called low-grade serous ovarian carcinoma (LGSOC) that accounts for approximately 5% of all ovarian cancer cases. Unlike the other types of ovarian cancer, LGSOCs are diagnosed at a much earlier age (43.0 versus 62.6 years, respectively), and standard ovarian chemotherapy drugs do not work. After initial chemotherapy treatment, the cancer will return in 69% of patients, with the majority of women dying from disease within 5 years of diagnosis. Due to the rarity of this cancer, the lack of LGSOC tissue samples has impeded the cancer research communities' efforts to conduct research. Currently, three critical gaps in knowledge preventing us from significantly changing survival outcomes for these women include:

1. What changes in tumor DNA makes this specific type of ovarian cancer different from other ovarian cancer types in which chemotherapy works well?

2. How do LGSOC turn genes "on" and "off" to allow them to distract the immune surveillance system and spread from the ovary to other sites within the body?

3. Are there proteins present on the cell surface of LGSOC cancer cells that we can target with specialized drugs or new technologies such as vaccines or immunotherapy to effectively kill them?

Due to our international and national collaborations with other leading scientists in the ovarian cancer field, our laboratory has access to the largest group of LGSOC tissue and patient-derived cell line samples for research purposes (more than 200 samples) in the world. Using this critical resource we will conduct scientific experiments to address all three areas stated above. In particular, we will assess whether specific changes in the DNA of LGSOC tumors alters the ability of genes to carry specific genetic messages which gives cancer cells a survival advantage. For example, the ability to grow faster or be resistant to drugs that should kill cancer cells. Using a new technology, we will also investigate what specific proteins are present on the surface of LGSOC cancer cells and not normal cells (for example bone, ovary, and heart cells) which will allow us to design new drugs that have less toxic side effects. In addition, using specialized equipment, our laboratory is currently looking at whether 6,700 drugs can effectively kill LGSOC cancer cells. Together, by understanding the basic biology and underlying vulnerabilities of LGSOC cells, we can use this information to match drugs that target specific changes in the DNA and/or surface proteins that are unique to LGSOC cells. Our powerful study has the ability to significantly influence the survival outcomes for women diagnosed with LGSOC, and our team of leading ovarian doctors will ensure that our results will be translated to the clinic where patients can benefit.

The goal of our research is to provide shifts in treatment protocols for LGSOC women by providing new insights into what drives the cancerous nature of these devastating cancers. As such, our research aligns with the mission of the OCRP to support patient centered research to treat and cure ovarian cancer in all women impacted by this disease including active Service Members/Veterans. Interestingly ovarian cancer is the most predominant gynecological cancer diagnosed in U.S. active-duty women (46.4% of cases). Hence, our research efforts have large implications for all women diagnosed with ovarian cancer, including active-duty and Veteran Service Women. Also, our study has been designed to align with several OCRP Area's of Emphasis, including (i) understanding the basic biology of ovarian cancer, (ii) developing novel therapeutic strategies for the treatment of ovarian cancer, and (iii) improving precision medicine.

For the very first time, we have the ability to transform LGSOCs from frequently incurable into survivable – to give women a future free from cancer. In addition, the large amount of new data and insights generated by this groundbreaking proposal can be used by other ovarian cancer researchers to accelerate significant scientific progress for this understudied cancer.

Proposal Title:	Development of an Orally Available and Low-Toxic Chemotherapy for Improved Ovarian Cancer Therapy
Log Number:	OC220028
Current PI Name:	Wei Li
Award Number:	HT9425-23-1-0216
Current Contracting Organization:	Tennessee, University of, Health Science Center
Current Performing Organization:	Tennessee, University of, Health Science Center
Web Approval Date:	09-13-2023

Background: The overall survival (OS) rate of ovarian cancer (OC) patients diagnosed with metastatic OC remains dismal. Chemotherapeutic drugs, including Taxol® (paclitaxel) and Platinol® (cisplatin or carboplatin), are often used to treat OC patients. While they show initial anticancer activity, the clinical efficacy of these currently FDA-approved chemotherapeutic drugs are limited by several factors. First, these drugs are often actively pumped out of cancer cells by proteins known as efflux pumps. Upon prolonged treatment with these drugs, cancer cells will produce excessive levels of drug efflux pumps and the body will effectively clear these drugs before they can kill the cancer cells, leading to therapeutic resistance and disease progression. Escalating the dose of these chemotherapy drugs is not currently possible, because it leads to high toxicity. Second, the use of these drugs is frequently associated with neurotoxicity, including persistent peripheral neuropathy after therapy ends, particularly if used with other agents that are neurotoxic. Third, these drugs must be administered using the intravenous route (i.v.) by infusion, and cannot be taken orally. This could increase patient exposures to potential environmental infections (e.g., COVID-19 as an extreme example). It is especially important since existing chemotherapies often weaken cancer patients' immune systems and thus increases their risk of infection. Therefore, there is an urgent need to develop new oral therapies that are not susceptible to drug resistance mechanisms (i.e., drug efflux) and are less toxic in order to improve quality of life and to reduce the morbidity and mortality associated with metastatic OC.

Rationale, Objectives, and Aims: Our overarching objective is to design novel therapies to make metastatic OC a manageable, chronic disease ("no evidence of disease" [NED]). We discovered a new class of tubulin inhibitors represented by our investigational new drug VERU-111 (Sabizabulin) that can be taken orally (PO [per oral] once per day under current clinical trials) and effectively bypasses many of the known resistance mechanisms associated with the prolonged use of existing chemotherapy for OC. VERU-111 also shows substantially less neurotoxicity in preclinical mouse and dog studies and the ongoing clinical trials. Preliminary evaluation using VERU-111 in highly metastatic, orthotopic human models of OC in mice clearly demonstrated that it suppresses ovarian tumor growth and metastases. Currently, VERU-111 is in late-stage human clinical trials for breast cancer and prostate cancer. A phase 3 trial for hospitalized COVID-19 patients was stopped early due to overwhelming efficacy and an Emergence Use Approval (EUA) application was submitted to the FDA on June 7. We have also developed a new generation of VERU-111, represented by our best new compound SHIP-216, which showed improved efficacy and maintains the ability to circumventing drug resistance to existing chemotherapy. Our technical goals in this project are to rigorously de-risk SHIP-216 as a potential future clinical candidate and comprehensively evaluate both VERU-111 and SHIP-216 in chemotherapy-resistant, well-characterized preclinical mouse models that develop lethal metastatic OC disease. If successful, such preclinical data will allow our industry partner, Veru, Inc., who licensed these patent portfolios, to have solid foundations and optimal designs to initiate phase 2 trials in OC patients within the next 4 years, with the goal to provide a more efficacious therapy for metastatic and chemoresistant OC patients.

Overarching Challenges Addressed: To revolutionize treatment regimens by overcoming current clinical limitations by replacing the use of existing FDA approved tubulin inhibitor treatment regimens with a new

generation of orally available tubulin inhibitors that are more effective and less toxic and will increase overall survival (OS). A second challenge is to eliminate the mortality associated with metastatic ovarian cancer (OC).

Clinical Benefits and Projected Timing of Impact: Our research plan will result in comprehensive preclinical data of VERU-111 and its improved compound SHIP-216 in clinically relevant OC animal models. VERU-111 has successfully completed a phase 1b/2 clinical trial for metastatic prostate cancer (NCT03752099), a second phase 2 clinical trial for COVID-19 (NCT04388826), and a phase 3 trial for hospitalized COVID-19 patients (NCT04842747, 300 patients) as a result of its anti-inflammatory activities similar to that of colchicine (colchicine is the FDA-approved anti-inflammatory drug for gout). It is currently under FDA consideration for possible EUA approvals. A phase 3 trial for metastatic prostate cancer patients who failed first-line androgen depletion therapy (NCT04844749, VERACITY trial, 245 patients) and a phase 2 trial for taxane-resistant metastatic triple negative breast cancer with 156 patients are currently ongoing. Preclinical data resulted from this project will allow our committed industry partner (Veru, Inc.) to establish the confidence and optimal designs for initiating phase 2 clinical trials for OC patients with VERU-111. Due to the high attrition rate of early-stage drugs during trials and the potential development of acquired drug resistance, it is highly risky to rely on only one compound in a drug pipeline. Therefore, the proposed preclinical de-risk and development of SHIP-216 that shows great promises in overcoming drug resistance to existing chemotherapy as well as the anticipated drug resistance to VERU- 111, which will increase the likelihood of the clinical successes. Within 4 years (2026) or less, it is feasible that we will bring a safer and more effective tubulin inhibitor into the clinic to benefit patients with metastatic OC. Since tubulin inhibitors are widely used in other types of metastatic solid tumors, including prostate, breast, pancreatic, and lung cancers, project success could have a broader impact to improve the clinical outcomes for these cancer patients and to reduce toxicities.

Proposal Title:	Target Validation and Inhibition of RHNO1 Function in Ovarian Cancer
Log Number:	OC220035
Current PI Name:	Adam Karpf
Award Number:	HT9425-23-1-0238
Current Contracting Organization:	Nebraska, University of, Medical Center
Current Performing Organization:	Nebraska, University of, Medical Center
Web Approval Date:	05-18-2023

Rationale and Objective: DNA replication stress (RS) is a defect in the process that duplicates DNA. RS occurs at high levels in cancer cells and low levels in normal cells and makes cancer cells dependent on the cellular RS response. The RS response protects cancer cells from DNA damage and increases cancer cell survival. Thus, drugs that inhibit the RS response can be effective treatments for cancer in that they kill cancer cells but spare the normal cells of the body. Investigating new ways to inhibit the RS response in cancer cells is thus backed by a strong rationale.

RHNO1 is a protein that plays a role in the cellular response to RS. We showed that RHNO1 is greatly increased in high-grade serous ovarian cancer (HGSC) compared to normal tissues and is present in the vast majority of HGSC cases (~85%). In addition, we showed that RHNO1 promotes both the RS response and HGSC cell survival. The first objective of the proposed study is to determine whether RHNO1 promotes HGSC tumor growth in vivo, using mice. The second objective of the proposed study is to develop drugs that prevent RHNO1 from activating the cellular RS response. Such drugs have high potential as new therapeutics for the treatment of HGSC patients.

Critical Problem in Ovarian Cancer: HGSC is the most common and deadly subtype of ovarian cancer, leading to the deaths of ~15,000 women per year in the United States. A major clinical problem in HGSC is that, after completion of the initial therapy, many women experience disease recurrence, and a large proportion of the relapsed patients ultimately die from HGSC. This sobering fact emphasizes the critical need for improved therapies to treat women with recurrent HGSC. The proposed study directly addresses this critical problem by working to validate a new therapeutic target and by developing new drugs to treat HGSC. This is further supported by the fact that RHNO1 is overexpressed in chemoresistant, recurrent HGSC and that RHNO1 helps to mediate chemoresistance. Thus, drugs that target RHNO1 are anticipated to be helpful to patients with recurrent HGSC.

New Paradigms, Insights, Technologies, and Applications in Ovarian Cancer: This study will provide new insight into the mechanisms of HGSC by investigating a newly discovered contributor to the disease, the oncoprotein RHNO1. In addition, this study will investigate new treatments for HGSC by developing drugs to inhibit the function of RHNO1 in the cellular RS response, which will target cancer cells and spare normal cells.

FY22 OCRP Area of Emphasis: Develop novel therapeutic strategies for treatment and prevention.

Individuals Helped: The study has the potential to help most women with HGSC because RHNO1 overexpression is common in HGSC, occurring in ~85% of HGSC cases. In addition, increased RS is a defining characteristic of most HGSC cases.

Impact on the Health And Well-Being of Service Members, Veterans, Their Family Members, and All Women Impacted by Ovarian Cancer: By providing information about the biological causes of HGSC and

developing novel treatment approaches for HGSC, this study has the potential to benefit military Service Members, their families, and all other women who are diagnosed with HGSC.

Proposal Title:	Interferon Epsilon: A New Localized Immunotherapy for Ovarian Cancer
Log Number:	OC220041
Current PI Name:	Nicole Campbell
Award Number:	HT9425-23-1-0268
Current Contracting Organization:	Monash University
Current Performing Organization:	Monash University
Web Approval Date:	07-12-2023

High-grade serous ovarian carcinoma (HGSOC) is the most prevalent and deadly form of ovarian cancer. Most patients are diagnosed late in the course of disease development, with advanced disease that has spread beyond the original site into the peritoneal cavity. The standard treatment is surgery to remove most of the visible tumor plus chemotherapy, or targeted therapies known as PARP inhibitors, to kill residual tumor cells. However, most (90%) of those women receiving chemotherapy eventually develop resistance to the treatment, and PARP inhibitors are only effective for a subset of tumors. Therefore, survival rates for patients with HGSOC are very low, and it is important to develop new therapies that can treat this disease. A new type of cancer therapy is immunotherapies, which target the body's immune system to improve its ability to fight the tumor. Cancer immunotherapies have been very successful in the treatment of other types of cancer, but they have had limited success in ovarian cancer. This is in part because HGSOC tumors recruit and activate "immunosuppressive" cells that prevent anti-tumor immune cells from killing tumor cells, and promote tumor growth. Therefore, there is a critical need to develop new therapeutics which can reverse this immunosuppressive environment in HGSOC and improve patient survival.

Interferons (IFN) are a type of signaling protein, or cytokine, that can kill tumor cells and activate anti-tumor immune responses. We discovered a new IFN, called IFN epsilon, which is found in the female reproductive tract where it protects against infection. In a previous project, funded by a U.S. DOD Pilot Award, we performed studies in mice and found that IFN epsilon also protects against ovarian cancer. Since then, we have performed follow-up mouse studies that revealed that IFN epsilon controls ovarian cancer progression through its activities on immune cells, not tumor cells. Importantly, IFN epsilon significantly reduced the numbers and activation of immunosuppressive immune cells in the peritoneal cavity and activated anti-tumor immune cells. Based on these exciting and very promising results, we are now well placed to begin a new study to understand which immune cells are important to the anti-tumor activity of IFN epsilon and to test whether it is effective on human cells. As the discoverers of this protein, we are in a unique position to fully study its role on immune cells in ovarian cancer and determine whether we can use it as a new therapy in the future.

In this project, we plan to characterize the immune response to IFN epsilon in the peritoneal cavity and determine which cell types are the main "targets" of IFN epsilon in ovarian cancer. These studies will be performed in mice, which will allow us to experiment with different immune cell populations in ways that are not possible in humans. We will complement our mouse studies through analysis of IFN epsilon activity on human peritoneal immune and tumor cells that are isolated from ascites fluids from ovarian cancer patients; these ascites are ordinarily drained to reduce patient discomfort and are otherwise discarded. We will also create a new mouse model of ovarian cancer that allows us to test the effects of IFN epsilon therapy on human peritoneal cells. In our studies, we will use powerful technologies that allow us to measure the expression of different genes and proteins at the level of a single cell, allowing us to compare the responses to IFN epsilon observed in mouse and human cells. This research will generate new knowledge that will increase our understanding of how IFN epsilon works to protect against ovarian cancer and provide important preclinical data to support "first-in-human" clinical trials, ultimately enabling development of IFN

epsilon as a new immunotherapy for ovarian cancer. We will also make all of our data and new mouse model publicly available to other researchers to support their work in identifying the causes and potential treatments for ovarian cancer.

This proposal is relevant to the OCRP's vision to eliminate ovarian cancer through addressing a major limiting factor in successful treatment of this disease: immunosuppression in the peritoneal cavity. In particular, our research addresses multiple FY22 OCRP Areas of Emphasis, namely, to develop novel therapeutic strategies for the treatment of ovarian cancer and to increase understanding into the basic biology and etiology of ovarian cancer initiation, progression, metastasis, and recurrence, particularly as to how these events relate to the peritoneal immune system. This year in the U.S., 19,880 women will be diagnosed with ovarian cancer, and 12,810 women will die of this deadly disease; this includes U.S. Service members, Veterans, and their family members in ever-increasing numbers as more and more women take up military careers. Successful completion of this research will impact all women diagnosed with ovarian cancer through progressing development of a new immunotherapy with potential to revolutionize patient care for this disease, thereby improving survivorship and quality of life and taking us a step closer towards eliminating ovarian cancer.

Proposal Title:	Epigenetic Reprogramming to Target Senescent Ovarian Cancer Cells and Overcome Therapeutic Resistance
Log Number:	OC220042
Current PI Name:	Katherine Aird
Award Number:	HT9425-23-1-0436
Current Contracting Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Current Performing Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Web Approval Date:	09-13-2023

Scientific Rationale and Objectives: Ovarian cancer is the deadliest gynecological cancer with over 19,880 new diagnoses and 12,810 deaths in 2022 alone. The standard-of-care therapies for ovarian cancer are platinum-based drugs and poly(ADP-ribose) polymerase (PARP) inhibitors. Resistance to both therapies remains a major clinical issue. Therefore, new therapies are needed to discover a long-term cure for these women. This proposal seeks to address this critical problem in ovarian cancer.

Studies have shown that, if ovarian cancer cells are not killed by therapy, they may undergo a process called “cellular senescence.” Cellular senescence has both good and bad effects on tumor progression, and recent work has indicated that decreasing the bad effects of senescence can alleviate therapy resistance. Two of the most important bad parts of senescence are (1) increased inflammation and (2) increased anti-cell death signaling. We discovered that a protein called DOT1L is increased in senescent ovarian cancer cells after therapy treatment, and high DOT1L activity subsequently increases both the inflammation and anti-cell death signaling. Therefore, inhibition of DOT1L could be a one-two punch to fully eliminate these bad effects of senescence and overcome therapy resistance. In this proposal, we aim to determine how to kill the resistant ovarian cancer cells using inhibition of DOT1L that hits two resistance mechanisms, namely increased inflammation and anti-cell death signaling. The DOT1L inhibitor pinometostat is currently in clinical trials for acute myeloid leukemia, providing the opportunity to repurpose pinometostat for ovarian cancer.

Areas of Emphasis: This proposal is directly related to (1) understanding the basic biology of ovarian cancer progression and response to therapy and (2) developing novel therapeutic strategies for treatment. The proposed studies are highly relevant to the vision and mission of the OCRP. These studies will study resistance of ovarian cancer, one of the ongoing missions of the OCRP, in disease-relevant ovarian cancer models. The proposed animal studies will identify whether inhibition of DOT1L after treatment with either platinum-based or PARP inhibitor therapy leads to a durable, long-term cure for therapy-resistant ovarian cancer.

What Individuals Will This Research Help? These studies have the potential to help ovarian cancer patients with initial or acquired resistance to therapy, which is the majority of women. While the current studies focus on high-grade serous ovarian cancer, future work based on these studies will determine the applicability of the combination of clinically relevant DOT1L inhibitors after standard-of-care treatment in all subtypes of ovarian cancer.

Clinical Applications, Benefits, and Risks: The potential clinical applications are implementation of a clinically relevant DOT1L inhibitor after treatment of ovarian cancer patients with either platinum-based on PARP inhibitor therapies. DOT1L inhibitors are already being tested in phase 1/2 clinical trials. If successful, the proposed studies could provide proof of principle to use these DOT1L inhibitors for ovarian cancer. Preclinical studies like the ones proposed here will be critical towards minimizing the risk (for instance, side effects) of the drug in solid tumor models. Overall, these studies have the potential to lead to

an investigator-initiated clinical trial of clinically relevant DOT1L inhibitors after treatment with standard-of-care therapy.

Ultimate Applicability and Impact for Military Service Members, Their Families, and Other Military Beneficiaries: The physical and mental cost of ovarian cancer on military health is clear. A staggering number of female Service Members and wives and adult daughters of active-duty military will be diagnosed with ovarian cancer (~11,900 of 850,000 women). Additionally, women continue to die, with a very grim ~30% 5-year survival rate for advanced-stage patients. Therefore, the proposed research to inhibit DOT1L in ovarian cancers treated with either platinum-based or PARP inhibitor therapies to overcome drug resistance may have a potential significant impact on the welfare of military Service Members, their Families, and other beneficiaries.

Impact on Ovarian Cancer Field and Patient Care: This innovative study will address both an important basic science question in the ovarian cancer field and a critical issue in the treatment of ovarian cancer patients. The newly discovered pathway of DOT1L's role in promoting two important resistance mechanisms will lead to a new understanding of cellular senescence in ovarian cancer cells and the impact of this on therapeutic response. Therefore, these studies will have an impact on the ovarian cancer field. We anticipate that this work could ultimately benefit all ovarian cancer patients, regardless of BRCA or HR status. DOT1L inhibitors are currently in phase 1/2 clinical trials for acute myeloid leukemia. Therefore, if successful, the proposed studies could lead to an investigator-initiated clinical trial for ovarian cancer patients. This would lead to a new paradigm for treating therapy-resistant ovarian cancer patients.

Proposal Title:	Replication-Driven Vulnerabilities in Cyclin E-Overexpressing Ovarian Cancers
Log Number:	OC220059
Current PI Name:	Priyanka Verma
Award Number:	HT9425-23-1-0133
Current Contracting Organization:	Washington University in St Louis
Current Performing Organization:	Washington University in St Louis
Web Approval Date:	07-11-2023

Hypothesis and Rationale: A major subset of highly deadly ovarian cancers is associated with an increase in the amount of a protein called cyclin E1. Ovarian cancer patients with a high abundance of cyclin E1 show poor overall survival and have no curative treatment options. Therefore, there is a critical clinical need for new therapies to treat these ovarian cancers. We are developing ways to specifically target cyclin E1-high ovarian cancer cells, while leaving normal cells unaffected.

DNA replication is important for cell survival. Our results suggest that, unlike normal cells, cancer cells with more cyclin E1 protein face multiple problems during DNA replication. These observations suggest that cancers with high cyclin E1 expression should be more dependent on repair pathways that allow them to fix problems during DNA replication. Therefore, inhibiting these pathways will selectively kill cyclin E1-high cancer cells. In this grant, we will identify (1) how cyclin E-high cancers deal with problems in DNA replication and manage to survive and (2) why cyclin E1-high cancers do not respond to chemotherapies. These efforts will help address key knowledge gaps on the etiology and chemotherapeutic response of CyclinE1-high ovarian cancers.

Principal Investigator's (PI) Career Goal in Ovarian Cancer: As an independent investigator, I am committed to leveraging my postdoctoral training at the interface of DNA repair biology and BRCA-mutant ovarian cancers to expand the scope of my research to other ovarian cancers subtypes that are highly lethal and have no defined cure. I therefore chose to study cyclin E1-high ovarian cancers. DNA damage has an integral role in the etiology of this ovarian cancer, and hence my training and expertise position me well to pursue the proposed research question. To catalyze my efforts in developing clinically meaningful research, I chose my mentor to be Dr. Dineo Khabele, who has extensive translation and clinical research experience with cyclin E1-high gynecological malignancy. Furthermore, I aim to position myself at the forefront of ovarian cancer research by (1) attending conferences that link DNA repair to cancer etiology and therapy, (2) attending workshops on the use of cutting-edge tools to study ovarian cancers, (3) developing fruitful collaborations with researcher having complementary expertise (e.g., in vivo mouse modeling, medical geneticist), and (4) actively engaging with the OCA community.

PI's participation in and contribution to the growth of the OCA. I strongly believe that a highly collaborative environment that enables the exchange of expertise and resources creates the strongest foundation for breakthrough discoveries. By actively participating in the monthly webinars and annual workshops organized by OCA, I will engage with leaders and peers in the field of ovarian cancer where our complementary expertise can catalyze seminal discoveries for the prevention, diagnosis, and cure of this deadly disease. I also look forward to contributing to the review of grant applications and engaging with patient advocates, with the ultimate goal of supporting the OCRP mission toward developing innovative platforms to combat ovarian cancer.

Ultimate Applicability of the Research:

Type of Patients. Ovarian cancer patients with amplification or copy number gain in gene CCNE1, which encodes for cyclin E1 protein. This includes 20-30% of all cases with high- grade serous ovarian carcinoma, the most deadly and common ovarian cancer. Potential clinical applications, benefits, and risks.

Applications and Benefits:

1. Identification of new therapeutic targets that selectively kill ovarian cancer cells without harming normal healthy cells.

2. Approaches to mitigate cancer progression.

3. Development of a biomarker platform to predict responses to therapy.

Risks: Our initial studies are based on patient-derived cell line models of ovarian cancer. Existing literature provides strong support that most findings from cell line models hold true in-patient tumors. However, to fully establish that our findings are valid in clinic settings, we will rigorously validate our findings in patient tumors. My mentor is a practicing gyno-oncologist and her guidance and collaboration will be imperative for the clinical studies.

Timeline for Patient Outcome:

Short-Term: A biomarker platform to predict chemotherapy response in patients can be achieved within 5-6 years.

Long-Term: While our proposed studies will establish relevant drug targets, pharmaceutical interventions and assessment by clinical trials will add another 5-7 years before realizing the benefits of our discoveries in patients. However, this effort also represents one of the most exciting aspects of the proposal given the paucity of targeted therapeutic strategies for cyclin-E1 high ovarian cancers.

Advancement in Knowledge: It remains enigmatic (1) how cyclin E1-high cancers continue to divide despite facing multiple problems during DNA replication and (2) why these cancers do not respond to multiple types of chemotherapies. Our mechanistic studies will reveal how cancer cells can endure challenges during replication and develop resistance to therapies. These advancements are vital to developing innovative strategies to combat ovarian cancers.

Impact: Our results will identify new ways to prevent and treat the highly aggressive and one of the most chemo-resistant forms of ovarian cancers. Therefore, our studies will profoundly benefit the high- risk group of women with CCNE1-amplification who currently have little to no preventive or curative options.

Proposal Title:	Combination Intraperitoneal Local Delivery of PARPi Implants and Anti-PDL1
Log Number:	OC220064
Current PI Name:	Needa Brown
Award Number:	HT9425-23-1-0274
Current Contracting Organization:	Northeastern University
Current Performing Organization:	Northeastern University
Web Approval Date:	07-12-2023

More than 85% of OC patients relapse with a higher-grade, often metastatic disease with limited treatment options. While each case is expected to present a very different disease profile, there is emerging evidence that specific genetic mutations can render a large subset of these tumors more sensitive to treatment. The most commonly known are the BRCA1 and BRCA2 genes, which normally help repair damaged DNA or kill cells when the DNA cannot be repaired. Mutation or inactivation of these genes results in uncontrolled DNA repair and continued survival of cancer cells. Thus, inhibition of DNA repair through the use of drugs that block PARP activity promotes cancer cell death. The goal of the proposed work is to develop a novel treatment for high grade serous metastatic ovarian cancer that will target the DNA repair pathway in combination with immunomodulation, lower systemic toxicity, and synergization.

The PARP inhibitor (PARPi) Talazoparib (TLZ) that blocks DNA repair has proven antitumor activity in patients with both inherited BRCA1/2 mutations and non-inherited inactivation of BRCA1/2 genes. TLZ has been approved for clinical use in breast cancer and is being tested in clinical trials for many other cancers, including ovarian cancer. TLZ has shown to be 100-fold more potent at PARP trapping leading to stronger synthetic lethality and induces DNA damage and STING modulation in ovarian cancers regardless of BRCAness, thus expanding the scope of TLZ PARPi beyond BRCA-patients. However, up to 80% of patients suffer significant side effects associated with PARPi systemic administration, including myelosuppression, nausea, and fatigue, forcing a reduction in the dose below the clinically therapeutic level. To overcome this toxicity, my team has prepared a sustained delivery depot of TLZ called InCeT-TLZ and showed excellent therapeutic efficacy with minimal toxicity for treating established tumors in metastatic ovarian cancer (mOC) and breast cancer models. The proposed InCeT-TLZ will be able to deliver TLZ released over several days directly to the peritoneal cavity to treat mOC rather than distributing systemically.

PARPi resistance is a key manifestation in patients, often constraining treatment efficacy. Recent clinical trials highlighted the increased potential for overlapping toxicities of myelosuppression when combining systemic PARPi and immunomodulators or small molecule inhibitors. The highly toxic nature of systemic PARPi limits its potential for therapeutic efficacy as a first-line monotherapy and a combinatorial drug. With the use of InCeT- TLZ we avoid systemic toxicity and can evaluate the combinatorial approach of a dual innate and adaptive modulatory nanoformulation (NanoSTING-PDL1). This formulation combines a STING agonist liposomal formulation with anti-PDL1 targeting as a multi-pronged approach. The proposed study seeks to develop effective combinations of sustained release InCeT-TLZ with immunomodulatory factors, anti-PDL1 and STING agonist, to synergize and minimize systemic toxicity in a patient-mimicking, peritoneal, metastatic, high-grade serous ovarian cancer model.

Our central hypothesis is that InCeT-TLZ administered as a sustained release implant intraperitoneally will minimize toxicities to non-target organs, improve drug bioavailability, delay or suppress tumor progression, and allow for safer combinatorial treatment options. The InCeT-TLZ implants has several innovative features that allow for a tailored release profile to accommodate different disease stratifications, can be modulated to include other drugs or used in combination with conventional therapy, and can treat the

metastatic sites locally while avoiding systemic toxicity. Additionally, to our knowledge, the approach to administer a nanoformulation of NanoSTING-PDL1 combining the immune modulation of the innate and adaptive system in one delivery is unique. The delivery of a nanoformulation approach allows for targeting of both aspects of the immune system as well as tumor specific delivery of a higher payload compared to free drug. The successful outcome of this proposal will expand the scope of TLZ PARPi for local delivery and improve combination therapy efficacy with reduced adverse toxicity in high-grade serous mOC patients who have limited treatment options.

Proposal Title:	Targeted Therapeutic Opportunities for Ovarian Clear Cell, Small Cell, and Endometrioid Carcinomas
Log Number:	OC220066
Current PI Name:	Christina Woo
Award Number:	HT9425-23-1-0263
Current Contracting Organization:	Harvard College, President & Fellows of
Current Performing Organization:	Harvard College, President & Fellows of
Web Approval Date:	07-31-2023

Ovarian cancer is one of the most lethal gynecological malignancy in the United States. Currently, at least 85% of ovarian cancer patients do not have viable targeted therapy options. Some of these patients are subject to highly aggressive ovarian cancer subtypes, including clear cell, small cell, and endometrioid carcinomas. These carcinomas can be characterized in part by damage to tumor suppressor genes ARID1A or related members of the SWI/SNF chromatin remodeling complex, which results in dysregulation and promotion of oncogenesis. Mutations in these genes may also present tumor vulnerabilities that can be exploited by the development of new therapeutic strategies. The central problem our proposal addresses is the development of novel therapeutic strategies to treat ovarian cancer patients who are in critical need of a targeted therapeutic strategy to mitigate disease burden.

Here, we will explore an exciting small molecule therapeutic approach, termed a degrader, for their ability to exploit specific biomarkers as tumor vulnerabilities to provide a targeted therapeutic strategy for ovarian clear cell, small cell, and endometrioid carcinomas. Degraders act by controlled removal of a desired protein target, many of which are typically otherwise considered “undruggable” by more traditional inhibition mechanisms. Degradation of a protein can have stronger biological outcomes than more traditional inhibition of the protein, as removal of the protein is often catalytic and the degradation event is irreversible. We have developed a class of dual degraders that target two proteins: casein kinase 1 alpha (CK1) and IKZF2. Degradation of these therapeutic targets can promote selective and desirable apoptosis and differentiation in cancer cells. Our prior efforts have shown that these compounds work well to mitigate a form of blood cancer, acute myeloid leukemia. In the course of our studies, we found that ovarian cancer cell lineages are also particularly sensitive to our therapeutic targets and that sensitivity in these cell lines correlated strongly with the presence of a damaging mutation to ARID1A. We therefore hypothesized that ovarian cancer lineages with damaging mutations to tumor suppressor genes like ARID1A or the related SWI/SNF chromatin remodeling complex would be particularly sensitive to a targeted therapeutic strategy that promotes apoptosis and differentiation.

We will test this hypothesis by systematically establishing a connection between the mechanism of action of our small molecule degraders in promoting apoptosis to damaging mutations in tumor suppressor genes in ovarian cancer. First, we will test specific ovarian cancer subtypes for sensitivity to our therapeutic strategy, followed by evaluation of CK1 and downstream apoptosis as a primary driver of that sensitivity. Second, we will use the existence of a damaging mutation to a tumor suppressor gene, like ARID1A, to predict the efficacy of our degraders and evaluate the mechanistic drivers that promote sensitivity in the best model systems. These studies will enable us to conclusively connect specific biomarkers to stratify patient populations who would respond to our therapeutic target in the long-term. Finally, we will undertake rigorous efforts to develop new small molecule degraders with superior biological and chemical properties in order to bring these compounds to the clinic. In sum, these aims will provide a critical connection between a biomarker of relatively untreatable ovarian cancer subtypes to a therapeutic target that is accessed through a small molecule degrader.

The successful outcome of these studies will benefit ovarian cancer patients by providing them with new targeted therapy options that are currently not available for these patient populations. In the short term, we will identify a novel targeted therapeutic strategy for ovarian small cell, clear cell, and endometrioid carcinomas using models of these tumor types. In the long term, these studies may lead to the development of new degrader modalities to treat patients in the clinic and open new avenues for related efforts to target these tumor vulnerabilities beyond a small molecule degrader mechanism. The proposed research therefore will have wide-ranging impact on the health and well-being of Service Members, and women more broadly, who have been afflicted with ovarian cancer, who will have increasing access to targeted treatment options in the clinic.

Proposal Title:	The Role of Distress-Related Metabolic Dysfunction in Ovarian Cancer Development
Log Number:	OC220079
Current PI Name:	Shelley Tworoger
Award Number:	HT9425-23-1-0231
Current Contracting Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Current Performing Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Web Approval Date:	07-31-2023

Ovarian cancer is the fifth leading cause of cancer death for U.S. women and the seventh most deadly cancer worldwide. Preventing ovarian cancer is challenging because, among women without genetic mutations that increase risk, it is difficult to identify women at high-risk of developing ovarian cancer. Also, although some risk factors are known, such as never giving birth and later age at menopause, they are not easy to modify. Therefore, an important area of research is discovering new risk factors that can be used to identify high-risk women so that interventions can be targeted to them. Growing evidence suggests long-term stress and distress (for example, depression and anxiety) cause changes in the body, such as increasing inflammation and suppressing the immune system, that make it more likely that a growing ovarian tumor escapes the body' s ability to eliminate it at an early stage. In our research, we found that women with depression or anxiety could be distinguished from women without depression or anxiety by the levels of certain chemicals in the blood, called metabolites. The metabolites that differed between women with and without distress also were associated with risk of ovarian cancer. We combined the various metabolites into a score, called the Metabolic Distress Score (MDS), so that we can conduct research to better understand how distress influences development of ovarian cancer. This is a potentially impactful area of research since many women have conditions of distress and interventions can be given to reduce distress.

The central questions our study will address are (1) do women with more distress-related metabolites in their blood have a higher risk of being diagnosed with ovarian cancer than women with fewer distress-related metabolites? and (2) are distress-related changes in metabolites associated with characteristics in the ovarian tumor, such as markers of inflammation and poor immune function? For both study questions, we will explore whether the timing of when the MDS was measured before ovarian cancer diagnosis is important; this will provide clues into the stage of cancer development most impacted by distress. We will address the first question using data from 560 women with ovarian cancer who provided blood samples before they were diagnosed matched to 560 women without ovarian cancer who also provided blood samples. We will address the second question using data from a subset of women with ovarian cancer with both pre-diagnostic blood samples and ovarian tumor tissue. These research questions address two FY22 OCRP Areas of Emphasis: (1) "Understand the basic biology and etiology of ovarian cancer initiation, progression, metastasis, recurrence, genetics and other critical events," and (2) "Identify and develop new strategies for screening, early-stage detection, prevention, accurate diagnosis and prognosis."

This research project is relevant to many women since conditions of distress, such as PTSD and depression, frequently develop after trauma, and trauma is a common experience among women. Exposure to trauma is particularly common among U.S. women Veterans, due to war zone exposure, combat-related injuries, military sexual trauma, and traumas experienced before enlistment. Also, over half of U.S. adult civilian women will have exposure to trauma during their lifetime. Understanding the ways distress impacts inflammation in the ovarian tumor and the body's immune response to the tumor could lead to discovery of new targets for prevention and treatment that will be relevant for many women. Our findings will also lay the groundwork for future studies to examine whether common medications that impact metabolites, such as aspirin and statins, may help reduce risk of developing ovarian cancer among women with conditions of

distress. Other types of interventions that can lower distress and distress-related metabolites, such as meditation, exercise, and healthy diet, could also be explored. Such interventions are low-cost and have few side effects.

If we confirm that distress-related metabolites (a biologic “signature” of distress) increase ovarian cancer risk, this could lead to several long-term outcomes: (1) better ability to predict which women are at high-risk who may benefit from surgery to remove their ovaries and/or fallopian tubes; (2) better understanding of the ways distress impacts chemicals in the body and contributes to ovarian tumor development; and (3) identification of chemicals in the blood that could be targeted by medications to reduce the risk of ovarian cancer development among women experiencing long-term stress and distress. Our research on tumor characteristics related to the MDS will point toward targets for treatments to reduce inflammation in the tumor and improve the immune system’s ability to fight the tumor.

Proposal Title:	The Role of Distress-Related Metabolic Dysfunction in Ovarian Cancer Development
Log Number:	OC220079P1
Current PI Name:	Oana Zeleznik
Award Number:	HT9425-23-1-0236
Current Contracting Organization:	Brigham and Women's Hospital, Inc.
Current Performing Organization:	Brigham and Women's Hospital, Inc.
Web Approval Date:	07-31-2023

Ovarian cancer is the fifth leading cause of cancer death for U.S. women and the seventh most deadly cancer worldwide. Preventing ovarian cancer is challenging because, among women without genetic mutations that increase risk, it is difficult to identify women at high-risk of developing ovarian cancer. Also, although some risk factors are known, such as never giving birth and later age at menopause, they are not easy to modify. Therefore, an important area of research is discovering new risk factors that can be used to identify high-risk women so that interventions can be targeted to them. Growing evidence suggests long-term stress and distress (for example, depression and anxiety) cause changes in the body, such as increasing inflammation and suppressing the immune system, that make it more likely that a growing ovarian tumor escapes the body' s ability to eliminate it at an early stage. In our research, we found that women with depression or anxiety could be distinguished from women without depression or anxiety by the levels of certain chemicals in the blood, called metabolites. The metabolites that differed between women with and without distress also were associated with risk of ovarian cancer. We combined the various metabolites into a score, called the Metabolic Distress Score (MDS), so that we can conduct research to better understand how distress influences development of ovarian cancer. This is a potentially impactful area of research since many women have conditions of distress and interventions can be given to reduce distress.

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distress. Other types of interventions that can lower distress and distress-related metabolites, such as meditation, exercise, and healthy diet, could also be explored. Such interventions are low-cost and have few side effects.

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Proposal Title:	The Ovarian Cancer Observatory: Prevention, Impact, and Learning from Opportunistic Salpingectomy
Log Number:	OC220082
Current PI Name:	David Huntsman
Award Number:	HT9425-23-1-0956
Current Contracting Organization:	Provincial Health Services Authority
Current Performing Organization:	Provincial Health Services Authority
Web Approval Date:	09-13-2023

The vast majority of ovarian cancers are epithelial in origin. These are not a single disease, but rather five distinct diseases (referred to as histotypes). These five histotypes, in order of frequency, are high-grade serous (HGSC), endometrioid (ENOC), clear cell (CCOC), low-grade serous (LGSC), and mucinous ovarian cancer. The ovary is frequently the site of the dominant tumor mass when these cancers present, which led to the belief that ovarian cancer arises from transformation of the ovarian surface cells. However, in the past 20 years, it has become apparent that many HGSCs (the most common and lethal histotype) arise in the fallopian tube. The origin of each histotype of ovarian cancer remains an area of active debate and is important to understand to inform prevention, diagnostics, and treatment of each histotype.

The recognition that the fallopian tube is where many HGSCs originate has led to an important prevention strategy, removal of the fallopian tubes during other pelvic surgeries. Opportunistic salpingectomy (OS) collectively refers to the removal of fallopian tubes (salpingectomy) during the time of other common surgical procedures (opportunistic), in particular at hysterectomy (removal of the uterus) or instead of tubal ligation (tying tubes) for sterilization. OS is now recommended practice in at least nine different countries.

Research has shown that it is safe, feasible, and cost-effective. Recent data from British Columbia (BC), Canada, has also shown that it is likely effective in preventing HGSCs. There has not been a single case of HGSC following OS in BC, where it has been commonly performed since 2008. This is significantly fewer than were expected if cancers were arising at the same rate in the OS group as in the control group (people who had hysterectomy alone or tubal ligation), where there were 15 cases of HGSC.

While no evidence to date has directly addressed whether OS reduces risk for ENOC and CCOC (the two next most common histotypes), we hypothesize that it will be at least partially effective in preventing many of these cancers as well. This is because these forms of cancer are believed to originate in the uterus and reach the ovary through the fallopian tube, which is supported by evidence showing that tubal ligation (tying tubes) decreases ENOC risk by 52% and CCOC risk by 48%.

Herein, we propose to create the Ovarian Cancer Observatory, which will track the impact of OS and use this unique opportunity to better understand the origins of ovarian cancer. We will do this by using large, population-based datasets, as well as by generating new case-based data through a broad-based international collaboration to answer important questions about the etiology of different histotypes of ovarian cancer. We will examine whether ovarian cancers differ qualitatively when they arise following fallopian tube removal than when they arise in people with fallopian tubes. We hypothesize that OS will preferentially prevent specific histotypes of ovarian cancer (i.e., those that arise or travel through the fallopian tubes). We also hypothesize that HGSCs that arise following OS will be genomically and molecularly distinct from those that arise in people with intact fallopian tubes. To test this hypothesis, we propose two aims:

Aim 1. Determine whether and by how much OS reduces the incidence of HGSC, ENOC and CCOC.

Aim 2. Determine whether ovarian carcinoma cases occurring post-OS are qualitatively distinct from control cases of the same histotype.

We will work with the population-based administrative datasets in the Canadian provinces of BC and Ontario to generate effectiveness data on OS for HGSC, ENOC, and CCOC. These data on an underlying population of 20 million people will be large enough to assess the incidence of each of these histotypes following OS. We have also engaged an international network of pathologist researchers from large volume hospitals/cancer centers all over the world to help us generate a database of cases and controls consisting of ovarian cancers arising after an OS and controls where fallopian tubes were still intact at the time of the cancer diagnosis. These cases and controls will be used to conduct the molecular and genomic work to understand whether and how ovarian cancers are qualitatively different in people with and without fallopian tubes.

Because we are unable to effectively screen for ovarian cancer, and because data suggests that OS is an effective primary prevention approach, studying and promoting OS is a powerful opportunity to save lives and reduce the burden of ovarian cancer worldwide. It is also important to address the considerable debate around the proportion of HGSCs that arise outside of the fallopian tube in human research. This research project will provide this needed evidence.

Proposal Title:	The Ovarian Cancer Observatory: Prevention, Impact, and Learning from Opportunistic Salpingectomy
Log Number:	OC220082P1
Current PI Name:	Gillian Hanley
Award Number:	HT9425-23-1-0957
Current Contracting Organization:	British Columbia, University of
Current Performing Organization:	British Columbia, University of
Web Approval Date:	09-13-2023

The vast majority of ovarian cancers are epithelial in origin. These are not a single disease, but rather five distinct diseases (referred to as histotypes). These five histotypes, in order of frequency, are high-grade serous (HGSC), endometrioid (ENOC), clear cell (CCOC), low-grade serous (LGSC), and mucinous ovarian cancer. The ovary is frequently the site of the dominant tumor mass when these cancers present, which led to the belief that ovarian cancer arises from transformation of the ovarian surface cells. However, in the past 20 years, it has become apparent that many HGSCs (the most common and lethal histotype) arise in the fallopian tube. The origin of each histotype of ovarian cancer remains an area of active debate and is important to understand to inform prevention, diagnostics, and treatment of each histotype.

The recognition that the fallopian tube is where many HGSCs originate has led to an important prevention strategy, removal of the fallopian tubes during other pelvic surgeries. Opportunistic salpingectomy (OS) collectively refers to the removal of fallopian tubes (salpingectomy) during the time of other common surgical procedures (opportunistic), in particular at hysterectomy (removal of the uterus) or instead of tubal ligation (tying tubes) for sterilization. OS is now recommended practice in at least nine different countries.

Research has shown that it is safe, feasible, and cost-effective. Recent data from British Columbia (BC), Canada, has also shown that it is likely effective in preventing HGSCs. There has not been a single case of HGSC following OS in BC, where it has been commonly performed since 2008. This is significantly fewer than were expected if cancers were arising at the same rate in the OS group as in the control group (people who had hysterectomy alone or tubal ligation), where there were 15 cases of HGSC.

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Because we are unable to effectively screen for ovarian cancer, and because data suggests that OS is an effective primary prevention approach, studying and promoting OS is a powerful opportunity to save lives and reduce the burden of ovarian cancer worldwide. It is also important to address the considerable debate around the proportion of HGSCs that arise outside of the fallopian tube in human research. This research project will provide this needed evidence.

Proposal Title:	A sUPR Driver of T-Cell Metabolic Dysfunction in Ovarian Cancer
Log Number:	OC220110
Current PI Name:	Juan Cubillos-Ruiz
Award Number:	HT9425-23-1-0204
Current Contracting Organization:	Joan and Sanford I Weill Medical College of Cornell University
Current Performing Organization:	Joan and Sanford I Weill Medical College of Cornell University
Web Approval Date:	07-14-2023

Ovarian carcinoma is the most lethal cancer of the female reproductive tract and is the fifth leading cause of cancer-related death in American women. While basic knowledge on cancer cell biology has markedly increased over the last decades, current treatments for metastatic carcinoma, the stage at which most ovarian cancers are diagnosed, lead to a 5-year survival rate of less than 30%. These classical treatments based on surgery and chemotherapy seek to eliminate tumors that are initially sensitive to drugs. However, the usual failure of this approach to eradicate all malignant cells promotes the development of drug resistance cancer and aggressive systemic relapse. Therefore, novel and more effective approaches are urgently needed in the clinic to complement the standard treatments and improve the dismal prognosis of ovarian cancer patients.

Specific cells in our immune system, called T cells, exhibit a remarkable ability to recognize and kill cancer cells. Harnessing their capacity to fight tumors represents the most promising anti-cancer strategy since the development of chemotherapy, as evidenced by the dramatic shrinkage of human melanoma and lung cancer in response to various immunotherapeutic agents. Notably, ovarian cancer patients with high accumulation of T cells inside the tumor survive longer than those with reduced numbers of intra-tumoral T cells. These observations indicate that the immune system can exert pressure against ovarian cancer progression and suggest that therapies capable of unleashing anti-tumor T cells could offer a strategy to support the standard treatments. Nevertheless, ovarian cancers develop potent mechanisms to prevent and counteract the anti-tumor effects of immune T cells. Our Investigator Initiated Research Project seeks to uncover and characterize the dominant mechanisms through which ovarian tumors restrain the anti-cancer activity of our immune system.

Supported by our initial Ovarian Cancer Academy - Early-Career Investigator Award of the Department of Defense, our group discovered that immune cells within ovarian tumors are “stressed out” due to the adverse environmental conditions that these malignant masses create, such as decreased oxygen availability and lack of nutrients. These harsh conditions, and the ensuing cellular stress provoked, render the immune system inactive and unable to spot and kill the cancer cells. Therefore, we postulate that identifying, understanding, and disabling the sensors of stress in immune T cells could be exploited as a novel approach to unleash protective and durable immune responses against ovarian cancer. Hence, our project will comprehensively characterize the tactics that ovarian cancers utilize to induce cellular stress in the immune system and will unveil the functional processes that are affected in stressed out immune cells. Moreover, our project will test the novel translational hypothesis that overcoming immune stress within the tumor environment could be used as a new strategy to restrain ovarian cancer progression. Therefore, our project is intimately connected with the following areas of emphasis relevance: “understand the basic biology and etiology of ovarian cancer initiation, progression, metastasis, recurrence, genetics, and other critical events”, as well as “develop novel therapeutic strategies for ovarian cancer treatment.”

Successful completion of this project will unveil new classes of fundamental mechanisms and novel therapeutic targets for ovarian cancer. As such, this project is expected to provide a strong mechanistic rationale for targeting cellular stress pathways in the clinic as a novel approach to unleash protective immunity against, and prevent recurrence of, ovarian cancer. Since multiple pharmaceutical companies are

currently developing drugs to target cellular stress pathways in other indications, our findings could have rapid clinical applicability towards eliminating ovarian cancer, thus positively impacting the wellness of thousands of patients and their families.

Proposal Title:	Role and Regulation of ZNF217 in Ovarian Cancer Metastasis and Drug Resistance
Log Number:	OC220131
Current PI Name:	Achuth Padmanabhan
Award Number:	HT9425-23-1-0351
Current Contracting Organization:	Maryland, University of, Baltimore County
Current Performing Organization:	Maryland, University of, Baltimore County
Web Approval Date:	09-13-2023

Ovarian cancer continues to be the most lethal gynecologic cancer and the fifth leading cause for cancer-associated deaths among women in the United States. Due to the absence of obvious symptoms and the lack of reliable methods to identify ovarian cancer early, the majority of women are diagnosed at an advanced stage. At this stage, the cancer is no longer confined to the ovary and unfortunately, the existing drugs are ineffective. This limitation of extant drugs and therapeutic strategies is a major reason for the high mortality rate associated with ovarian cancer. This clinical reality highlights the urgent need to develop new and more effective ways to treat this deadly disease and translate them rapidly to the clinic. My long-term goal is to develop treatment strategies for advanced stage metastatic ovarian cancer and eliminate ovarian cancer associated deaths in women. Thus, through my research, I aim to benefit Service Members, Veterans, retirees, their Family members, and all women impacted by this deadly disease. In this proposal, I outline my plan to achieve this goal by characterizing a novel therapeutic target in ovarian cancer cells and determining its utility in addressing the deficiencies in extant treatment options.

We discovered that a protein called Zinc Finger Protein 217 (or ZNF217) has great utility as a therapeutic target in metastatic ovarian tumors. ZNF217 is overexpressed in ~60% metastatic ovarian tumors and is associated with poor prognosis. Our data shows that ZNF217 promotes proliferation, metastasis, and drug resistance in ovarian tumors. However, the ZNF217's utility as a therapeutic target in ovarian cancer has remained unexploited. We hypothesize that ZNF217 depletion will impair tumor progression and sensitize drug-resistant ovarian tumors to clinically approved chemotherapeutics. As drug resistance is the major challenge that limits the effectiveness of extant drugs, we believe our studies will address a critical need in ovarian cancer therapeutic development.

In addition to promoting chemotherapeutic resistance, we discovered a link between ZNF217 and another key ovarian cancer risk factor, estrogen. Ovarian cancer cells that overexpress ZNF217 exhibited increased metastatic potential in the presence of estrogen. While estrogen is known to impact ovarian cancer incidence and progression, drugs that target estrogen signaling pathway have not been successful as a therapeutic strategy for ovarian cancer so far. We believe the limited effectiveness of FDA approved estrogen targeting drugs to be largely due to our inability to distinguish estrogen responsive ovarian tumors from non-responsive ones. Biomarkers that will allow us to make this important distinction will enable us to identify patients who would benefit from estrogen targeting therapeutics. Based on our exciting data, we hypothesize that ZNF217 levels in ovarian tumors has utility to serve as a predictive biomarker for identifying estrogen-responsive ovarian tumors. We will test this hypothesis using clinically relevant models of metastatic ovarian cancer.

Interestingly, we found that ZNF217 also regulates several factors that are known to induce exclusion of immune cells from ovarian tumors. Such immune cell deficient tumors are highly prevalent in ovarian cancer and are associated with poor prognosis. The lack of immune cells in these tumors are also believed to be the major reason for the ineffectiveness of immunotherapy in ovarian cancer. To improve immunotherapy's efficacy in ovarian cancer, we must convert these immune cell deficient tumors into one that is infiltrated

with immune cells. Strategies that achieve this goal will define the success of immunotherapy in ovarian cancer and profoundly impact clinical outcome in patients. Based on our exciting data, we hypothesize that ZNF217 depletion will remodel ovarian tumors and convert them into tumors that is infiltrated with immune cells. Therefore, we propose combining ZNF217 depletion with immunotherapeutic agents as a strategy to improve immunotherapy's effectiveness in ovarian cancer and achieve enhanced and targeted killing of metastatic ovarian cancer cells.

Thus, ZNF217 impacts ovarian cancer progression and clinical response through many distinct processes. Despite these compelling data, we still know absolutely nothing about the mechanisms that regulates ZNF217 protein stability and turnover in cells. Understanding these mechanisms are critical to develop new clinically translatable strategies to target ZNF217 in cancer cells. This proposal we will address this knowledge gap as well.

Overall, successful completion of studies described in this proposal will significantly advance our understanding of how ZNF217 impacts ovarian cancer progression and will pave the way towards the development of new, effective, and clinically translatable therapeutic strategies for metastatic ovarian cancer. As all the strategies described in our proposal aims to overcome challenges faced by drugs that are already clinically approved, we believe the outcome of our project has extremely high translational value. Given that ZNF217 is overexpressed in several human tumors, the impact of our work will likely extend well beyond ovarian cancer.

Proposal Title:	Genomics and Biophysical Strategies to Overcome Cisplatin Resistance in Ovarian Cancer
Log Number:	OC220133
Current PI Name:	Sreejith Nair
Award Number:	HT9425-23-1-0265
Current Contracting Organization:	Georgetown University
Current Performing Organization:	Georgetown University
Web Approval Date:	07-10-2023

The mainstay therapy for ovarian cancer is surgery followed by chemotherapy. Platinum-based anticancer drugs like cisplatin are the most commonly used chemotherapeutic agents against ovarian tumors. Almost all ovarian cancer patients receive platinum-based drugs as a treatment regimen. Although initially effective in the vast major of cases, most tumors acquire resistance to the drug and stop responding to all forms of therapies. Therefore, strategies to overcome chemoresistance will extend the survival rates of millions of ovarian cancer patients.

Objective and Rationale of the Proposed Work: Although we have a good understanding of how cisplatin works by causing DNA damage in cancer cells, it is evident that better strategies through “out of the box” thinking are needed to overcome chemoresistance. The goal of this project is to focus on poorly understood but promising observations on the cellular behavior of cisplatin to better understand the molecular action of this drug and develop innovative strategies to overcome therapy resistance. It has been known for decades that, in addition to binding to genomic DNA where the genetic information is stored, cisplatin also interacts with another class of cellular nucleic acids known as RNAs. The significance of this interaction in the anti-cancer property of cisplatin is unknown. Based on the recent findings, we propose that the association of cisplatin to cellular RNAs plays a crucial role in its anticancer action and also in the development of therapy resistance.

Critical Problems in Ovarian Cancer Addressed in the Proposed Research: As the key chemotherapeutic agents that all ovarian cancer patients depend on, it is essential to devise strategies that make the platinum drugs most effective for the patients. Even after decades of intense research on this topic, chemoresistance is still a persistent problem faced by a significant fraction of ovarian cancer patients. Therefore, new strategies have to be explored to overcome this problem. The proposed research aims to investigate the poorly understood but promising recent observations on cisplatin biology to develop innovative strategies to overcome chemoresistance. To achieve this goal, we have assembled a multi-disciplinary team composed of cell biologists, genomic experts, chemists, physicists, and ovarian cancer biologists to apply modern tools to unravel the mysteries surrounding the mechanisms of action of platinum drugs. By focusing on the role of RNAs, an unconventional target of cisplatin, this study will set new paradigms for the molecular action of cisplatin and the emergence of chemoresistance.

Relevance of the Project to the Ovarian Cancer Research Program (OCRP) Mission and Vision: According to the OCRP funding opportunity announcement, innovative projects are those that introduce a new paradigm, challenge current paradigms, look at existing problems from a new perspective, or provide new insights and technologies. By focusing on the unconventional mode of action of platinum-drug targets, using novel reagents and innovative multi-disciplinary strategies, this study fulfills all the criteria for innovation. We will develop novel cisplatin-derivatives and apply them to develop new technologies to provide insights into cisplatin biology and chemoresistance. This proposal addresses two FY22 areas of emphasis:

Develop Novel Therapeutic Strategies for Treatment and Prevention: This project will identify novel RNAs that cause cisplatin therapy resistance and, therefore, promises to provide novel therapeutic targets. We will test the use of targeted RNA degradation (e.g., antisense oligo therapy) to improve the efficacy of cisplatin therapy, which will be a novel strategy to overcome therapy resistance in a patient-specific manner. In the long term, RNA targeting has the potential to develop into a personalized therapeutic approach.

Identify and Develop New Strategies for Screening, Early-Stage Detection, Prevention, Accurate Diagnosis, and Prognosis: The RNA targets confirmed to cause cisplatin resistance will help predict the patient's response to cisplatin and the chances of developing chemoresistance. It will lead to improved survivorship of ovarian cancer patients who are initially responsive to cisplatin therapy.

Impact: By focusing on an unconventional, poorly understood cellular target of cisplatin, this study differentiates itself from most of the ongoing research aimed at solving the problem of chemoresistance in ovarian cancer. This basic research grant aims to unravel novel modes of action of the most-used chemotherapeutic agent in ovarian cancer. Since platinum drugs are used in treating almost half of all cancer patients, any discoveries made in the study will have a broad impact on cancer research. RNAs and cellular condensates, the two main foci in this study, are emerging as powerful drug targets. As appropriate for a pilot award, these studies are in the early phase of idea development. This means that the clinical translation of our work will require long-term effort. Our immediate goal is to publish the findings by the end of this project and attract more funding support for a much larger study.

Proposal Title:	Elucidating and Targeting Clonal Lineages Influencing Resistance to Therapy in Distinct Mutational Subtypes of High-Grade Ovarian Serous Carcinoma
Log Number:	OC220134
Current PI Name:	James Brenton
Award Number:	HT9425-23-1-0276
Current Contracting Organization:	Cambridge, University of
Current Performing Organization:	Cambridge, University of
Web Approval Date:	07-03-2023

Over the last decade, it has become increasingly clear that tumors are very complex and made up of lots of different kinds of cells, including cancer cells and healthy cells. Importantly, not all the cancer cells are the same, even within a single tumor, and it is this diversity among the cancer cells that often causes therapy to be unsuccessful. When the tumor is treated, the majority of the cancer cells are successfully destroyed, the tumor shrinks, and the therapy appears to be successful. But, in reality, there may be a small number of other types of cancer cells present in the tumor that are able to survive the treatment and then regrow, often more vigorously than they did before, causing a relapse. Resistance to treatment is the main reason someone's cancer may return and become incurable. We need to understand why and how a particular cancer becomes resistant to specific drugs and how we can stop this from happening.

Ovarian tumors are characterized by particularly high levels of cancer cell diversity because they often have mutations in genes which normally prevent errors occurring in our DNA. This leads to an accumulation in errors or mutations in the DNA, the pattern of which can be used to categorize a patient's tumor. Work from the Brenton lab has shown that these patterns or signatures can predict whether a patient will have poor prognosis and whether they will benefit from therapy or not. However, we do not understand why these predictions work at the cellular level, which is critical if we want to improve therapy and identify alternative drugs for treatment of tumors that do not respond to standard therapy. A major challenge to being able to investigating this is that it can be very hard for researchers to determine how each type of cancer cell is behaving within a complex mixture. The Hannon lab have developed a new approach to tackle this problem that allows us to unambiguously identify each type of cancer cell before and after treatment within tumors in a dish or grown in mice. Each cell type is given a unique genetic barcode that we can use to identify it. These barcodes are used alongside cutting-edge technologies that allow us to measure the patterns of genes which are turned on and off within each cell and dictate the cell's identity and behavior. This enables us to follow each type of cancer cell simultaneously over the course of treatment, determine whether it was killed by or survived treatment, measure the characteristics of the cells, and determine how those characteristics change when it is treated with drug. Recent improvements to this barcoding approach now allow us to additionally purify a single type of cancer cell of interest from a complex mixture by using its unique barcode to activate a fluorescent gene in only that cell type.

We will use these barcoding technologies in clinically relevant ovarian cancer models established by the Brenton lab. Specifically, the Brenton lab have isolated cells directly from ovarian cancer patients at Addenbrookes hospital and then grow them as 3D tumors in a dish, called organoids. When these organoids are treated with chemotherapy, they respond in the same way as the original tumor in the patient, making them ideal models to study chemotherapy response in the lab. We have organoids derived from patients with different subtypes of ovarian cancer, meaning that we can determine whether our results are applicable to all patients or a specific subset. In these models, we will determine which types of tumor cells are killed by or

survive chemotherapy, what determines this behavior, and how the cells are altered by treatment. Having identified cell types that survive treatment, we will purify these and test them against a large panel of drugs to identify drugs that preferentially kill these resistant cells. Those drugs are then good candidates to be used in combination with standard chemotherapy, as the chemotherapy will kill the majority of the cancer cells and the new drug will kill the remaining resistant cells.

In summary, through this research, we will increase our understanding of how complex ovarian tumors respond to chemotherapy, enabling us to improve the identification of patients most likely to respond to treatment, provide critical knowledge for new drug development, and uncover novel, more effective drug combinations. Ultimately, this study will pave the way towards a future where a patient can receive a specifically selected combination of two or more drugs that will each target different types of cancer cells within that patient’s tumor so that all of the cancer cells can be killed effectively at the same time.

Proposal Title:	Elucidating and Targeting Clonal Lineages Influencing Resistance to Therapy in Distinct Mutational Subtypes of High-Grade Ovarian Serous Carcinoma
Log Number:	OC220134P1
Current PI Name:	Gregory Hannon
Award Number:	HT9425-23-1-0277
Current Contracting Organization:	Cambridge, University of
Current Performing Organization:	Cambridge, University of
Web Approval Date:	07-03-2023

Over the last decade, it has become increasingly clear that tumors are very complex and made up of lots of different kinds of cells, including cancer cells and healthy cells. Importantly, not all the cancer cells are the same, even within a single tumor, and it is this diversity among the cancer cells that often causes therapy to be unsuccessful. When the tumor is treated, the majority of the cancer cells are successfully destroyed, the tumor shrinks, and the therapy appears to be successful. But, in reality, there may be a small number of other types of cancer cells present in the tumor that are able to survive the treatment and then regrow, often more vigorously than they did before, causing a relapse. Resistance to treatment is the main reason someone's cancer may return and become incurable. We need to understand why and how a particular cancer becomes resistant to specific drugs and how we can stop this from happening.

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survive chemotherapy, what determines this behavior, and how the cells are altered by treatment. Having identified cell types that survive treatment, we will purify these and test them against a large panel of drugs to identify drugs that preferentially kill these resistant cells. Those drugs are then good candidates to be used in combination with standard chemotherapy, as the chemotherapy will kill the majority of the cancer cells and the new drug will kill the remaining resistant cells.

In summary, through this research, we will increase our understanding of how complex ovarian tumors respond to chemotherapy, enabling us to improve the identification of patients most likely to respond to treatment, provide critical knowledge for new drug development, and uncover novel, more effective drug combinations. Ultimately, this study will pave the way towards a future where a patient can receive a specifically selected combination of two or more drugs that will each target different types of cancer cells within that patient’s tumor so that all of the cancer cells can be killed effectively at the same time.

Proposal Title:	Tubal Immune Milieu and Tumor Precursor Evolution in the Development of High-Grade Serous Carcinoma
Log Number:	OC220138
Current PI Name:	Thing Soong
Award Number:	HT9425-23-1-0442
Current Contracting Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Current Performing Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Web Approval Date:	09-28-2023

Hypothesis and Rationale for the Proposed Project: Women with germline mutations in BRCA1 and BRCA2 have increased lifetime risk of ovarian high grade serous carcinoma (HGSC). To date, there is no effective early HGSC screening method, and women with BRCA germline mutations are recommended to undergo risk-reducing salpingo-oophorectomy, a procedure which is often associated with long-term health consequences due to premature menopause. The early pathogenesis of HGSCs remains poorly understood, hampering our efforts to optimize screening and preventive strategies of HGSC.

Our research data suggest that early serous proliferations (ESPs) in the fallopian tubes (FTs), which comprise TP53 mutant clones with nil to mild atypia, may act as early direct HGSC precursors. Understanding is extremely limited on the impact of the local immune microenvironment on the progression of these mutant clones. We previously sampled FTs from healthy women and women with tubal lesions as well as FTs from mouse models that developed HGSC, and showed in initial analyses that unique immune signatures in the FTs might differentially correlate with tubal pathology status.

We hypothesize that (1) TP53-mutated FT epithelial lesions are associated with an increasingly proinflammatory immune phenotype and higher TP53 mutational pathogenicity as these FT lesions develop into malignancy, and (2) disruption of host immune response accelerates the progression of these lesions into carcinoma. In this proposed study, we will utilize multiplex immunofluorescence studies and RNA-based multiplex profiling to examine the local immune profiles in human and murine FTs with and without ESPs and FT HGSC, and evaluate the impact of immune cell depletion on clonal expansion and HGSC development in transgenic mouse models.

Principle Investigator's Career Goals in Ovarian Cancer Research: I am a board-certified pathologist with expertise in gynecologic pathology and is fervently devoted to investigating the pathogenesis of ovarian cancer, particularly the prevention and detection of high-grade serous carcinoma (HGSC), which is the most common subtype and deadliest form of ovarian cancer. I had Ph.D. training in epidemiology, and subspecialized training in gynecologic pathology. My prior studies on ovarian cancer showed data supporting a novel paradigm for HGSC development, in which mutated cells could disseminate from FTs or clonally expand in the peritoneal cavity, which may give rise to carcinoma as direct precursors. The present study proposal is an expansion of my current research work and collaborations with co- investigators to further examine risk factors that promote the malignant transformation of these mutant clones. I have a track record of publications and short-term independent funding in ovarian cancer research. My long-term career goal is to become an independent clinician-scientist and establish an integrative molecular, pathological and epidemiologic research program to study the interactions between the local microenvironment and host exposures in the setting of HGSC. The aim is to identify factors influencing the development of HGSC, with translation of findings to population-based studies to inform better strategies for HGSC prevention and detection.

I believe my rare combination of training backgrounds in pathology and epidemiology would contribute to the diversity of expertise and perspectives in the ovarian cancer research community. The proposed research and the early-investigator award will provide me with the perfect venue to acquire the necessary training, mentorship and funding to achieve my career goal while addressing a critical question in the pathogenesis and early detection of HGSC.

Applicability of the Research Study: The proposed research study is based on a novel paradigm uniting the host immune milieu with TP53 clonal evolution in the FTs and addresses the understudied question on how the local immune microenvironment co-evolve with mutant clonal proliferation during the pre-cancer time window of HGSC. The data from this study will be critical in uncovering immune biomarkers that correlate with early disease changes in the FTs several years before cells become committed to the fate of carcinoma.

The information from this study thus has great potential of being translated into larger-scale population-based studies for biomarker testing and validation. The study will have important implications on risk stratification and early HGSC disease detection in women with or without family history of ovarian cancer.

Proposal Title:	Taking Ovarian Cancer Maintenance Therapy to the Next Level
Log Number:	OC220161
Current PI Name:	Doris Benbrook
Award Number:	HT9425-23-1-0175
Current Contracting Organization:	Oklahoma, University of, Health Sciences Center
Current Performing Organization:	Oklahoma, University of, Health Sciences Center
Web Approval Date:	06-22-2023

Women in the general public and military service who are treated for ovarian cancer can be cleared of their cancer with surgery and chemotherapy, but most often their cancer comes back. After completion of surgery and chemotherapy, doctors treat patients with a treatment called maintenance therapy to increase the time it takes for their cancer to come back. Once the cancer returns during maintenance therapy, patients are treated again with chemotherapy, but the cancer keeps coming back and the chemotherapy is less effective on each subsequent round of treatment. Often additional rounds of maintenance therapy are no longer able to keep the cancer at bay. Even before the cancer comes back, patients often experience severe side effects from the maintenance therapy and have to stop. Thus, new and less-toxic maintenance therapies are needed. This is an area of high unmet need in which our novel investigational drug, SHetA2, has demonstrated much promise as an active and well-tolerated intervention.

In laboratory studies, SHetA2 did not cause side effects or toxicity at doses 50-fold above doses shown to prevent and inhibit cancer growth. Thus, it is expected that SHetA2 will cause no or less side effects to current drugs being used in ovarian cancer maintenance therapy. Since SHetA2 capsules can be taken by mouth, ovarian cancer patients on SHetA2 maintenance therapy can take their daily drug in the comfort of their own homes, without having to go into the clinic for IV infusions of drug.

We anticipate that SHetA2 maintenance therapy will, without causing side effects, increase the amount of time before ovarian cancer patients experience cancer recurrence. This is because SHetA2 was able to do this in a mouse model of ovarian cancer maintenance therapy. With this preliminary finding, we now need to understand more about how SHetA2 is doing this and what doses are most effective. The information of how SHetA2 works and how much is needed to prevent cancer recurrence is needed in order to design clinical trials testing SHetA2 in ovarian cancer maintenance therapy.

This proposed research project is designed to develop this needed understanding of how SHetA2 inhibits ovarian cancer recurrence. Recurrence is believed to be caused by clumps of ovarian cancer cells in fluid called ascites that builds up inside the belly of patients. Ovarian cancer cell clumps will be collected from ascites specimens donated by ovarian cancer patients, treated with SHetA2 in the lab and studied for how they react to this treatment at the molecular and cellular levels. Normally, these specimens are looked at by pathologists and then thrown away. This study will allow these discarded specimens to be put to good use in research. This study will compare how SHetA2 changes molecular and cellular aspect of the ovarian cancer cells with this drug's effect on the cell's abilities to form metastases (additional tumor growth sites in the body). These molecular and cellular effects that are altered by SHetA2 inhibition of metastases will be identified. These identified molecular effects will then be studied to confirm their role in SHetA2-inhibition of metastases by testing whether preventing the molecular effect also prevents the inhibition of metastases by SHetA2.

This project will also study ways that SHetA2 can be combined with other drugs to be even more effective at increasing the time to ovarian cancer recurrence without causing side effects. Findings will be also studied in mouse models of ovarian cancer maintenance therapy to confirm that the results we observe in cell cultures also occur inside the body.

Another goal of this project is to optimize a technology called “single spheroid mass spectrometry” that will allow measurement of drug concentrations inside clumps of ovarian cancer cells. At the same time, this technology will allow the measurement of SHetA2 effects on fat burning inside the ovarian cancer cells. Fat burning is an important part of ovarian cancer metastases. One of the main sites where ovarian cancer sets up metastatic tumors is fat cells, where it eats the fat for nutrition and growth. Published studies have shown that ovarian cancer patients have higher levels of fat in their blood and that SHetA2 can inhibit fat metabolism.

Areas of Emphasis and Relevance: The most relevant areas for this proposal are (1) understand the basic biology and etiology of ovarian cancer initiation, progression, metastasis, recurrence, genetics and other critical events and (2) develop novel therapeutic strategies for treatment and prevention.

Proposal Title:	Taking Ovarian Cancer Maintenance Therapy to the Next Level
Log Number:	OC220161P1
Current PI Name:	Anthony Burgett
Award Number:	HT9425-23-1-0176
Current Contracting Organization:	Oklahoma, University of, Health Sciences Center
Current Performing Organization:	Oklahoma, University of, Health Sciences Center
Web Approval Date:	06-22-2023

Women in the general public and military service who are treated for ovarian cancer can be cleared of their cancer with surgery and chemotherapy, but most often their cancer comes back. After completion of surgery and chemotherapy, doctors treat patients with a treatment called maintenance therapy to increase the time it takes for their cancer to come back. Once the cancer returns during maintenance therapy, patients are treated again with chemotherapy, but the cancer keeps coming back and the chemotherapy is less effective on each subsequent round of treatment. Often additional rounds of maintenance therapy are no longer able to keep the cancer at bay. Even before the cancer comes back, patients often experience severe side effects from the maintenance therapy and have to stop. Thus, new and less-toxic maintenance therapies are needed. This is an area of high unmet need in which our novel investigational drug, SHetA2, has demonstrated much promise as an active and well-tolerated intervention.

In laboratory studies, SHetA2 did not cause side effects or toxicity at doses 50-fold above doses shown to prevent and inhibit cancer growth. Thus, it is expected that SHetA2 will cause no or less side effects to current drugs being used in ovarian cancer maintenance therapy. Since SHetA2 capsules can be taken by mouth, ovarian cancer patients on SHetA2 maintenance therapy can take their daily drug in the comfort of their own homes, without having to go into the clinic for IV infusions of drug.

We anticipate that SHetA2 maintenance therapy will, without causing side effects, increase the amount of time before ovarian cancer patients experience cancer recurrence. This is because SHetA2 was able to do this in a mouse model of ovarian cancer maintenance therapy. With this preliminary finding, we now need to understand more about how SHetA2 is doing this and what doses are most effective. The information of how SHetA2 works and how much is needed to prevent cancer recurrence is needed in order to design clinical trials testing SHetA2 in ovarian cancer maintenance therapy.

This proposed research project is designed to develop this needed understanding of how SHetA2 inhibits ovarian cancer recurrence. Recurrence is believed to be caused by clumps of ovarian cancer cells in fluid called ascites that builds up inside the belly of patients. Ovarian cancer cell clumps will be collected from ascites specimens donated by ovarian cancer patients, treated with SHetA2 in the lab and studied for how they react to this treatment at the molecular and cellular levels. Normally, these specimens are looked at by pathologists and then thrown away. This study will allow these discarded specimens to be put to good use in research. This study will compare how SHetA2 changes molecular and cellular aspect of the ovarian cancer cells with this drug's effect on the cell's abilities to form metastases (additional tumor growth sites in the body). These molecular and cellular effects that are altered by SHetA2 inhibition of metastases will be identified. These identified molecular effects will then be studied to confirm their role in SHetA2-inhibition of metastases by testing whether preventing the molecular effect also prevents the inhibition of metastases by SHetA2.

This project will also study ways that SHetA2 can be combined with other drugs to be even more effective at increasing the time to ovarian cancer recurrence without causing side effects. Findings will be also studied in mouse models of ovarian cancer maintenance therapy to confirm that the results we observe in cell cultures also occur inside the body.

Another goal of this project is to optimize a technology called “single spheroid mass spectrometry” that will allow measurement of drug concentrations inside clumps of ovarian cancer cells. At the same time, this technology will allow the measurement of SHetA2 effects on fat burning inside the ovarian cancer cells. Fat burning is an important part of ovarian cancer metastases. One of the main sites where ovarian cancer sets up metastatic tumors is fat cells, where it eats the fat for nutrition and growth. Published studies have shown that ovarian cancer patients have higher levels of fat in their blood and that SHetA2 can inhibit fat metabolism.

Areas of Emphasis and Relevance: The most relevant areas for this proposal are (1) understand the basic biology and etiology of ovarian cancer initiation, progression, metastasis, recurrence, genetics and other critical events and (2) develop novel therapeutic strategies for treatment and prevention.

Proposal Title:	Clinically Translatable Strategies to Improve Therapeutic Response in Metastatic Ovarian Cancers
Log Number:	OC220162
Current PI Name:	Achuth Padmanabhan
Award Number:	HT9425-23-1-0232
Current Contracting Organization:	Maryland, University of, Baltimore County
Current Performing Organization:	Maryland, University of, Baltimore County
Web Approval Date:	07-10-2023

Ovarian cancer continues to be the most lethal gynecologic cancer and the fifth leading cause for cancer-associated deaths among women in the United States. Due to the absence of obvious symptoms and the lack of reliable methods to identify ovarian cancer early, the majority of women who have the disease are diagnosed at an advanced stage. At this stage, the cancer is no longer confined to the ovary, and unfortunately, the existing drugs are ineffective. This limitation of extant drugs and therapeutic strategies is a major limitation and the cause for the high mortality rate associated with ovarian cancer. This clinical reality highlights the urgent need to develop new and more effective ways to treat this deadly disease and translate them rapidly to the clinic. This research proposal outlines the plan to achieve this goal by developing and testing a new therapeutic strategy that addresses key deficiencies in current treatment options and promises to significantly improve the clinical outcome.

To achieve this goal, we propose to target two major factors that are associated with disease progression, drug resistance, and poor clinical outcome in ovarian cancer. The first factor is mutations in a gene called p53. While p53 is known to suppress tumor formation, the mutations in this gene, such as the ones seen in about 70 percent of ovarian cancers, convert p53 into a tumor promoting protein that accumulates in cancer cells. The second factor we will target is the tumor-promoting microenvironment prevalent within metastatic ovarian tumors. The tumor-promoting microenvironment prevalent in a majority of ovarian tumors that is believed to be the major reason for the ineffectiveness of immunotherapy in ovarian cancer. Both these factors are extremely common in ovarian cancer and, therefore, by targeting them simultaneously, we aim to develop a therapeutic approach that will benefit a broad spectrum of ovarian cancer patients.

Our approach builds on two very exciting recent discoveries made in our laboratory. First, we discovered using mouse models that mimic metastatic ovarian cancer progression that mutations in p53 is a key factor responsible for establishing the tumor-promoting microenvironment prevalent in ovarian cancer patients. This discovery is very important not just from the point of understanding the biology of the disease but also from a therapeutic standpoint. Our data suggest that, if we can develop a strategy to selectively deplete mutated p53 proteins, that are exclusive to cancer cells, we will not just target cancer cells to die and make them sensitive to extant drugs by changing properties that are intrinsic to the cancer cells, but will also induce their killing by recruiting immune cells into the tumors. Moreover, by recruiting more immune cells into the tumor, mutant p53 depletion will remodel the ovarian tumor microenvironment from a tumor promoting one to one that suppresses tumor growth and is permissive to the action of immunotherapy. Thus, combining mutant p53 with immunotherapy will help make immunotherapy effective in metastatic ovarian cancer patients.

While extremely attractive, a major challenge in achieving these objectives is identifying therapeutics that will selectively deplete mutant p53 proteins in ovarian cancer cells in a clinically translatable manner. Despite being a highly sought-after therapeutic target mutant p53, targeting mutant p53 in the clinic has been difficult to achieve. Our second exciting discovery promises to overcome this challenge. We discovered that a combination two FDA-approved drugs, metformin and statin (or MET-STAT), both of which have been

used in the clinic for decades to treat type-2 diabetes and hyperlipidemia, achieves potent and selective depletion of mutant p53. In this proposal, we will determine MET-STAT's utility as a therapeutic: (1) by itself, (2) in combination with extant clinically approved chemotherapeutics, and (3) in combination with immune checkpoint inhibitors, in metastatic ovarian cancer. Successful completion of these studies will significantly advance our understanding of how mutations in p53 impact progression of advanced-stage ovarian cancers and will pave the way towards the development of new, effective, and clinically translatable therapeutic strategies for metastatic ovarian cancer. As our strategy uses drugs that are already clinically approved for human use, it will also significantly reduce the time and effort needed to translate our studies to human patients once the merits of our methods are established. Thus, we expect the outcome of proposed research to significantly benefit Service Members, Veterans, retirees, their family members, and all women impacted by this deadly disease.

Proposal Title:	Metabolic Reprogramming of CAR-Macrophages for Ovarian Cancer Immunotherapy
Log Number:	OC220173
Current PI Name:	Marion Curtis
Award Number:	HT9425-23-1-0328
Current Contracting Organization:	Mayo Clinic and Foundation, Scottsdale
Current Performing Organization:	Mayo Clinic and Foundation, Scottsdale
Web Approval Date:	07-31-2023

Ovarian cancer treatment is an uphill battle in crucial need of new approaches and treatment options. Current treatments are often able to initially control ovarian cancer growth. However, most of the time, ovarian cancer will grow back and will become resistant to therapy. Many cancer types have benefited from immunotherapy, which is an approach that stimulates the immune system to attack and kill the tumor cells while leaving normal cells untouched, just as it would a virus or bacteria that had infected the body. Unfortunately, this type of treatment has not worked well for women with ovarian cancer. The long-term goal of this proposed work is to develop new treatment strategies and discover new treatment targets for all American women with ovarian cancer in order to improve quality of life and survival.

One type of immunotherapy takes cells from the patient's blood, activates those cells to target the tumor, and then gives those cells back to the patient through the blood stream. This kind of treatment has worked well for types of cancer that grow in the blood, but it has not worked well for patients with ovarian cancer. One roadblock for these activated cells is traveling through tissue to find the cancer. Recently, some studies have shown that a cell type called a macrophage can make it into ovarian tumors and are a safe therapy. We also now know that cells that are part of the immune system need certain metabolites, or nutrients, in order for the cells to function properly. In tumors, cancer cells take up those nutrients and prevent immune cells from gaining access to them, which results in the immune cells not functioning properly to target the tumor.

Our hypothesis is that we can load macrophages with an important nutrient called succinate that will enhance the cell's ability to fight ovarian cancer. Our proposal seeks to use a combination of human blood cells, ovarian cancer cell lines, and mouse models to understand how certain nutrients affect macrophage metabolism and how they may enhance tumor cell killing. As part of this proposal, we will use the Human Cell Therapy Lab (HCTL) at Mayo Clinic Arizona to produce these cells, called chimeric antigen receptor-macrophages (CAR-Ms).

The rationale for the proposed research is that, once it is known whether succinate can successfully enhance the killing ability of CAR-Ms, we can use this information to develop novel therapies. Importantly, because we will use the HCTL we will have critical insight into how to generate a clinical grade therapy that we could use in phase 1 clinical trials to benefit women with ovarian cancer that may lack additional therapeutic options.

Proposal Title:	Structural Biology-Informed Optimization of Antibody-Drug Conjugates for Ovarian Cancer with an Emphasis on Minimizing Toxicity
Log Number:	OC220207
Current PI Name:	Oladapo Yeku
Award Number:	HT9425-23-1-0241
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	07-11-2023

Hypothesis and Rationale: In contrast to other solid tumor malignancies such as breast and bladder cancer, there are no FDA-approved antibody-drug conjugates (ADC) for the treatment of ovarian cancer (OvCa). Despite decades of research, ADCs for ovarian cancer have been hobbled by lack of clinically meaningful efficacy and toxicity. OvCa has had few new therapy approvals in the past 20 years and immunotherapy has had limited efficacy in OvCa, likely because OvCa reflects a state of insufficient or inadequate target antigens. The epitope CA-125 or MUC16 is present in 95% of high-grade serous ovarian cancer (HGSOC) and is related to worse clinical outcomes. The overexpressed MUC16 protein is generally found anchored on cell surface and undergoes proteolytic cleavage over time into two independent fragments. A cleaved portion (Shed form) bearing the CA-125 antigen, is released into the blood circulation and can serve as a serum biomarker for diagnosing or monitoring response to therapy. However, there is a membrane tethered retained portion, herein termed the ectodomain, or MUC16ecto, that could be targeted by ADC. ADC against the shed CA-125 have been developed; however, because these antibodies bind to the circulating CA-125 that is not attached to the tumor, there have been limited efficacy and increased toxicity. The antibody we propose for development in this proposal binds portion of MUC16 that is retained on the tumor (MUC16ecto). This approach improves effectiveness and minimizes toxicity.

Using the structure of the retained portion of MUC16 (MUC16ecto) that we have solved in our group, we will optimize the binding properties of the antibodies we use for ADC development in the first aim, and we will rigorously test our best candidates for specificity and toxicity in our second aim.

At the completion of this project, we will have designed and evaluated ADC that can be used to treat high-grade serous ovarian cancer. Our lead candidates will have been rigorously evaluated for both toxicity, efficacy, and mechanisms of resistance. Once we have concluded all the preclinical experiments outlined in this proposal, our ADC will be ready for evaluation in clinical trials.

Areas of Emphasis: This proposal covers multiple components that are critical to the mission of the DOD OCRP award. In the first aim, this proposed work will harness fundamental structural and molecular biology principles to design a novel precision-medicine strategy for patients with ovarian cancer; in the second aim, we will validate these therapeutic ADC with an eye on clinical development as the next step.

The potential impact of the proposed research on the health and well-being of all women impacted by this disease. Women with ovarian cancer who have relapsed will eventually develop multidrug-resistant disease. Harnessing ADC to treat these cancers is a potential solution to this problem because these drugs can home to the tumor directly and deliver cytotoxic compounds directly to the tumor. This approach minimizes systemic side effects and, because different “payloads” can be attached to these ADC, if the cancer progresses on the first ADC, a second ADC with a different tumor toxin can be used.

Proposal Title:	Targeting DCLK1-Driven Chemoresistance in Ovarian Cancer
Log Number:	OC220211
Current PI Name:	Bethany Hannafon
Award Number:	HT9425-23-1-0223
Current Contracting Organization:	Oklahoma, University of, Health Sciences Center
Current Performing Organization:	Oklahoma, University of, Health Sciences Center
Web Approval Date:	07-03-2023

High-grade serous ovarian cancer (HGSOC) is the most lethal gynecologic malignancy and is characterized by repetitive disease recurrences due to the development or persistence of chemotherapy and targeted therapy-resistant tumors. There is an urgent need to develop new therapeutic strategies that target key pathways in the evolution of chemoresistance and drive HGSOC recurrence.

It is hypothesized that drug-resistant features associated with HGSOC recurrence patterns occur due to the evolution and enrichment of cancer stem cell (CSC)-like cells in recurrent/persistent tumors. CSCs retain the ability to self-renew and activate plasticity pathways to propagate new tumors and are especially resistant to chemotherapy. The CSC-related protein, doublecortin-like kinase 1 (DCLK1), is overexpressed in various solid tumors, including high-grade epithelial ovarian cancer, and is a major regulator of “stemness” programs, epithelial-to-mesenchymal transition - which drives tumor invasion and metastasis, drug resistance, and tumor cell dormancy in this highly lethal neoplasm. Our preliminary data implicate DCLK1 in the regulation of acquired resistance to platinum-based therapy in HGSOC and indicate that inhibition of DCLK1 enhances sensitivity to platinum-based chemotherapy by working in synergy to kill resistance cells. We hypothesize that DCLK1 drives cisplatin resistance in HGSOC by promoting cancer-cell stemness programs and that targeting DCLK1 in combination with platinum-based chemotherapy will combat resistance in HGSOC.

This proposal directly addresses two of the Ovarian Cancer Research Program’s Areas of Emphasis:

1. Understand the basic biology and etiology of ovarian cancer initiation, progression, metastasis, recurrence, genetics, and other critical events.

2. Develop novel therapeutic strategies for treatment and prevention.

At the completion of this study, we will have evaluated the mechanism by which DCLK1 drives resistance to platinum-based chemotherapy, optimized the various therapeutic strategies to target DCLK1, and determined the efficacy and mechanism of targeting DCLK1 to overcome resistance in preclinical models. We expect that this research will reveal new treatment strategies that can be evaluated in the near term to improve overall outcomes for ovarian cancer patients. Specifically, therapeutic targeting of DCLK1 could be used clinically in combination with front-line chemotherapy to enhance tumor cell killing at initial treatment and may also be used as a “maintenance” therapy by specifically targeting CSC-like cells to prevent recurrences.

The long-term outcomes of this study are the identification of a new therapeutic target that can be applied directly to the clinic to improve therapeutic outcomes and extend life in patients with OC. Improving therapeutic outcomes and extending life for ovarian cancer patients will directly impact the health and well-being of Service Members, Veterans, their Families, and all women impacted by this highly lethal disease.

Proposal Title:	Targeted Chemoimmunotherapy of Drug-Resistant Ovarian Cancer
Log Number:	OC220216
Current PI Name:	Arash Hatefi
Award Number:	HT9425-23-1-0191
Current Contracting Organization:	Rutgers, New Jersey, State University of
Current Performing Organization:	Rutgers, New Jersey, State University of
Web Approval Date:	04-18-2023

FY22 OCRP Area of Emphasis: Develop novel therapeutic strategies for treatment and prevention

Background: Ovarian cancer has the highest mortality rate among all gynecologic malignancies. When a normal ovarian cell turns cancerous, it freely spreads throughout the peritoneal cavity. The low rate of survival for ovarian cancer patients is mainly due to two factors: (1) the advanced stage of the disease at diagnosis, wherein the cancer cells have metastasized into the peritoneal cavity and (2) the inadequate efficacy of the available treatment options for recurrent disease. The standard of care for patients with primary ovarian cancer is cytoreductive surgery (removal of ovaries and visible tumors), followed by chemotherapy with platinum-based drugs (e.g., cisplatin) and/or paclitaxel. Although most ovarian tumors respond to standard of care at diagnosis, approximately 90% of patients after suboptimal surgery and 70% of patients after optimal surgery will experience a relapse within 18–24 months; this course for ovarian cancer follows the cancer stem cell model, which considers cancer stem-like cells with self-renewal capacity to be responsible for cancer recurrence and metastatic spread. While rapidly proliferating cancer cells are sensitive to chemo/radiation therapy, cancer stem-like cells are slow-growing, which helps them resist therapy. Therefore, to prevent relapse and provide survival benefits, it is crucial to eradicate both high-proliferating metastatic ovarian cancer cells as well as cancer stem-like cells. The objective of this research is “to develop a targeted therapy that kills both rapidly proliferating ovarian cancer cells and slow-growing/dormant drug-resistant cancer stem-like cells and to demonstrate not only the eradication of metastasis, but also the inhibition of relapse.” To achieve this goal, we have designed a novel approach that combines targeted chemotherapy with natural killer (NK) cell-based immunotherapy. The targeted chemotherapy is designed to not only target and kill the rapidly proliferating ovarian cancer cells without toxicity to normal cells, but also make the cancer stem-like cells visible and vulnerable to the NK cells. The NK cell-based therapy is designed to hunt for the remaining cancer stem-like cells that survived targeted chemotherapy.

Our laboratory has engineered and isolated a unique adipose-derived stem cell clone that actively and rapidly migrates towards ovarian tumors making it possible to target and deliver potent cytotoxic drugs specifically to ovarian tumors eliminating potential toxicity to normal tissues. For NK cell-based immunotherapy, we will use NK92 cells which is an FDA-approved cell line and suitable for human use. Clinical trials data show that allogenic NK92 cells can be injected into patients safely without adverse effects. This is especially helpful to patients who have gone through multiple rounds of therapy and lost a significant number of their lymphocytes.

If successful, the developed therapeutic protocol will be a pioneer in overcoming drug-resistance in recurrent metastatic ovarian cancer and in providing survival benefits for ovarian cancer patients who currently do not have any viable therapeutic option. Consequently, the success of this research project will have a significant impact on mortality in veterans, service women and their families, as well as in the general population.

Proposal Title:	Disabling Metastasis by Targeting the Ferroptosis Protection Mechanism of Serum
Log Number:	OC220231
Current PI Name:	Jen Tsan Chi
Award Number:	HT9425-23-1-0498
Current Contracting Organization:	Duke University
Current Performing Organization:	Duke University
Web Approval Date:	09-13-2023

Most women diagnosed with epithelial ovarian cancer (OVCA) first go through surgical removal and chemotherapy and enter a period of remission. However, this initially promising response is often followed by recurrence with metastasis to the peritoneal cavity (belly) or other distant organs. Such unfortunate events often occur within months, but sometimes even years later. Metastatic tumors are often unresponsive to the same chemotherapy initially effective for the original tumor, leading to unfortunate suffering and death. Therefore, we urgently need to identify new treatment approaches to reduce metastasis.

During metastasis, tumor cells need to dissociate from their neighboring cells, migrate and travel long distances to metastasize. While the traditional view is that these metastatic cells travel via ascites and in the belly, several recent studies have shown that many metastasis OVCA actually occur through the bloodstream to settle in the final destination organs. However, very little is known about how we can eliminate these traveling cells to reduce metastasis. We and others have found that these detached and traveling cancer cells, even when they have become resistant to chemotherapy, are very sensitive to a special kind of cell death called “ferroptosis.” An obvious question is: if these traveling tumor cells are so sensitive to ferroptosis, how can they survive ferroptosis and make it all the way to their destinations and establish metastasis? We think we have found the secrets. Our research has found that blood and serum protect traveling cancer cells from ferroptosis and explain why these cells don’t die during their travel and can form metastasis. However, our research has found that two FDA-approved drugs, auranofin (for joint pain) or bezafibrate (for high lipids), can abolish this protection by blood or serum, making them sensitive to ferroptosis again. Therefore, we will test whether we can push these traveling tumor cells toward ferroptosis to reduce metastasis as new treatments.

Our objective is to identify novel treatment strategies to prevent or eliminate metastatic ovarian cancer, which is highly relevant to the vision and mission of OCRP to “eliminate ovarian cancer” and “develop and validate models to understand the metastasis, treatment response, and recurrence of ovarian cancer.”

What Types of Patients Will It Help and How Will It Help Them? Most women diagnosed with ovarian cancer are at risk for metastasis. Therefore, our efforts to reduce or potentially eliminate metastatic OVCA will benefit the majority of women with advanced epithelial ovarian cancer for whom there are currently no such types of treatment.

What Are the Potential Clinical Applications, Benefits, and Risks? We will find out whether triggering ferroptosis, a new form of cell death, can be used to eliminate metastatic OVCA, especially when the protective effects of blood are disabled by two FDA-approved drugs, auranofin or bezafibrate used by billions of people. If successful, this will lead to an entirely new regimen of treatment that targets metastatic tumor cells’ survival. Several ferroptosis-inducing agents are in early clinical development. Therefore, depending on the progress of drug development and regulatory procedures, they could be available in 5-10 years. Risks include the possibility of undesirable side effects, as is true with any drug. Potential benefits are the ability to reduce metastatic and treatment-resistant OVCA.

What Is the Potential Impact on the Health and Welfare of Military Service Members and Their Families? Military Service Members, of which nearly 15% are women, as well as the women in their Families, are all vulnerable to ovarian cancer, with potentially increased risk from exposure to ionizing radiation and toxic chemicals during their service. The novel therapies will potentially benefit the health and welfare of these Service Members and their families by reducing and hopefully even preventing metastatic disease altogether.

Proposal Title:	Development of New Therapeutic Strategies of Chemobrain for Ovarian Cancer Survivors
Log Number:	OC220235
Current PI Name:	Mi-Hyeon Jang
Award Number:	HT9425-23-1-0364
Current Contracting Organization:	Rutgers, New Jersey, State University of
Current Performing Organization:	Rutgers, New Jersey, State University of
Web Approval Date:	07-31-2023

Ovarian cancer is the sixth most common cancer in women with more than 20,000 individuals diagnosed in the United States each year. Taxane and platinum-based chemotherapies are standard treatment for ovarian cancer and these treatments help avoid death from this disease. Nearly 70% of ovarian cancer survivors suffer from chemotherapy-induced cognitive impairment (CICI or chemobrain) and, unfortunately, the cognitive (memory and mood-anxiety) dysfunction continues well after chemotherapy has ended. Since the median survival for patients with ovarian cancer exceeds 5 years and about one third of patients are cured, CICI is of significant medical concern, as it negatively affects quality of life. Therefore, our research hopes to establish an understanding of the pathological mechanisms that drive CICI and, consequently, find effective therapies to help cancer survivors cope with these negative side effects. Given the critical importance that military Service Members, their families, as well as Veterans, provide to the security and overall well-being of the United States general population, it is imperative to find ways to improve quality of life for past (Veterans) and presently active U.S. Service Members or their families who are cancer survivors. Overall, by improving survivorship, our proposal will improve defense readiness for active members of the military so that they can perform their duties in service of our country.

In our recent studies, we revealed that cisplatin significantly increases the levels of the adenosine A2A receptor (A2AR) protein in the adult mouse hippocampus, a brain region known for its control of learning and memory. In addition, our preliminary results show that this phenotype is shared by paclitaxel and methotrexate chemotherapies, indicating that A2AR induction may be a common pathological mechanism mediating CICI. Most importantly, istradefylline, a drug that targets the A2AR and prevents it from increasing in function, significantly prevents cisplatin-induced cognitive deficits without promoting tumor growth or interfering with cisplatin’s anti-tumor activity.

Based on our observations, we will investigate whether chemotherapy causes cognitive impairment due to robust A2AR induction and that, conversely, by inhibiting A2AR, we can show that this is a safe and effective therapeutic strategy against CICI. To test this novel hypothesis, we will elucidate whether the FDA-approved A2AR inhibitor istradefylline has a prolonged preventative effect against CICI and/or rescues symptoms after onset of CICI in aged mice. Furthermore, given the importance of A2AR inhibition in preventing tumor growth as observed in multiple tumor bearing mouse models, we will further evaluate the impact of istradefylline-mediated A2AR inhibition on tumor growth and istradefylline’s ability to avoid interfering with chemotherapy’s anti-neoplastic activity using ovarian cancer tumor-bearing mouse models. Subsequently, by using mice which have had genetic deletion of the A2AR in specific cell types (e.g., cell type-specific conditional A2AR knockout mice), we will determine which neural cell type increases A2AR expression due to chemotherapy treatment.

Additionally, using these mouse models, we will determine whether increased A2AR abundance in these cell types is responsible for CICI. Last, to achieve long-lasting A2AR inhibition in a cell type-specific manner, we will develop an innovative delivery system where we genetically modify an inert, non-pathogenic viral sequence that silences the A2AR (siRNA-A2AR) and package this payload in nanoparticles which we will

deliver directly in the brain of our mouse models to determine whether this treatment can maximize therapeutic efficacy in preventing CICI.

Taken together, the short-term impact of our proposed work is to provide critical pathological mechanisms mediating CICI. Our long-term impact is to pave the way for transformative clinical interventions to prevent and/or mitigate the cognitive disabilities associated with chemotherapy, ultimately improving the quality of life for ovarian cancer survivors, ranging from active-duty Service Members and Veterans, as well as their respective family members, who are negatively impacted by CICI. We believe that, by repurposing istradefylline and applying nano-technology, our proposed study will help elucidate the novel therapies that can be quickly and safely moved forward to clinical trials for CICI as well as conceptually advance the fields of cancer research, regenerative medicine, and nano medicine. Therefore, by improving survivorship through our innovative research, our proposal will improve defense readiness for active members of the military so that they can perform their duties in service of our country.

Proposal Title:	Development of New Therapeutic Strategies of Chemobrain for Ovarian Cancer Survivors
Log Number:	OC220235P1
Current PI Name:	Ki Bum Lee
Award Number:	HT9425-23-1-0365
Current Contracting Organization:	Rutgers, New Jersey, State University of
Current Performing Organization:	Rutgers, New Jersey, State University of
Web Approval Date:	07-31-2023

Ovarian cancer is the sixth most common cancer in women with more than 20,000 individuals diagnosed in the United States each year. Taxane and platinum-based chemotherapies are standard treatment for ovarian cancer and these treatments help avoid death from this disease. Nearly 70% of ovarian cancer survivors suffer from chemotherapy-induced cognitive impairment (CICI or chemobrain) and, unfortunately, the cognitive (memory and mood-anxiety) dysfunction continues well after chemotherapy has ended. Since the median survival for patients with ovarian cancer exceeds 5 years and about one third of patients are cured, CICI is of significant medical concern, as it negatively affects quality of life. Therefore, our research hopes to establish an understanding of the pathological mechanisms that drive CICI and, consequently, find effective therapies to help cancer survivors cope with these negative side effects. Given the critical importance that military Service Members, their families, as well as Veterans, provide to the security and overall well-being of the United States general population, it is imperative to find ways to improve quality of life for past (Veterans) and presently active U.S. Service Members or their families who are cancer survivors. Overall, by improving survivorship, our proposal will improve defense readiness for active members of the military so that they can perform their duties in service of our country.

In our recent studies, we revealed that cisplatin significantly increases the levels of the adenosine A2A receptor (A2AR) protein in the adult mouse hippocampus, a brain region known for its control of learning and memory. In addition, our preliminary results show that this phenotype is shared by paclitaxel and methotrexate chemotherapies, indicating that A2AR induction may be a common pathological mechanism mediating CICI. Most importantly, istradefylline, a drug that targets the A2AR and prevents it from increasing in function, significantly prevents cisplatin-induced cognitive deficits without promoting tumor growth or interfering with cisplatin’s anti-tumor activity.

Based on our observations, we will investigate whether chemotherapy causes cognitive impairment due to robust A2AR induction and that, conversely, by inhibiting A2AR, we can show that this is a safe and effective therapeutic strategy against CICI. To test this novel hypothesis, we will elucidate whether the FDA-approved A2AR inhibitor istradefylline has a prolonged preventative effect against CICI and/or rescues symptoms after onset of CICI in aged mice. Furthermore, given the importance of A2AR inhibition in preventing tumor growth as observed in multiple tumor bearing mouse models, we will further evaluate the impact of istradefylline-mediated A2AR inhibition on tumor growth and istradefylline’s ability to avoid interfering with chemotherapy’s anti-neoplastic activity using ovarian cancer tumor-bearing mouse models. Subsequently, by using mice which have had genetic deletion of the A2AR in specific cell types (e.g., cell type-specific conditional A2AR knockout mice), we will determine which neural cell type increases A2AR expression due to chemotherapy treatment.

Additionally, using these mouse models, we will determine whether increased A2AR abundance in these cell types is responsible for CICI. Last, to achieve long-lasting A2AR inhibition in a cell type-specific manner, we will develop an innovative delivery system where we genetically modify an inert, non-pathogenic viral sequence that silences the A2AR (siRNA-A2AR) and package this payload in nanoparticles which we will

deliver directly in the brain of our mouse models to determine whether this treatment can maximize therapeutic efficacy in preventing CICI.

Taken together, the short-term impact of our proposed work is to provide critical pathological mechanisms mediating CICI. Our long-term impact is to pave the way for transformative clinical interventions to prevent and/or mitigate the cognitive disabilities associated with chemotherapy, ultimately improving the quality of life for ovarian cancer survivors, ranging from active-duty Service Members and Veterans, as well as their respective family members, who are negatively impacted by CICI. We believe that, by repurposing istradefylline and applying nano-technology, our proposed study will help elucidate the novel therapies that can be quickly and safely moved forward to clinical trials for CICI as well as conceptually advance the fields of cancer research, regenerative medicine, and nano medicine. Therefore, by improving survivorship through our innovative research, our proposal will improve defense readiness for active members of the military so that they can perform their duties in service of our country.

Proposal Title:	Drugging the Undruggable: Programming Ovarian Cancer Cells as the Next-Generation Immunotherapy
Log Number:	OC220238
Current PI Name:	Ming-Ru Wu
Award Number:	HT9425-23-1-0455
Current Contracting Organization:	Dana-Farber Cancer Institute
Current Performing Organization:	Dana-Farber Cancer Institute
Web Approval Date:	09-13-2023

Scientific Objective: Our goal is to develop a transformative new strategy—Tumor Immunotherapy by Gene-circuit Engineered Response (TIGER) for ovarian cancer. Ovarian cancer is the most lethal gynecological malignancy in the USA and usually relapses after standard treatment. TIGER forces tumors to recruit immune cells to kill primary tumors and metastasis. To achieve this goal, we have designed artificial gene circuits activated explicitly in ovarian cancer cells. These gene circuits will command ovarian cancer cells to secrete immune modulators that attract immune cells to target the tumors for destruction. This effect will also in-duce long-term immune memory against tumor relapses. Here, we will develop, optimize, and validate the effectiveness of TIGER within in vitro and in vivo mouse models of ovarian cancer.

Rationale: The immune system has been harnessed to treat a variety of blood cancers, including acute leukemia and multiple myeloma, via cell-based therapies. These strategies require isolation, engineering, and expansion of immune cells from each patient, which is expensive and labor-intensive. Furthermore, these approaches have not yet been applied successfully against ovarian cancer, which poses additional therapeutic challenges due to the tumor heterogeneity and immune suppressive microenvironment. Thus, there is an urgent need for novel, safe, and effective therapies.

We aim to develop novel therapies that act from within tumors to recruit and activate immune cells into tumors— Trojan horse approach. Specifically, we will design genetic circuits that can be delivered locally or systemically, sense when they are inside cancer cells, and respond by producing combinations of complementary immune modulators from within tumors. These immune modulators will condition the tumor microenvironment to favor immune response and recruit immune cells into the tumors, thus harnessing the immune system to target primary tumors and establishing long-lasting protection against metastasis and recurrence. TIGER can be modulated and shut off if needed, thus providing controllable safety switches. Furthermore, TIGER does not require custom cellular engineering for every patient, thus enabling greater patient access and reduced burden on healthcare infrastructure. TIGER can also be used with other cancer therapies to achieve enhanced efficacy.

Specific Aims: In Aim 1, we will identify the optimal therapeutic output combination that confers the strongest efficacy. We will also determine the minimal percentage of cancer cells that need to be targeted by TIGER to achieve therapeutic efficacy in various clinically-relevant mouse models. In Aim 2, we will elucidate the immune response triggered by TIGER. Furthermore, we will optimize the capacity of TIGER to trigger epitope spreading that counteracts tumor heterogeneity and immune memory to prevent tumor relapses. This work will establish key parameters for successful immunotherapy against ovarian cancer and enable the optimization of designs for future preclinical and clinical trials.

Area of Emphasis: We aim to address the following FY22 OCRP Area of Emphasis: develop novel therapeutic strategies for treatment and prevention.

Which Individuals Will It Help and How Will It Help Them? This work will benefit ovarian cancer patients, especially ones with metastatic or recurrent diseases, with a new and potentially powerful and long-lasting therapy.

What Are the Potential Clinical Applications, Benefits, and Risks? Our strategy can potentially become a new clinical therapy for ovarian cancer. The potential benefits of this technology include providing highly effective treatment for ovarian cancer and protection against future relapse. The potential risks of this technology include the challenge of targeting heterogeneous solid tumors, although we have outlined a comprehensive plan to minimize this risk with a set of alternative strategies.

What Is the Potential Impact on Service Members, Veterans, Family Members, and All Women Impacted by this Disease? We anticipate that, by the end of the 3 years of this grant, we will have optimized our therapeutic circuits and validated their therapeutic efficacy in multiple mouse ovarian cancer models. If successful, we anticipate that preclinical and clinical development can commence immediately after that, thus accelerating the time scale to impact patient health. If successful, this research will provide a novel therapeutic strategy for ovarian cancer, with the potential to replace existing treatments that have toxicities, and to treat metastatic and recurrent diseases, which are the major causes of mortality. It will also reduce the cost, labor, and infrastructure needed for therapeutic application.

Proposal Title:	Analysis of Host Obesity and Therapeutic Response in Ovarian Cancer
Log Number:	OC220251
Current PI Name:	Mary Stack
Award Number:	HT9425-23-1-0781
Current Contracting Organization:	Notre Dame, University of
Current Performing Organization:	Notre Dame, University of
Web Approval Date:	09-28-2023

Rationale: The majority of women with epithelial ovarian cancer (OvCa) are diagnosed with metastatic disease, resulting in a poor 5-year survival due to painful complications that result from widely disseminated intra-peritoneal (i.p., abdominal cavity) metastases. Obesity is increasingly widespread in the U.S. and worldwide. A number of studies have shown that there is a link between obesity and incidence of OvCa (serous, endometrioid, and mucinous types). Obesity is also associated with poor survival. A particularly interesting study looking at genes expressed in the tumors of women with high-grade serous OvCa (HGSOC) found that those women whose tumors expressed a large number of obesity- and fat metabolism-related genes had worse survival when compared to women whose tumors did not express these genes. This prompted us to develop experimental models to better understand the relationship between obesity and OvCa.

We have used mouse models to explore the link between obesity and OvCa. In these experiments, mice are fed a high fat diet (HFD) and are compared to control mice fed a low-fat diet (LFD). We found that the HFD mice had much larger and more numerous metastatic lesions. These lesions were loaded with fat droplets and expressed a protein called “sterol regulatory element binding protein 1” (SREBP1) that is a “master regulator” in the cell, instructing the tumor to transport fats into the cell and use them for energy and rapid growth. These tumors from HFD mice also contained different types of immune cells, with more “tumor-promoting” immune cells and fewer “tumor-killing” immune cells relative to tumors in the LFD mice. We found the same pattern when we analyzed tumors from women with HGSOC who have a high body mass index (BMI>30). These results show that the mouse model faithfully reproduces what is found in women with OvCa. We then treated these groups of mice with standard of care (SOC) chemotherapy (carboplatin and paclitaxel), with the dose adjusted to their weight, and showed that HFD mice respond poorly to treatment, with significantly more residual disease and faster recurrence. Interestingly, if we block SREBP1 together with SOC, we see a much longer time to recurrence, indicating that this novel combination therapy will likely enhance overall survival.

Central Problem: We plan to evaluate the effect of several SREBP1-blocking drugs on tumor progression in HFD/LFD mice treated with standard-of-care chemotherapy. We will also evaluate the influence of obesity on immune cells, the cancer stem cell population, and metastatic “tumorspheres” that participate in recurrence.

Area of Emphasis: Proposed studies address two FY22 OCRP areas of interest: (i) understand the basic biology of ovarian cancer progression, metastasis, recurrence, and other critical events and (ii) develop novel therapeutic strategies for treatment.

We propose to use our preclinical mouse models of obesity (HFD/LFD) to (i) gain a molecular-level understanding of how and why obesity promotes tumor growth and metastatic spread; (ii) understand why standard-of-care chemotherapy is less effective in the context of obesity; (iii) test drugs that block the

activity of molecules we identified as important in HFD models (like SREBP1) to see whether they improve the response to chemotherapy in HFD subjects; and (iv) reverse the immune landscape to more of a “tumor-killing” scenario.

Potential Impact: U.S. active-duty Service Women, women Veterans, and women spouses/partners of Service Members exhibit the same incidence rates of gynecological cancers as the general population; namely a 1 in 78 incidence rate of OvCa and a 1 in 108 lifetime chance of death from OvCa. Obesity has an adverse effect on survival of women with OvCa, implicating a link between host obesity, metastatic success, and response to therapy. Our preliminary data support the hypothesis that therapeutic targeting of SREBP1 in combination with SOC delays disease recurrence, indicating that this new therapeutic approach may enhance overall survival. Importantly, the SREBP1-targeting agent used in the preliminary data is already FDA-approved. Thus, pending successful completion of the proposed pre-clinical studies, results could be immediately translated to the clinic to improve outcomes of women with ovarian cancer in the context of obesity.

Proposal Title:	miR-195 Re-Expression: A Therapeutic Strategy for Chemoresistant Ovarian Cancer
Log Number:	OC220252
Current PI Name:	Shailendra Dwivedi
Award Number:	HT9425-23-1-0772
Current Contracting Organization:	Oklahoma, University of, Health Sciences Center
Current Performing Organization:	Oklahoma, University of, Health Sciences Center
Web Approval Date:	09-28-2023

Scientific Objective and Rationale: Around 85-90% of ovarian cancer (OvCa) patients initially respond to the therapy, but in most of the patients, the tumor reoccurs and does not respond to the therapy; these tumors are known as drug-resistant and are the main reasons for the higher mortality rate in OvCa. However, the mechanism responsible for the drug-resistant phenotype in OvCa is still evolving, and further research is urgently needed to overcome the therapeutic resistance and poor prognosis. In my previous research, I have shown that a small RNA (18-22 nucleotide long) known as micro-RNA-195 (miR-195), which does not code for any protein, is under-expressed in OvCa. Our results show that re-expressing in OvCa cells reduced their cancer-causing properties by regulating the expression of mitochondrial calcium uptake 1 (MICU1) protein. Using a mouse model of ovarian cancer, we reported that miR-195 re expression significantly reduces tumor growth, increases tumor doubling times, and enhances the overall survival of the tumor-bearing mouse. However, the role of miR-195 in the drug resistance of OvCa has not been elucidated and is the goal of the proposed research. Interestingly, by analyzing the publicly available database we found that miR-195 may target WNT7A, one of the key proteins responsible for Wnt/beta-catenin singling. WNT7A is upregulated in OvCa and is not detected in normal ovaries. The upregulation of WNT7A is responsible for the activated Wnt /beta-catenin singling, known for cancer recurrence by regulating stem-like properties in cancer cells with drug-resistant phenotype. Our preliminary data show that miR-195 negatively regulates Wnt/beta-catenin signaling pathway in OvCa cells. Based on these results, we hypothesize that the under-expression of miR-195 in OvCa is responsible for WNT7A upregulation and evolution of the drug resistance phenotype. We will use following specific aims to test the hypothesis and accomplish overall objectives:

Aim 1. Investigate the role of miR-195 expression in drug-resistant OvCa.

Aim 2. Evaluate the effect of miR-195 on metastasis and drug sensitivity in an OvCa model using an auroliposome-mediated miR-195 delivery system.

Successful completion of the project will establish miR-195 as a regulator of drug resistance in OvCa and a potentially translatable strategy, by our recently developed highly efficient auroliposome delivery method that can deliver miRNA and siRNA with almost no side effects.

PI’s Career Goals: An inadequate understanding of adaptive signaling coupled with limited treatment options for a chemo-resistant tumor is likely a cause of poor outcomes. A thorough understanding of drug resistance mechanisms is needed, as this remains the main obstacle in treating patients with recurrent disease. Therefore, to improve outcomes in OvCa, the identification and development of new targets is a priority and motivation behind my ongoing research in OvCa. I have been working on various aspects of OvCa and non-coding RNA since Sept 2013 and have acquired a unique understanding of OvCa and microRNA biology, coupled with my doctoral research experience in drug development along with the experience of nanoliposome delivery systems gives me a unique ability to decipher the detailed role of miR-195 in drug-resistant OvCa and targeting these signaling pathway for the overall benefit of OvCa patients. The protected time afforded by the Ovarian Cancer Academy – Early-Career Investigator Award (OCA-

ECIA) will not only allow me to evaluate my hypothesis, it will also provide me an opportunity for collaborative research and acquire skills and specific knowledge that will help me become established as an independent investigator. The collaborative mentorship from OCA and my mentors will provide an ideal platform to develop my career as a scientist and become an independent investigator.

Applicability of the Research: OvCa is typically diagnosed at advanced stages or at the time when the tumor has already spread to other parts of the body (metastasized), because complete remission is infrequent and is not achieved in almost half of the women diagnosed with ovarian cancer. Consequently, management and treatment of this disease is challenging as many patients are faced with tumor recurrence further complicated by drug resistance. The objective of the proposed research is to understand the process and to make them respond to the therapy by miR-195 re-expression. In addition, these results and methods developed during the research will apply to other cancer as well where miR-195 is known to be down-regulated.

Relevance to Military: Women in the military are at increased risk of developing OvCa, in part due to elevated exposure to carcinogens present in diesel exhaust, ionizing radiation, and cigarette smoke. Thus, developing novel innovative therapeutic strategies that can also provide early prognosis and prevent drug resistance has major implications for both the high-risk individuals, including military personnel as well as the general population.

Proposal Title:	RBP-Mediated MLO Formation in Chemoresistance and Cancer Recurrence
Log Number:	OC220267
Current PI Name:	Pradeep Chaluvally-Raghavan
Award Number:	HT9425-23-1-0311
Current Contracting Organization:	Wisconsin, Medical College of
Current Performing Organization:	Wisconsin, Medical College of
Web Approval Date:	07-13-2023

High-grade serous epithelial ovarian cancer (HGSOC) is the most common, aggressive and lethal form of ovarian cancer. Our research identified that an RNA binding protein, Fragile X Mental Retardation gene 1 (FXR1), is highly expressed in more than 40% of ovarian cancer patients. Notably, FXR1 expression is associated with poor overall survival in ovarian cancer patients.

Our data demonstrate that FXR1 is a key regulator of translation blocking (protein synthesis blocking) mechanisms of a set of important tumor suppressors in normal epithelial cells. Protein synthesis is required to be tightly regulated for the synthesis of an accurate amount of proteins for normal cellular functions. Therefore, disruption of normal translation (protein synthesis) by FXR1 leads to a deficiency of several tumor suppressor genes, which in turn causes aggressive cell proliferation, cancer cell invasion, metastasis, and resistance to chemotherapy.

Strikingly, our data demonstrated that FXR1 inhibits the protein synthesis of several tumor suppressors such as cyclin-dependent kinase inhibitors CDKN1A, CDKN1B, CDKN2A, and CDKN2B mRNA due to their condensation into membrane-less organelles (MLOs) in normal epithelial cells. Cyclin-dependent kinase inhibitors such as CDKN1A, CDKN1B, CDKN2A and CDKN2B are required to prevent cell cycle progression by normalizing the functions of cyclins in both normal and cancer cells. Therefore, our studies uncovering novel mechanisms that cause translational blockade of tumor suppressors will provide novel therapeutic opportunities to treat ovarian cancer.

We found that the MLOs formed by FXR1 protein serve as condensed units of protein-mRNA, complex where tumor suppressor genes are trapped, and their translation (protein synthesis) is inactivated. As a consequence, FXR1 promotes oncogenic transformation of normal cells to tumor cells. Therefore, the main objective of this proposal is to uncover the mechanism of how does FXR1 causes translational blockade of tumor suppressor genes for the survival of tumor cells and resistance to cell death mechanisms. Our preliminary data proved that FXR1 causes the condensation of protein-tumor suppressor mRNAs through liquid-liquid phase separation (LLPS) and MLO formation.

We hypothesize that the binding of FXR1 to the key tumor suppressor genes on the AU-Rich element (ARE) leads to the change in the secondary structure of mRNA. We also hypothesize that the binding of FXR1 on AREs causes changes in the secondary structure that will allow the condensation of FXR1-bound tumor suppressor mRNAs and lead to MLO formation. Therefore, the binding of FXR1 to a selective set of mRNAs results in a deficiency in the levels of tumor suppressor proteins in ovarian cancer cells, which causes resistance to chemotherapy and recurrence after therapy. We will test our hypothesis using the following aims: (1) delineate the mechanism of how FXR1 interacts with its target mRNAs for membrane-less organelles formation; (2) determine the mechanism of how FXR1 promotes membrane-less organelles formation; and (3) characterize the effect of FXR1 mediated MLO formation on ovarian cancer aggressiveness and recurrence.

Ovarian Cancer Advocacy Plans: A highly experienced ovarian cancer advocate, Ms. Jody Elliot, will participate as the ovarian cancer advocate in this research (Letter of Support attached). Ms. Elliot is an active participant in the Ray of Hope for Ovarian Cancer Cure, Inc. Ray of hope is a non-profit organization raise awareness and funds for the fight against ovarian cancer and to provide support for the warriors and their families. Ms. Elliot will bring the approaches she has used successfully in ovarian cancer advocacy to this grant. She will provide the patient perspective when research projects are designed and implemented and have been involved in all phases of the development of this proposal. Ms. Elliot will be actively involved in all aspects of the proposed research program, including planning and oversight, program evaluation, and/or dissemination of information to the public through her advocacy programs in Wisconsin, Illinois, and Minnesota.

Expected Outcome: The scientific question and the proposed research are significant because we expect that the completion of this proposal will identify novel mechanisms adopted by tumor cells to evade cell death and apoptosis for tumor cell recurrence when therapy stops. We expect that our studies will identify novel biomarkers that predict the outcome of ovarian cancer and identify novel targets for treating ovarian cancer. FXR1 is also amplified in other cancers such as endometrial, cervical and breast cancers, which are the other lethal cancers in women. Therefore, we expect that our results can be broadly applicable to other women's cancers that exhibit FXR1 amplification.

Military Relevance: Ovarian cancer afflicts Warfighters and Warfighters' partners, as well as other family members. On this background, our research on identifying novel biomarkers that predict the outcome of ovarian cancer and novel strategies to identify novel targets for treating ovarian cancer will lessen their suffering and enable them to return to active duty faster.

Proposal Title:	Next-Generation Chimeric Antigen Receptor T Cells Targeting FOLR1 for the Treatment of Ovarian Cancer
Log Number:	OC220273
Current PI Name:	Preet Chaudhary
Award Number:	HT9425-23-1-0506
Current Contracting Organization:	University of Southern California
Current Performing Organization:	University of Southern California
Web Approval Date:	09-28-2023

Ovarian cancer is the leading cause of cancer mortality in the United States. A majority of patients with ovarian cancer show high level expression of a protein called Folate Receptor (FR). Chimeric antigen receptor T (CAR-T) is a novel approach to genetically engineer immune cells to kill cancer cells. Despite the success of CAR-T in blood cancers, there are several limitations to this approach including lack of efficacy against solid tumor and toxicities. To overcome the above design limitations of the current generation CARs, we have developed a next generation platform, designated Synthetic Immune Receptor (SIR). The main objective of the proposal is to generate a panel of SIRs targeting FR for the treatment of ovarian cancers and to demonstrate their efficacy and safety using preclinical studies. Thus, the project's area of emphasis is to develop novel therapeutic strategies for treatment and prevention of ovarian cancer. At the end of the project period, we hope to have demonstrated the safety and efficacy of FR SIRs using pre-clinical studies and selected the optimal SIR construct which will lay the foundation for confirmatory studies before the initiation of human clinical trials in 5-6 years. In the long term, the FR SIR developed by this project could potentially revolutionize the treatment of ovarian cancers as a single injection of FR SIR could potentially result in long-term remission and perhaps cure of even advance metastatic disease. The FR SIR of the current proposals could be also combined with other forms of immunotherapy and/or targeted therapies to further improve the response rate or duration of remission. The project is relevant for military health as, according to Military Health Service data, over 20,000 DOD beneficiaries were diagnosed with ovarian cancer.

Proposal Title:	Targeting Tissue Transglutaminase to Enhance Antitumor Immunity in Ovarian Cancer
Log Number:	OC220291
Current PI Name:	Daniela Matei
Award Number:	HT9425-23-1-0997
Current Contracting Organization:	Northwestern University, Chicago, Illinois
Current Performing Organization:	Northwestern University, Chicago, Illinois
Web Approval Date:	09-13-2023

Central Problem: Ovarian cancer tends to come back after surgery and standard chemotherapy. The recurrent tumors are generally resistant to further chemotherapy. Immunotherapy harnesses the power of the immune system to attack and eliminate cancer cells. While immunotherapy is very active in many solid tumors, its efficacy remains modest in ovarian cancer. Thus, to improve the outcomes of women afflicted with this deadly cancer, studies to understand the causes of resistance to immunotherapy and to develop new combination treatments are very much needed.

Rationale: We have recently discovered a protein that promotes weakening of the immune system, which permits ovarian cancer to grow undisturbed in the peritoneal cavity. This protein, called TG2, has other well-studied functions that promote ovarian cancer growth and metastasis by directly affecting the cancer cells. We recently showed that inside immune cells, TG2 causes weakened killer T cells and active suppressive cells. We observed that blocking TG2 through genetic methods delays cancer progression. New drugs blocking TG2 are starting to be developed for use in the clinic. We reasoned that this represents an opportunity to study such drugs in combination with immunotherapy. In this project, we propose to study how TG2 interferes with the function of immune cells in animal models of ovarian cancer and in human tumor specimens. These studies will provide the foundation for new types of treatment.

Research Objectives: Armed with this knowledge, we propose to figure out: (1) how the protein TG2 weakens the function of killer immune cells, (2) how do immune cells in human tumors react around cancer cells that secrete TG2? and (3) do drugs that inhibit TG2 make immunotherapy work better in ovarian cancer ? If our project is successful, we can further develop TG2 inhibitors for clinical use along with immunotherapy. Furthermore, we can start other directions of research looking at more effective ways of blocking TG2 to attack ovarian cancer cells and to augment immune responses.

Relevance of the Research Project to the Mission of the OCRP: This research project is directly applicable to women with ovarian cancer, and the proposed combination treatment can make a difference in their battle with this fatal illness. Thus, the proposal fits well the mission of the OCRP to “support patient-centered research to treat and cure ovarian cancer” and will address “fundamental biological questions related to tumor progression” and “development of novel therapeutics” topics of high relevance to the 2022 OCRP.

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New Paradigms in Ovarian Cancer: This research project proposes a new idea to explain the lack of response to immunotherapy in ovarian cancer and a new target that could be exploited to remove this natural break. The success of this project will lead to a potential intervention for women with ovarian cancer aiming to enhance the natural anti-tumor immune defense. The new therapy could help women who have failed standard chemotherapy and have limited options to extend their lives, therefore addressing a key unmet need in ovarian cancer.

Research aiming to improve the outcome of fatal diseases affecting women, such as ovarian cancer, is also of particular significance to the military beneficiaries, because the number of women in the U.S. Armed Forces increased substantially from 2% in 1973 to 15% in 2002. In 2004, of 485,500 active Soldiers, 71,400 were women. Therefore, this translational project is highly significant to service members and their Families.

Proposal Title:	Targeting AXL Receptor Tyrosine Kinase with a Humanized Anti-AXL Monoclonal Antibody as a Novel Therapy for Ovarian Cancer
Log Number:	OC220297
Current PI Name:	Xinyan Wu
Award Number:	HT9425-23-1-0752
Current Contracting Organization:	Mayo Clinic
Current Performing Organization:	Mayo Clinic
Web Approval Date:	09-13-2023

High-grade serous ovarian cancer (HGSOC) is the most common subtype of ovarian cancer and accounts for most of the ovarian cancer mortality. There is an urgent need to identify novel and effective targeted therapies for patients with HGSOC. Our preliminary studies found that ~80% of HGSOCs produce a protein called AXL on the outside of cancer cells. Higher levels of this protein are significantly associated with worse clinical outcomes, such as shorter. AXL protein is a receptor tyrosine kinase that plays a critical role in regulating many important cellular processes that are also used by cancer cells to proliferate, migrate to new areas, and invade into neighboring tissues. Overproduction of AXL by cancer cells can enhance these processes and promote tumor growth and metastasis. AXL's innate feature as a cell surface protein makes it an ideal target for antibody-based therapy, like other antibody-based therapies with existing FDA approvals.

Our group has studied AXL protein for more than 8 years. Our previous studies on breast cancer discovered that AXL protein is overproduced in triple-negative breast cancer (TNBC). In order to specifically target AXL, we have developed a humanized anti-AXL monoclonal antibody (hMAb173) that can induce the rapid degradation of AXL and reduce cancer cell growth and tumor formation. Due to the tremendous translational potential of hMAb173, our research on TNBC received the Breakthrough Award from the Department of Defense Breast Cancer Research Program. The knowledge and therapeutic agents that developed in our study on breast cancer can be quickly applied to HGSOC and will greatly facilitate this proposed project.

In this study, we will evaluate the potential of targeting AXL with hMAb173 as a novel therapeutic agent for HGSOC. Two forms of hMAb173 will be tested, the free antibody and the same antibody conjugated to a chemotherapeutic agent (also known as an antibody-drug conjugate). We will validate the therapeutic efficacy by treating preclinical ovarian cancer patient-derived xenograft (PDX) animal models with hMAb173. We will also use a novel immunocompetent transgenic animal model to investigate the therapeutic advantage of combining hMAb173 with FDA-approved immunotherapy antibody drugs. In addition, we will use cutting-edge proteomics and transcriptomics to dissect the molecular mechanism of AXL in promoting ovarian cancer initiation and progression. Our proposed studies, if successful, will help define targeting AXL with our novel humanized antibody as a promising therapeutic agent for HGSOC. New clinical trials based on hMAb173 could result from our proposal for evaluating its efficacy in patients with HGSOC.

Proposal Title:	Targeting AXL Receptor Tyrosine Kinase with a Humanized Anti-AXL Monoclonal Antibody as a Novel Therapy for Ovarian Cancer
Log Number:	OC220297P1
Current PI Name:	Saravut Weroha
Award Number:	HT9425-23-1-0754
Current Contracting Organization:	Mayo Clinic
Current Performing Organization:	Mayo Clinic
Web Approval Date:	09-13-2023

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Proposal Title:	RAS Inhibitors for NF1-Deficient HGSOC
Log Number:	OC220311
Current PI Name:	Geoffrey Clark
Award Number:	HT9425-23-1-0283
Current Contracting Organization:	University of Louisville Research Foundation, Inc.
Current Performing Organization:	University of Louisville Research Foundation, Inc.
Web Approval Date:	07-10-2023

Objective: A significant sub-set of high-grade serous ovarian cancers (HGSOC) exhibit loss of function of the NF1 tumor suppressor. The NF1 protein serves as an important negative regulator of a notorious oncoprotein called RAS. Thus, when NF1 activity is lost, RAS loses negative regulation and becomes stabilized in the active form, even though it is not mutated itself. Experiments with cell lines and transgenic mice have shown this loss of NF1 function has a potent effect on HGSOC malignancy. Because RAS is such a common oncoprotein many attempts have been made to develop targeted inhibitors for it. These attempts have mostly failed. We appear to have succeeded in developing a direct RAS inhibitor than binds to and inhibits the function of all forms of RAS. We have shown our current best agent is active in vivo against tumor systems derived from patients with defects in NF1. We are sponsored by Biotech to produce enhanced activity variants of the compound for pancreatic cancer. We expect some of these compounds will also have enhanced activity against NF1 loss-driven ovarian cancers. In this project, we seek to obtain proof of principal that RAS inhibition can make a valuable contribution to HGSOC treatment.

Research: HGSOC has no good targeted-therapy options. This contributes to the dismal patient outcomes typically associated with the disease. Identifying excess RAS activity as potentially actionable therapeutic opportunity for treating HGSOC is a new paradigm that could be applicable to more than 20% of HGSOC. It may lead to the development of enhanced activity variants of our anti-RAS drug that are optimized for inhibition of NF1 loss driven cancers.

Relevance: It is highly relevant to the vision of the OCRP as it involves both developing new therapeutic strategies and improving precision medicine options.

Areas of Emphasis:

1. Develop novel therapeutic strategies for treatment and prevention.

2. Improve precision medicine.

Who Will It Help: If successful, the project will help HGSOC patients with defects in the NF1 tumor suppressor. This is at least 20% of HGSOC patients and probably considerably higher.

Impact: The project has the potential to have a positive impact in the short to medium term because we are funded to develop the compounds for a mutant RAS pancreatic cancer indication by a biotech company. Their goal is to have a compound ready for an IND application withing 18 months. It is likely that the final compounds will also have activity against wild-type RAS driven tumors, which would include NF1-ovarian cancers.

Proposal Title:	Image-Guided Bidirectional EphB4 Agonist-Based Therapy for Ovarian Cancer
Log Number:	OC220321
Current PI Name:	Yunfei Wen
Award Number:	HT9425-23-1-0500
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	07-31-2023

Ovarian cancer affects more than 22,000 women in the United States each year. Despite progress in surgery and chemotherapy, the disease still proves lethal to most patients. Most ovarian cancers have a positive response to initial chemotherapy, but over 70% of ovarian cancer patients ultimately develop metastasis, which inevitably becomes resistant to the existing chemotherapy drugs. These patients then succumb to the disease. Evaluation of multiple chemotherapeutic agents in several combinations and schedules has not yielded a significant overall improvement in outcome over the last 20 years. Clearly, new, effective therapies are urgently needed.

High-grade serous ovarian cancer (HGSC), the most aggressive subtype of ovarian cancer, accounts for two-thirds of all ovarian cancer deaths. In most cases of HGSC, the level of the protein EphB4 is high. EphB4 in ovarian cancer cells interacts with another protein, EFNB2. The interaction between EphB4 and EFNB2 has both a positive impact and a negative impact in terms of tumor control. The positive impact is that interaction between the two proteins can suppress the proliferation and metastasis of ovarian cancer cells. The negative impact is that interaction between the two proteins can stimulate angiogenesis (formation and growth of new blood vessels), which supports tumor growth. EphB4-based therapy has raised more attention clinically and has been tested as monotherapy or in combination with immunocheckpoints such as pembrolizumab in several solid cancers, including small cell lung cancer and advanced bladder cancer in phase II trials.

Recently, in a series of experiments designed to identify potential new drugs, we discovered a small molecule, which we called BIDEN-AP, that simultaneously promotes the positive impact and suppresses the negative impact of interaction between EphB4 and EFNB2. In mouse models of human cancer, BIDEN-AP suppressed tumor growth and metastasis. Furthermore, in mouse models of human cancer, BIDEN-AP also inhibited a process called epithelial-mesenchymal transition (EMT), which contributes to metastasis. In this proposal, we hypothesize that BIDEN-AP-based agents inhibit EMT and angiogenesis, suppress tumor growth, and overcome acquired resistance to anti-angiogenic therapies in HGSC. We further hypothesize that molecules based on BIDEN-AP can be used in combination with noninvasive medical imaging techniques to determine EphB4 expression status in HGSC tumors and tumor-associated blood vessel cells. The ability to determine EphB4 expression status in this way would help physicians select patients most likely to benefit from BIDEN-AP-based agents and monitor the response to treatment with such agents. On this ground, the proposed work is a direct response to address two of the FY22 W81XWH-22-OCRP-IIRA Areas of Encouragement: (a) Develop novel therapeutic strategies for treatment and (b) prevention and Improve precision medicine. To test this hypothesis, we will pursue three specific aims: (1) determine the effects of BIDEN-AP-based agents on inhibiting EMT and angiogenesis; (2) investigate the mechanisms of action for BIDEN-AP-based agents suppressing tumor growth and overcoming acquired resistance to anti-angiogenic therapy; and (3) determine the pharmacokinetics and pharmacodynamics of BIDEN-AP-based agents using positron emission tomography/computed tomography. We expect the proposed work to show that BIDEN-AP has strong potential to improve the clinical outcomes of patients with ovarian cancer that is not responding to current therapies and to show that noninvasive medical imaging is a useful tool for both

determining EphB4 expression level prior to therapy and re-assessing EphB4 expression status after treatment. Such a tool will greatly facilitate selection of patients for personalized therapy and monitoring of response to molecularly targeted therapies.

Proposal Title:	Image-Guided Bidirectional EphB4 Agonist-Based Therapy for Ovarian Cancer
Log Number:	OC220321P1
Current PI Name:	Chun Li
Award Number:	HT9425-23-1-0501
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	07-31-2023

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determining EphB4 expression level prior to therapy and re-assessing EphB4 expression status after treatment. Such a tool will greatly facilitate selection of patients for personalized therapy and monitoring of response to molecularly targeted therapies.

Proposal Title:	A Multifaceted Approach to Biomarker Discovery for BRCA-Associated Fallopian Carcinoma
Log Number:	OC220326
Current PI Name:	Kate Lawrenson
Award Number:	HT9425-23-1-0361
Current Contracting Organization:	Cedars-Sinai Medical Center
Current Performing Organization:	Cedars-Sinai Medical Center
Web Approval Date:	07-31-2023

The Central Problem: Survival from a diagnosis of ovarian cancer continues to be significantly affected by the frequent late stage of diagnosis of the disease. When ovarian cancers are detected early enough, the chances of survival in patients with the disease increase dramatically—5-year survival rates are more than 90% for patients at this stage. However, there are currently no clinical biomarkers that can detect the disease at the earliest, most treatable stages.

Addressing FY22 OCRP Areas of Emphasis: This proposal focuses on two FY22 OCRP priorities, specifically “understanding the basic biology and etiology of ovarian cancer initiation and progression” and “identifying and developing new strategies for screening, early-stage detection, prevention, accurate diagnosis and prognosis.” Using our experimental approaches, we expect to identify high-probability biomarkers for early-stage ovarian cancer detection based on unique cell types of normal fallopian tubes (FTs) and serous tubal intraepithelial carcinomas (STIC) precursors of ovarian cancer. In this study, we hypothesize that the development and characterization of human avatars of FT created from induced pluripotent stem cell (iPSC) models of women carrying germline mutations in genes known to cause ovarian cancer (BRCA1 and BRCA2) can identify biomarkers of early-stage pathogenesis of ovarian cancers. This will be further complemented using innovative single cell profiling of normal FT and STIC tissues from subjects with and without BRCA1/BRCA2 mutations. Single cell methods allow us to resolve cell-specific heterogeneity in tissues and help us find both cell types and specific molecular markers that are particularly associated with early-stage disease. Finally, one of the main reasons for the lack of success in this area in the past has been our inability to develop accurate and successful screening strategies for the detection of early-stage ovarian cancer. To address this challenge, we have developed “tissue-informed” methods for the analysis of cell free DNA to diagnose cancers in the blood (liquid biopsies). This validated method represents a novel approach to detect early- stage cancers in women before they can develop advanced ovarian cancer, which is without a doubt a more effective way to prevent deaths from ovarian cancer.

Patient Cohort: The research described in the proposal is based on a unique, clinically annotated biorepository of specimens collected over more than 30 years from subjects at high risk of ovarian cancers (the Gilda Radner Hereditary Cancer Program, or GRHCP). Specimens and clinical and epidemiological data have been collected for ~2,000 subjects screened for mutations in high and moderate penetrant susceptibility genes, including BRCA1 and BRCA2. More than 900 BRCA1/BRCA2 carriers have been identified and more than 60,000 liquid biopsy samples have been collected from these subjects following annual visits both prior to any diagnosis of ovarian cancer and in other subjects following a disease diagnosis. This biorepository is ideal for the identification of putative clinical biomarkers in BRCA1/BRCA2 carriers and non-carriers associated with early-stage diagnosis of ovarian cancer.

Clinical Implications: We expect this work to identify hundreds to thousands of previously unknown biomarkers that are associated with the development of ovarian cancer in patients with and without BRCA1 and BRCA2 mutations. Because of the novelty of the methods we are using, none of which have been used before in this context, we anticipate we will find several novel biomarkers that can detect ovarian cancers at

their earliest and most treatable stages. If the project is successful, it is likely that these strategies and/or the biomarkers we identify will be effectively used in clinical practice to screen for early-stage ovarian cancer as a prevention measure against further development to late-stage disease when outcomes are so poor.

Clinical Impact: Stage I ovarian cancer has 5-year survival rates of more than 90%, but this reduces to less than 30% for Stage IV ovarian cancer. However, because of challenges in understanding the early signs and symptoms of ovarian cancer, most cases are found in the late stages. Thus, these studies may have a substantial impact on reducing deaths due to ovarian cancer in Service Members, Veterans, and their family members, particularly if they carry a BRCA1/BRCA2 mutation, and in all women in the general population, by identifying biomarkers that can detect ovarian cancers early. Women comprise 15- 20% of the military and as the number of military Servicewomen and their operational scope increase, it is imperative to understand and manage health issues uniquely affecting women that challenge readiness.

Proposal Title:	A Multifaceted Approach to Biomarker Discovery for BRCA-Associated Fallopian Carcinoma
Log Number:	OC220326P1
Current PI Name:	Simon Gayther
Award Number:	HT9425-23-1-0362
Current Contracting Organization:	Cedars-Sinai Medical Center
Current Performing Organization:	Cedars-Sinai Medical Center
Web Approval Date:	07-31-2023

The Central Problem: Survival from a diagnosis of ovarian cancer continues to be significantly affected by the frequent late stage of diagnosis of the disease. When ovarian cancers are detected early enough, the chances of survival in patients with the disease increase dramatically—5-year survival rates are more than 90% for patients at this stage. However, there are currently no clinical biomarkers that can detect the disease at the earliest, most treatable stages.

Addressing FY22 OCRP Areas of Emphasis: This proposal focuses on two FY22 OCRP priorities, specifically “understanding the basic biology and etiology of ovarian cancer initiation and progression” and “identifying and developing new strategies for screening, early-stage detection, prevention, accurate diagnosis and prognosis.” Using our experimental approaches, we expect to identify high-probability biomarkers for early-stage ovarian cancer detection based on unique cell types of normal fallopian tubes (FTs) and serous tubal intraepithelial carcinomas (STIC) precursors of ovarian cancer. In this study, we hypothesize that the development and characterization of human avatars of FT created from induced pluripotent stem cell (iPSC) models of women carrying germline mutations in genes known to cause ovarian cancer (BRCA1 and BRCA2) can identify biomarkers of early-stage pathogenesis of ovarian cancers. This will be further complemented using innovative single cell profiling of normal FT and STIC tissues from subjects with and without BRCA1/BRCA2 mutations. Single cell methods allow us to resolve cell-specific heterogeneity in tissues and help us find both cell types and specific molecular markers that are particularly associated with early-stage disease. Finally, one of the main reasons for the lack of success in this area in the past has been our inability to develop accurate and successful screening strategies for the detection of early-stage ovarian cancer. To address this challenge, we have developed “tissue-informed” methods for the analysis of cell free DNA to diagnose cancers in the blood (liquid biopsies). This validated method represents a novel approach to detect early- stage cancers in women before they can develop advanced ovarian cancer, which is without a doubt a more effective way to prevent deaths from ovarian cancer.

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Clinical Implications: We expect this work to identify hundreds to thousands of previously unknown biomarkers that are associated with the development of ovarian cancer in patients with and without BRCA1 and BRCA2 mutations. Because of the novelty of the methods we are using, none of which have been used before in this context, we anticipate we will find several novel biomarkers that can detect ovarian cancers at

their earliest and most treatable stages. If the project is successful, it is likely that these strategies and/or the biomarkers we identify will be effectively used in clinical practice to screen for early-stage ovarian cancer as a prevention measure against further development to late-stage disease when outcomes are so poor.

Clinical Impact: Stage I ovarian cancer has 5-year survival rates of more than 90%, but this reduces to less than 30% for Stage IV ovarian cancer. However, because of challenges in understanding the early signs and symptoms of ovarian cancer, most cases are found in the late stages. Thus, these studies may have a substantial impact on reducing deaths due to ovarian cancer in Service Members, Veterans, and their family members, particularly if they carry a BRCA1/BRCA2 mutation, and in all women in the general population, by identifying biomarkers that can detect ovarian cancers early. Women comprise 15- 20% of the military and as the number of military Servicewomen and their operational scope increase, it is imperative to understand and manage health issues uniquely affecting women that challenge readiness.

Proposal Title:	Regulation of Ovarian Cancer Stem Cell Niche by Cancer-Associated Fibroblasts
Log Number:	OC220329
Current PI Name:	Anirban Mitra
Award Number:	HT9425-23-1-0605
Current Contracting Organization:	Indiana University
Current Performing Organization:	Indiana University
Web Approval Date:	09-28-2023

Ovarian cancer is the deadliest of all gynecologic cancers and the fifth leading cause of cancer-related deaths among women in the United States. While most patients respond well initially to the standard of care surgery and chemotherapy, a majority will suffer from disease relapse and will eventually develop chemoresistance. This is one of the main reasons for poor outcome of ovarian cancer patients. Disease relapse is triggered by residual tumors that are enriched in cancer stem cells, a population of cancer cells that are capable of seeding new tumors. Cancer stem cells constitute a small subpopulation in the tumor that are resistant to cytotoxic chemotherapy and, therefore, are a potential cause relapse and chemoresistance. Hence, targeting the cancer stem cells in combination with conventional chemotherapy would be an ideal approach to prevent recurrence. Most studies on cancer stem cells focus on cancer cells alone, while emerging evidence indicate that they exist in a complex microenvironment or niche. The cancer stem cell niche provides critical factors that are essential for the sustenance and growth of cancer stem cells. Poor understanding the reciprocal communications between cancer stem cells and their microenvironment remain a critical barrier to developing effective therapies targeting cancer stem cells to effectively control chemoresistance and relapse. Therefore, there is a need to identify and target the mechanism of reciprocal interactions with the microenvironment that maintains ovarian cancer stem cells and causes disease relapse.

We have identified cancer associated fibroblast (CAFs) as a key component of the tumor microenvironment that serve as an ovarian cancer stem cell niche. CAFs are a major constituent of the tumor and promote its growth and spread. It is well known that the residual tumors after neoadjuvant chemotherapy in ovarian cancer patients are enriched in fibroblasts, indicating their possible role in relapse. Recent research has shown that the presence of greater amounts of the non-cancer cells in the tumor microenvironment led to poor outcomes in ovarian cancer patients. Therefore, we studied the role of CAFs in promoting chemoresistance and relapse through providing an optimal environment for the growth of cancer stem cells. Using patient tumor-derived CAFs and 3D culture models mimicking the patient tumors, we have identified the mechanism by which CAFs can support ovarian cancer stem cells. Targeting this mechanism helped sensitize tumors to carboplatin in cell-based experiments and in a mouse model of ovarian cancer. Like cancer cells, CAFs in the tumors are also heterogeneous. We have now identified subpopulations of CAFs and cancer cells that productively talk to each other to maintain cancer stem cells. We propose to characterize these subpopulations and determine the mechanism of the crosstalk between them so that they can be effectively targeted to eliminate disease relapse.

The proposed research addresses the FY22 OCRP area of interest, “Understand the basic biology and etiology of ovarian cancer initiation, progression, metastasis, recurrence, genetics and other critical events.” Studying and targeting ovarian cancer stem cells in isolation is not the most efficient approach, as they exist in a specific microenvironment. Our studies indicate that this microenvironment can reprogram some bulk ovarian cancer cells back into cancer stem cells. Therefore, to effectively prevent relapse, our study will understand and target the crosstalk between the subpopulations of the microenvironment and ovarian cancer cells that maintain the cancer stem cell population. Since a vast majority of ovarian cancer patients suffer from disease relapse and development of chemoresistance, our study has the potential to strongly impact

their lives. Therefore, it is very relevant to the vision and mission of OCRP and will positively impact all women with ovarian cancer, including Service Members, Veterans, and their families.

Proposal Title:	Do Cytomegalovirus and Epstein-Barr Virus Infections Exacerbate Symptoms of Cognitive Decline Following Chemotherapy for Ovarian Cancer?
Log Number:	OC220359
Current PI Name:	Rachel Vogel
Award Number:	HT9425-23-1-0171
Current Contracting Organization:	Minnesota, University of, Twin Cities
Current Performing Organization:	Minnesota, University of, Twin Cities
Web Approval Date:	09-28-2023

Background: Cancer-related cognitive impairment (CRCI), often called “chemo brain,” is a frequent side effect of chemotherapy and can be devastating to individuals with cancer and affects quality of life (QOL). However, despite its importance for patients, CRCI is poorly understood. Recent research has linked virus infections and inflammation to cognitive decline in the general population. We have recently demonstrated that cytomegalovirus (CMV) and Epstein Barr virus (EBV) infections are common in ovarian cancer patients and may be associated with worse cognitive outcomes. Based on this preliminary evidence, the objective of the proposed study is to further assess and confirm the potential role of these viruses for CRCI and investigate the role of inflammation. Findings from this study could facilitate the development and testing of promising interventions targeted at these viruses, or inflammation, to prevent CRCI in those with ovarian cancer.

Area of Emphasis: This research project addresses the OCRP Area of Emphasis, “Identify and implement strategies to improve OCRP’s survivorship and quality of life.”

Approach: We will use data from two ongoing biorepository cohorts at the University of Minnesota (collected under the original OCRP award) and at the Moffitt Cancer Center (collected under the Ovarian Targeted Collection Project). We will analyze samples from 80 individuals with newly diagnosed with ovarian cancer.

These individuals will be selected based on the availability of CRCI data and available blood samples at three time points: at diagnosis, after frontline-line chemotherapy, and 6-12 months after the end of front-line chemotherapy. We will analyze the blood samples for CMV and EBV viral infection status and biomarkers of inflammation. We will then assess the associations between (1) CMV and EBV status and CRCI, (2) inflammation and CRCI, and (3) how viral infection status plays a role in the relationship between inflammation and CRCI.

Impact and Potential Clinical Significance: The proposed research will be relevant to military Service Members and other military beneficiaries and their family members who have either been diagnosed with ovarian cancer themselves or are otherwise affected by ovarian cancer. Linking cognitive outcomes in cancer survivorship to viruses and inflammation is novel. If we can confirm the hypothesized associations of CMV and EBV and inflammation with CRCI, this will directly lead to follow-up research studies in broader cancer populations who receive chemotherapy, including male cancer patients. Hence, this work will potentially have a far-reaching impact with implications for all cancers treated with chemotherapy, not just ovarian cancer. Our finding will also help to design future interventions to reduce CRCI. If targeting viruses such as CMV and EBC can disrupt CRCI from occurring after chemotherapy, steps could be taken to avoid CRCI.

Proposal Title:	Genomic Predictors of Ovarian Cancer Risk in the PLCO Trial
Log Number:	OC220361
Current PI Name:	Barbara Norquist
Award Number:	HT9425-23-1-0173
Current Contracting Organization:	Washington, University of
Current Performing Organization:	Washington, University of
Web Approval Date:	07-13-2023

Rationale: Ovarian cancer remains the deadliest gynecologic malignancy. Prior studies have indicated that around 20% of ovarian cancers are associated with identifiable inherited mutations (for example, mutations in the genes BRCA1 and BRCA2) that increase the risk of developing ovarian cancer, one of the highest proportions of any solid tumor. When these inherited risk mutations are identified in advance, patients have the opportunity to undergo highly effective and lifesaving interventions such as preventative removal of the fallopian tubes and ovaries. In addition to inherited mutations, the function of a gene can also be impacted by a process called promotor methylation, which effectively silences the gene in question and can lead to similar effects as a mutation. Constitutional promotor methylation of the BRCA1 gene has recently been identified as a possible risk factor for developing ovarian cancer and could be another indication to consider risk reduction strategies. Our group has also examined promotor methylation of the RAD51C gene (another ovarian cancer susceptibility gene) in ovarian tumors. This finding has not been assessed before in constitutional DNA and may represent another important cause of ovarian cancer risk.

The Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer screening trial is a population-based, randomized study of cancer screening in nearly 150,000 people aged 55-74. The participants from the PLCO trial offer a unique population to assess for inherited risk of ovarian cancer. Female subjects enrolled in the PLCO trial provided DNA samples and completed detailed surveys with their reproductive history, hormone use, and family history of malignancies. Around 400 women enrolled in PLCO went on to develop ovarian cancer during the study. Prior detailed examinations of ovarian cancer risk factors have been assessed in this population, but no study has been done defining inherited mutations or constitutional methylation in this group.

The central problem that this work addresses is the need to decrease ovarian cancer mortality through improving ovarian cancer prevention. Our goals are to (1) determine the frequency of inherited mutations in cases and controls in PLCO, (2) determine the frequency of constitutional promotor methylation of BRCA1 and RAD51C in cases and controls, (3) examine how these genomic changes (mutation and methylation) relate to clinical characteristics, and (4) see how inherited mutations and methylation interact with other known risk factors for ovarian cancer within the unique population of the PLCO trial. It is rare to have detailed information about hormone use, parity, age, race/ethnicity, and ovarian cancer screening results in a group of patients with OC who also have pre-diagnosis DNA samples. Our proposal is unique, in that other studies evaluating gene-environment interactions have focused on common variants and single nucleotide polymorphisms rather than our focus on pathogenic mutations and methylation. To further evaluate these findings, we will also assess how clinical ovarian cancer risk factors interact with inherited mutations within the large UK Biobank study.

Understanding constitutional promotor methylation of BRCA1 and RAD51C and ovarian cancer risk is likely to expand the population eligible for OC risk-reduction efforts, and our methods should provide the most accurate and sensitive detection of this finding thus far. The assessment of interactions between genomic risk factors and other potentially modifiable risk factors for ovarian cancer will help guide the health behaviors of patients at risk. Finally, we plan to create an enduring resource by providing the sequencing and methylation results back to the PLCO study group for use in future investigation.

Our multidisciplinary team is well positioned to carry out this project, combining expertise in inherited ovarian cancer and the clinical care of women who are at risk of ovarian cancer (Norquist/PI); expertise in molecular epidemiological studies from an investigator that has run multiple prior studies involving our target populations, PLCO and the UK Biobank (Trabert/Co-I); as well as our experienced biostatistician (Katz/Co-I). We are committed to reducing deaths from ovarian cancer through better prevention.

Proposal Title:	Understanding the Interplay of Aspirin and Other Commonly Used Medication and the Potential for Ovarian Cancer Chemoprevention
Log Number:	OC220370
Current PI Name:	Britton Trabert
Award Number:	HT9425-23-1-0247
Current Contracting Organization:	Utah, University of
Current Performing Organization:	Utah, University of
Web Approval Date:	07-13-2023

Rationale and Objective: Ovarian cancer is the most fatal cancer affecting women, largely due to the symptoms of the disease, like pain and pressure, being common and therefore leading to delays in diagnosis. Currently, there are no tests to detect early-stage ovarian cancer and very limited ways to prevent it. While women with higher risk of the disease because of genetic mutations in the BRCA1 or BRCA2 genes can have surgery to remove their ovaries, the disadvantages are that the surgery leads to infertility and early menopause and may not be acceptable to all who are eligible. Traditional cancer risk factors, like physical activity, body mass index, and diet are not associated with large increases in ovarian cancer risk. Oral contraceptive use for five years or longer can prevent ovarian cancer, but it is not suitable as a recommendation for all women due to harmful side effects in certain individuals (e.g., risk of venous thromboembolism in smokers) and undesirable side effects like weight gain. Thus, there is a great need to find prevention strategies to reduce the number of women being diagnosed with ovarian cancer. Further, given that all medications come with some risk of side effects, it is important to identify subgroups that will benefit most from ovarian cancer chemoprevention—the prevention of disease through medication use. The subgroups that will likely derive the most benefit of ovarian cancer chemoprevention are those who are at higher risk of ovarian cancer either through individual risk factors or due to genetic mutations, as the benefit of preventing ovarian cancer in this group likely offsets or outweighs the risk of side effects from the medication use.

Our group was funded previously to conduct a comprehensive analysis to understand whether aspirin could prevent ovarian cancer. As part of this research, we expanded the data resources in the Ovarian Cancer Cohort Consortium (OC3, including data from 13 large observational cohort studies) to include information on over-the-counter medications use (e.g., aspirin, Tylenol, ibuprofen), lifestyle factors, ovarian cancer risk factors, and ovarian cancers diagnoses. We found that aspirin reduced the risk of ovarian cancer and that women who are at higher risk of ovarian cancer due to the presence of one or more ovarian cancer risk factors (e.g., positive family history, never oral contraceptive use, no pregnancies) may be more likely to benefit from frequent aspirin use to reduce ovarian cancer risk. Unfortunately, we did not have the support to also collect the existing data on prescription medication use from all studies, and were not able to evaluate whether the benefit of aspirin was changed or altered by prescription medication use. Research evaluating statins and/or bisphosphonates as preventive agents for ovarian cancer have shown mixed results. However, encouraging preclinical and epidemiologic data, as well as the broad access, low cost, and low risk of major adverse side effects, makes the concept of repurposing these drugs for ovarian cancer chemoprevention appealing. It is also possible that prescription medication (i.e., statins or bisphosphonates) used alone or in combination with aspirin could also prevent ovarian cancer.

Problem to Be Addressed and How It Will Advance the Field: The proposed research both expands and extends our previous research as follows:

Aim 1. The project expands on our previous research to evaluate whether use of both aspirin and prescription medication use enhances or reduces the chemopreventive benefits of aspirin in reducing ovarian cancer risk.

Aim 2. The project extends our prior research to evaluate whether common prescription medications, particularly those that affect an important pathway of cellular and lipid metabolism (i.e., statins or bisphosphonates), may be used to prevent ovarian cancer and to identify groups of women that will receive the most benefit from these medications.

Aim 3. The project will also evaluate whether statins and/or bisphosphonates alter the tumor's ability to regulate lipids or alter the tumor immune microenvironment to help us understand the biologic ways in which these medications might prevent ovarian cancer. By addressing these important unanswered research questions we will be able to provide evidence to clinical and public health decision-making organizations that can inform prevention recommendations for ovarian cancer.

Clinical Applications, Benefits, Risks: Since aspirin, statins, and bisphosphonates have few major side effects, the potential public health impact of the proposed research is substantial. Identifying the characteristics of specific groups (e.g., number of ovarian cancer risk factors, age at medication use, etc.) that will receive the most benefit from medication used to prevent ovarian cancer, provides doctors with research that allows them to tailor their recommendations to individual patients. All of the medications we propose to evaluate are currently used to treat or prevent common diseases; aspirin and/or statins are used to prevent heart disease, and bisphosphonates are used to prevent or treat bone loss. If our results support that these medications also directly influence ovarian cancer risk, this will reinforce that they can prevent multiple diseases. Our research can also provide information to inform clinical trials of medications to prevent ovarian cancer in high-risk women. By understanding the biologic effects of these medications on the ovarian tumor environment, this work may identify other medications that could be tested for chemoprevention or to improve survival in ovarian cancer patients.

Impact of Proposed Research on Health and Well-Being of Individuals Impacted by This Disease: This innovative application combines epidemiologic and tumor tissue data to conduct a well-powered (large) investigation of whether prescription medication use can reduce the risk of ovarian cancer. This research will improve both the biologic understanding of how ovarian cancers grow and develop and the ability to make recommendations regarding the prevention of this fatal disease. The impact of our proposal is enhanced by its methodological innovation, rigor, and the demonstrated success of our previous Department of Defense-funded research on aspirin's ability to prevent ovarian cancer. The extensive resources we are leveraging combined with our experienced team and multidisciplinary focus will help us to better identify women who would be most likely to benefit from prescription medication repurposed for chemoprevention (i.e., "Personalized Medicine" or "Precision Prevention"). The proposed research will benefit all women, including military Service Members, their families, and other military beneficiaries.

Proposal Title:	Targeting Regulators of Outside-In Signaling in Ovarian Cancer
Log Number:	OC220373
Current PI Name:	Salvatore Condello
Award Number:	HT9425-23-1-0509
Current Contracting Organization:	Indiana University
Current Performing Organization:	Indiana University
Web Approval Date:	09-28-2023

Epithelial ovarian cancer is the most lethal of all gynecologic malignancies, with the majority of cases being diagnosed at an advanced stage. Current standard treatment of ovarian cancer, in both early and advanced stages, consists of complete cytoreductive surgery followed by chemotherapy, usually based on platinum /taxane combination. The initial response rate is high (70%-80%); but the majority of patients with advanced disease relapse within the first five years after diagnosis resulting in a cycle of repeated surgeries and additional rounds of chemotherapy. Recurrent ovarian cancer is not curable, due to the development of chemoresistance.

Both recurrence and spread have been linked to a small population of ovarian cancer cells called ovarian cancer stem cells, which are resistant to approved chemotherapy agents for the treatment of recurrent ovarian cancer, such as platinum-based drugs and PARP inhibitors. The accumulation of ascites in the abdomen provides a favorable environment, enriched in growth factors and proteins that protects cancer stem cells during chemotherapy and promotes their growth. To date, treatment strategies designed to eliminate the genesis of ovarian cancer stem cells still remain a significant challenge. One main obstacle toward a successful treatment option for ovarian cancer remains the molecular identification of these tumorigenic cells.

Our previous study demonstrated that tissue transglutaminase, an enzyme found to be active in ovarian tumors, protects cancer stem cells and stimulates their growth. We found that this enzyme is enriched at the membrane of cancer stem cells forming a complex with several receptors, such as integrins, which allow the stem cells to attach and grow in the peritoneal space. Additionally, we provided evidence that tissue transglutaminase binds to the cellular receptor frizzled 7 and that this interaction stimulates the oncogenic Wnt pathway in cancer stem cells. However, the exact mechanism by which tissue transglutaminase interaction with frizzled 7 promotes ovarian cancer progression and ovarian cancer stem cell survival after chemotherapy remains unknown. Our preliminary data discovered that the enzyme integrin-linked kinase, a signaling molecule recruited by beta 1 integrins upon binding to their extracellular-matrix ligands, such as fibronectin, is activated by the functional interaction between transglutaminase and frizzled 7, leading to Wnt aberrant signaling in ovarian cancer cells and cancer stem cells. In addition, our preliminary data demonstrate that integrin-linked kinase expression is upregulated in ovarian cancer cells and tumor specimens from ovarian cancer patients compared to the normal fallopian tube and ovarian epithelium, correlates with ovarian tumor grade, and promotes ovarian cancer stem cell survival and proliferation. Furthermore, ovarian cancer patients with higher integrin-linked kinase levels have worse survival rates. Integrin- linked kinase expression has already been linked to the cancerous behaviors of other cancer cells.

Based on the previous premises, the hypothesis is that tissue transglutaminase engages the Wnt receptor frizzled 7 and activates Wnt signaling through integrin-linked kinase. This in turn promotes ovarian cancer progression and ovarian cancer stem cell self/renewal. Demonstration of this concept will provide the rationale to pursue new strategies to disrupt this mechanism with the goal of eliminating these tumorigenic cells. We propose to address the hypothesis by pursuing three objectives: 1) Define the integrin-linked kinase interaction with tissue transglutaminase and frizzled 7 in high-grade serous ovarian cancer cells and patient-derived organoids; 2) Determine whether integrin-linked kinase activation by tissues transglutaminase and frizzled 7 is altered in platinum-resistant OC models and tumors and required for

maintaining the aggressive ovarian cancer stem cell phenotype. 3) Determine whether tissue transglutaminase and frizzled 7 axis with integrin-linked kinase can be target in ovarian cancer mouse model to block ovarian cancer stem cell self/renewal, reduce tumor burden, and metastatic progression.

This innovative study will address a new conceptual discovery with potential future therapeutic applications aimed to interfere with the establishment of a supportive environment for the chemo-resistant cancer stem cells. Integrin-linked kinase is proposed as new target and studying its crosstalk with tissue transglutaminase and frizzled 7 will provide new insights on how ovarian cancer stem cells survive after chemotherapy promoting tumor relapse.

Ovarian cancer is the deadliest gynecological malignancy and its aggressive manifestation impacts dramatically the life of diagnosed patients. A better understanding of the key regulators involved in OCSC survival is needed and the use of combination therapies should improve outcomes of women with OC. According to the American Cancer Society, ovarian cancer accounts for an estimated 21,750 new cases and 13,940 people will die of this disease in the U.S. in 2020. Currently the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute estimated that nearly 2,600 members of military or their families may be hospitalized for ovarian cancer or suspected ovarian cancer, over a five-year period, with a great cost in terms of patients' life and cancer care costs. Thus, this project is highly responsive to the Department of Defense providing a new potential therapeutic target to block tumor progression, enhancing the quality of life and welfare after cancer treatment for women in the military and their families.

Proposal Title:	Extension of an Artificial Chromosome-Based System as a Novel Delivery Strategy for CAR-T Cell Therapy of Ovarian Cancer to Human Lymphocytes
Log Number:	OC220375
Current PI Name:	David Spriggs
Award Number:	HT9425-23-1-0683
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	09-28-2023

Ovarian cancer remains one of the leading causes of cancer death among women in the United States. Unfortunately, most patients are diagnosed with advanced-stage disease, and recurrence after definitive therapy is high. Thus, new therapeutic strategies are needed to improve the lives of women with ovarian cancer.

Unfortunately, recent progress in immunotherapy and adoptive cellular therapy in other malignancies have not extended to ovarian cancer treatment, and innovative strategies are urgently needed. One form of adoptive cellular therapy that uses modified T-cells derived from the patient represents one such approach. A major limitation to this engineered T-cell strategy is suppression by the tumor microenvironment after these treatments have been administered. One way to overcome this limitation is to modify these engineered cells with protective or inflammatory proteins via expression of genes specifically designed to overcome the hostile tumor microenvironment. However, current engineering approaches limit the number of these protective genes to 2 or 3, a number insufficient to address the many suppressive factors in the tumor microenvironment.

In our completed pilot grant, we pioneered a gene-delivery system capable of encoding a potentially unlimited number of therapeutic genes, and we used a 5-gene platform as a proof of principle. We were able to demonstrate successful transfer of this gene platform to human T-cells at levels sufficient for therapeutic applications.

In this proposal, we plan to continue optimization of our gene delivery system to facilitate large-scale manufacturing. We also propose to increase the number of therapeutic genes to 8 and evaluate both the 4-gene and 8-gene platform for efficacy against several ovarian cancer cell lines and patient-derived organoid samples. We will select the construct with the minimum number of genes that show efficacy in validated preclinical models of ovarian cancer. We will also evaluate our engineered T-cells for safety, a key selection factor for our final cellular therapy candidate. As a precaution, we have engineered a suicide gene into our 4-gene and 8-gene platform for use as necessary to mitigate toxicity.

This approach to adoptive cellular therapy represents a generational leap over currently available methods. Just as importantly, we have overcome key obstacles in transferring our gene product to T-cells, a key prerequisite step for clinical trial testing. At the conclusion of the experiments outlined in this proposal, we will have designed, validated, and evaluated the efficacy and toxicity of a novel approach to adoptive cellular therapy for ovarian cancer. We anticipate preparations for clinical trial testing once we have selected an appropriate candidate.

Proposal Title:	Unleashing the Full Potential of Checkpoint Inhibitor Antibodies with T-Cell-Stimulating Oncolytic Adenoviruses for Treatment of Ovarian Cancer
Log Number:	OC220391
Current PI Name:	Akseli Hemminki
Award Number:	HT9425-23-1-0988
Current Contracting Organization:	TILT Biotherapeutics Oy
Current Performing Organization:	TILT Biotherapeutics Oy
Web Approval Date:	09-28-2023

Ovarian cancer stands out as one of the deadliest cancers in women with a yearly rising trend in new cases and deaths. In 2021, more than 20,000 American women are estimated to be affected by it, and expected 13,000 deaths will result from the disease. Over the next 5 years, an estimated 2,600 members of military families will be diagnosed with ovarian cancer, which will cost TRICARE approximately $971.2 million. Currently, the standard treatment includes surgery, chemotherapy, and/or other systemic therapies. While initial cancer shrinkage and, even cancer disappearance, is often achieved, it will eventually return more aggressive but resistant/refractory to platinum chemotherapy and often not effectively treatable with any of the aforementioned therapies. With the life expectancy of those patients remaining short, it is critical to develop new treatment options for ovarian cancer patients. Immunotherapies - which induce and/or enhance the patient's own immune system response against cancer – may offer the key for this therapeutic gap. The past decades produced reliable evidence that the approach can lead to cancer shrinkage, cancer disappearance and, importantly, long lasting cancer-free periods or cures. In advanced ovarian cancer, immunotherapy has led to promising anticancer responses, however the approach only works in a few patients.

Current treatments focus on surgery and platinum-based chemotherapy, which are not curative for most patients. A particular problem in OvCa is platinum-resistant patients, who progress sooner than six months after platinum- based chemotherapy. Platinum-resistance ultimately occurs in the majority of recurrent OvCa patients, and no therapy is considered standard for these patients because of poor response rates. Ongoing trials investigate new treatment approaches, such as immune checkpoint inhibitors (ICI), which show much promise in many indications, but the overall response rates in OvCa remain low: a bit over 10%. The unmet medical need requires development of more effective treatments.

Adenoviruses are a well-known family of viruses, which can be repurposed for cancer therapy. They can be easily genetically modified to selectively target, infect and destroy cancer cells. Oncolytic adenoviruses, as they are called, are inherently capable of stimulating the immune system and can be further engineered with the addition of genes that enhance anticancer immune responses. In particular, oncolytic adenoviruses engineered with immune stimulatory genes have been shown promising for the treatment of patients with ovarian cancer and beneficial in combination with a wide range of other immunotherapies in animal models.

TILT Biotherapeutics (TILT) proposes to develop a "double punch" therapeutic optimized for ovarian cancer, in this application. For this purpose, they will make use of a genetically-engineered common cold oncolytic adenovirus TILT-123 that (1) selectively kills cancer cells without harming healthy ones and (2) stimulates the immune system to kill cancer cells. The latter is achieved by the release of IL-2 variant, a growth factor that is known to increase the anticancer immune cells without stimulating regulator immune cells. In order to do so, TILT will utilize their novel technology, TILT-123 in combination with

pembrolizumab to make them effective enough to treat ovarian cancer that is resistant/refractory to platinum chemotherapy.

The proposed project is an open-label, phase 1, dose-escalation study evaluating the safety of TILT-123 when given in combination with pembrolizumab to subjects with platinum refractory/resistant ovarian cancer (NCT05271318). A total of 15-24 female subjects will be recruited into a maximum of three cohorts. The study will consist of screening, a treatment period (up to 2 years), and a safety follow-up period. TILT-123 dose will increase between each cohort. Finally, a cohort expansion at maximum tolerated dose will be added. The trial is currently open in two sites, one in Europe (Docrates Cancer Centre) and one in the U.S. (Mayo Clinic, Minnesota). In addition, the company expects to open additional sites in the U.S. This project will consist of the part conducted at Mayo Clinic, Minnesota.

The proposed development of this approach can potentially lead to cures in the aforementioned ovarian cancer population, instead of just palliating it. This could ultimately result in significant savings health care costs for Service Members, Veterans, or their family members, not to mention the humanistic and mental well-being gains.

Proposal Title:	Modeling and Cross-Sectional Analysis of Movement Quality with Osseointegrated Prostheses
Log Number:	OP220004
Current PI Name:	Ross Miller
Award Number:	HT9425-23-1-0103
Current Contracting Organization:	Maryland, University of, College Park
Current Performing Organization:	Maryland, University of, College Park
Web Approval Date:	01-31-2023

Loss of a lower limb and consequently walking with a prosthetic limb presents a variety of challenges that may affect the individual's risk for future health problems. The method of prosthesis attachment has a large influence on quality of life for individuals with limb loss, and potential a large influence on their mobility and their walking gaits. Currently, there are two ways a prosthesis can be attached to an amputated limb for individuals who have lost their limb above the knee: a traditional socket prosthesis where the prosthesis attaches compliantly to the body by wrapping a socket around the amputated limb or an "osseointegrated" prosthesis attached rigidly by anchoring the prosthesis attachment directly to the remaining bone of the amputated limb. Osseointegration has mostly been used as a secondary alternative for individuals who have a poor outcome with socket-based prostheses first, and studies to date on walking gaits in individuals before and after osseointegration suggest that it allows for smaller deviations in hip and pelvis motions and more efficient gait when walking.

These existing pre- vs. post-osseointegration surgery data support the effectiveness of osseointegration as a treatment for a particular population (individuals with poor outcomes using a traditional prosthesis), but it is difficult to draw general conclusions on the biomechanical effectiveness of osseointegrated prostheses from these data because they are limited to this particular population who often have low physical function and are affected by complicating factors such as the success of the surgery and the rehabilitation. Due to its suspected benefits, osseointegration is soon expected to become a more popular option for treating limb loss in high-functioning individuals. However, there is little scientific data supporting the benefits of osseointegration for these individuals, making it difficult to recommend with confidence. This gap in knowledge is important because osseointegration involves irreversible surgery that could limit future options for revision. We therefore propose two complementary studies to better isolate the effect of the rigid bone-anchored interface of an osseointegrated prosthesis vs. the compliant socket-based interface of a traditional prosthesis on walking gait. We will first examine the mechanics and energetics of walking in high-functioning individuals with above-knee limb loss and compare these variables between users of osseointegrated prostheses and users of socket-based prostheses. By focusing on high-functioning individuals, this comparison will provide a novel perspective on the benefits of osseointegration for key clinical outcomes such as symmetry, efficiency, and knee joint loading when walking, in the absence of the complicating factors from the existing pre- vs. post-surgery data.

The comparison between user groups will give a unique look at osseointegration, but still may not strictly isolate the effect of the prosthesis. Individuals with limb loss are a highly variable group and even groups of high-functioning individuals could have differences that affect their walking independent of whether they have osseointegration or not. To address this issue, we will also perform a second study using computer modeling and simulation of human movement to truly isolate the effect of the prosthesis interface. In our recent research, we have developed state-of-the-art computer models of the human body. "Virtual surgeries" can be performed on the model to assign it prostheses with various properties, and we have validated the ability of the model to perform realistic simulations of walking with an above-knee prosthesis. By having precise control over all variables of the model and simulation, such as muscle strength and other fitness-

related variables, the subjective goal of the walking movement, and properties of the prosthesis other than osseointegration, we can generate simulations of walking with a large number of virtual patients that differ only in the factor of having osseointegration or socket-based attachment. From these data, within the limits of the modeling assumptions, we can truly isolate the effect of the osseointegration of walking mechanics and energetics relevant for mobility and health.

Osseointegrated prostheses are not limited to the general public; osseointegration surgery will likely appeal to many military Service Members who want to retain a high level of mobility, physical activity, ability to train and exercise, and potential for return to duty. An osseointegrated prosthesis may allow such high-functioning individuals to achieve/retain a higher level of function than what would be possible with socket-based prostheses. However, objective data supporting clinicians and patients in a decision to undergo osseointegration based on a high expectation of achieving certain mobility-related outcomes is currently lacking. Therefore, the proposed project not only serves the general public, but will assist Service Members with limb loss by better informing their decision on a limb loss solution (osseointegration) that may benefit their long-term health and mobility.

Proposal Title:	Translating Outcomes That Matter Most to Individuals Living with Orthotics and Prosthetics to Shared Decisionmaking in the Practice Setting
Log Number:	OP220008
Current PI Name:	Leslie Wilson
Award Number:	HT9425-23-1-0249
Current Contracting Organization:	California, University of, San Francisco
Current Performing Organization:	California, University of, San Francisco
Web Approval Date:	04-02-2023

Objective and Rationale: Our overall objective is to better understand and measure the risk and benefit trade-off preferences of individuals with upper limb loss make when choosing a prosthesis (across a user-complexity spectrum) and to use their weighted preferences to create a decision aid that can be used at the point of care to advance shared decision-making and improve prostheses outcomes. We will test the feasibility of the patient preference-based decision aid use in Hanger prosthetic practices to advance the direct impact of this work. Our rationale is to address the problem that currently prosthetics are not meeting the needs of patients with upper limb loss. By 2020, 2.2 million people will be living with limb loss in the U. S., but up to 70% adopt but don't use their prosthetic device. There is rapid innovation in advanced upper limb prosthetic devices, and their adoption and use are highly sensitive to the preferences of upper limb amputees. If patient preferences and available treatment regimens are misaligned, persons purchasing new innovative prosthetic devices may not use them, diminishing their quality of life. Prosthetic devices have the ability to significantly improve a person's ability to function in the world, but their use has risks and takes user commitment. It is imperative to understand what those with upper limb loss want and how they weigh the risks and benefits of all levels of prosthetics currently available as well as a preference for no prosthetic. Currently, there is little research that focuses on patient preferences and how to use their voice to enhance patient/prosthetist communication in their clinic visits when making a prosthetic choice. We will provide a decision aid that defines patient's preferences that can be shared with the prosthetist to advance their shared choices, and improve patient's satisfaction, and outcomes and decrease their decisional conflict and regret and reduce prosthetic abandonment.

Applicability and Research Impact: This project is directly applicable to the goals of the Orthotics and Prosthetics Outcomes Research Program (OPORP) to enhance the outcomes that matter most to individuals living with prosthetic devices. The results can have a direct impact into practice by demonstrating a mechanism for shared decision-making through our developed preference-based decision aid.

Patient Group of Focus: We will focus on individuals with upper limb loss, as they have not received as much research attention as other types of limb loss. The major reasons for upper limb amputation are trauma (43%), congenital absence (18%), and cancer (14%). Exact figures on the incidence of upper limb amputations during conflict is not directly available, but both civilian and military populations are exposed with identical risk to crush injuries, road accidents, and other workplace trauma associated with upper limb loss. Our study will focus on understanding the prosthetic preferences of both civilian and Veterans seen nationally at Hanger clinics, University of California, San Francisco (UCSF) clinics, the Amputee Coalition members as well as expanding to the Palo Alto Department of Veterans Affairs (VA), if needed.

Clinical Applications: This develop to new measures of patient preference that will be used to construct a decision aid for patients to use before their prosthetic appointment. The use of these tools can have direct clinical applications to put the patient voice more directly into prosthetic practice by promoting shared decision-making, which has been shown to improve patient satisfaction and improve outcomes.

Time to Achieving Patient Outcomes: In the first year, we will define attributes important to those with upper limb loss in making a prosthetic choice create a tool to measure patient's preferences. By the end of that year, we will design the PULLTY preference tool and begin our recruitment of those with upper limb loss to take this measure. By year 3, we will have analyzed the preferences of this sample of 200 and will have information about how different people make prosthetic choices base on their preferences. During year 4, we will test the feasibility of using these preferences in a decision aid to measure its acceptability for use and ability to improve communication and satisfaction, achieving patient-related outcomes.

Benefits to Veterans and Others with Upper Limb Loss: Our study will focus on understanding the prosthetic preferences of both civilian and Veterans seen nationally at Hanger clinics, UCSF clinics, the Amputee Coalition members as well as expanding to the Palo Alto VA, if needed. Those with limb loss can be helped to understand how they weight the risks and benefits of a prosthetic decision, by taking our preference measure. In addition, through our decision aid, these individuals will learn their likelihood of choosing a particular complexity level of prosthetic given their own preferences and personal characteristics. These factors can them be discussed with the prosthetist to advance their communication and prosthetic choice. Personalizing this communication can improve satisfaction and prosthetic use in all these populations with upper limb loss.

Proposal Title:	Transfemoral Osseointegrated Prosthesis Limb-Load Symmetry Training
Log Number:	OP220013
Current PI Name:	Cory Christiansen
Award Number:	HT9425-23-1-0047
Current Contracting Organization:	Colorado, University of, at Denver
Current Performing Organization:	Colorado, University of, at Denver
Web Approval Date:	01-12-2023

Major limb amputations are among the most debilitating injuries that affect both active-duty Service Members and Veterans. Nearly 7.4% of all major extremity injuries suffered by U.S. military personnel during active duty end with major limb amputation. Unfortunately, nearly 85% of all military personnel who suffer limb loss will not return to active duty, resulting in a significant loss of highly trained Warfighters.

Furthermore, limb loss also has a profoundly negative effect on individual health in the Veteran population, primarily due to limited prosthesis use and physical activity. The low retention rates and negative impact on health in both populations are primarily caused by socket-related complications (e.g., poor fit, chronic blisters, volume fluctuations, etc.) and chronic joint injuries (e.g., low back pain, osteoarthritis).

Osseointegrated (OI) prostheses are a novel alternative to socket prostheses, as OI prostheses directly mount the prosthetic limb to the amputated limb through a medical implant into the bone. OI prosthesis implantation allows the amputated limb bone to be loaded more directly than a socket prosthesis. Within the University of Colorado Limb Restoration Clinic, this new prosthesis has been met with astoundingly positive improvements in mobility and quality of life. However, our pilot data also demonstrate asymmetrical joint loading 1 year after OI implantation, likely caused by persistently altered movement behaviors. This loading asymmetry is important because loads applied to the implant during daily activities are vital to long-term outcomes. Too high of loads, either short-term or over a prolonged period, may result in bone fracture, while too low of loads may not promote proper healing between the bone and implant. As such, the primary focus in rehabilitation is driven by tight restrictions on progressive loading during physical rehabilitation to stimulate osseointegration between the bone and implant to ready the limb for activities of daily living. However, due to its novelty, current standard of care rehabilitation protocols immediately following OI prosthesis implantation are based on limited evidence and lack validation. Furthermore, there is standard for intervention to promote and maintain healthy prosthesis and limb loading after acute rehabilitation. Therefore, our overarching objective is to test the feasibility of the first-of-its-kind limb-load biofeedback training intervention in patients with OI prostheses.

We propose a single-site, randomized, controlled Phase I clinical trial that will be integrated within the standard of care rehabilitation following OI prosthesis implantation. The intervention will be integrated into current standard of care in a manner that optimizes intervention delivery, provides direct user feedback with wearable technology, is safe, and is patient centered. This intervention will target improved between-limb load symmetry in patients with OI prostheses by using established behavior change methods and motor learning principles with instrumented insoles for limb-load biofeedback training. To accomplish this, this proposal will implement three specific aims:

Aim 1: Determine if the limb-load biofeedback intervention is feasible to implement and acceptable in its form for people with transfemoral OI prostheses. We will test feasibility in terms of patient participant

retention, the ability to deliver the intervention as prescribed (intervention fidelity), intervention acceptability rating by the patient participants, and the relative number of adverse events between the control and intervention groups.

Aim 2: Determine if this intervention effects limb loading. Between-group changes over a 64-week period (pre-OI to 52 weeks post-OI surgery) will be assessed for between-limb cumulative loading symmetry. We will also assess group change for between-limb joint biomechanics symmetry and clinical outcome measures (free-living daily step count, Colorado Limb Donning + Timed-Up and Go Test, 30-second Sit to Stand, Gait Speed, Prosthesis Limb User Survey-M, and World Health Organization Disability Assessment Scale 2.0).

Aim 3: Identify which movement patterns and functional tasks patient participants with OI prostheses prioritized when attempting to normalize limb loading within the biofeedback training. This will be accomplished through patient stakeholder exit interviews upon the completion of study participation and used to guide our transition into a Phase II clinical trial.

This study will take us 3 years to complete. Two years will be devoted to patient participant recruitment, enrollment, and intervention delivery. Study participation will occur over a 64-week period with three testing points, all of which are integrated within the standard of care. The active research intervention will occur over 12 sessions (1 in person, 11 telehealth) spread over 40 weeks. Study results will provide critical evidence needed to better inform patient-centered rehabilitation after OI prosthesis implantation.

Proposal Title:	Comparing the Attentional Demands and Functional Outcomes of Pattern Recognition and Direct Myoelectric Control in People with Transradial Amputation
Log Number:	OP220020
Current PI Name:	Benjamin Darter
Award Number:	HT9425-23-1-0259
Current Contracting Organization:	Virginia Commonwealth University
Current Performing Organization:	Virginia Commonwealth University
Web Approval Date:	03-22-2023

Objectives and Rationale: The human hand is a powerful tool, responsible for various sophisticated movements ranging from precision tasks to powerful grips. However, a significant proportion of individuals with upper limb loss use a prosthetic device in only a limited capacity or abandon it completely. Disuse or abandonment occur, in part, because users need to focus their attention on the limb while using a prosthesis and because they are not satisfied with the functional abilities of the device. Pattern recognition control of an upper limb prosthesis allows a user to more easily control the device and could improve the user's experience and reduce the likelihood of disuse or abandonment. A well-designed clinical trial to examine the potential benefits of pattern recognition control compared to standard-of-care direct control is needed to understand the benefits to pattern recognition control. Furthermore, a health survey to easily assess how much a user has to pay attention while using a prosthetic device would assist in this clinical evaluation.

Applicability and Impact of the Research: The proposed research is highly relevant to the care of Service Members and Veterans with upper limb loss. A rigorous clinical trial like that proposed for this project will provide important information about the advantages and disadvantages of different methods for controlling an upper limb prosthetic device. At the same time, a survey of attention for upper limb prosthesis users would assess function in a way other measures do not. As an extension to the widely used Prosthetic Limb Users Survey (PLUS) measurement system, the new survey could be easily adopted for use by the many clinics within the Amputee System of Care (ASoC), military treatment facilities, and in the general public to help in providing a more comprehensive assessment of users' functional capabilities than existing functional measures alone.

Type of Patients Helped by the Research: Approximately 41,000 persons in the United States were living with major upper limb loss in 2005. Well-designed clinical trials and assessments are essential to fully understanding the impact of emerging prosthetic technology on the abilities and needs of Service Members and Veterans with limb loss.

Clinical Applications, Benefits, and Risks: New knowledge about how different upper limb prosthetic control strategies affect users and a validated survey of attention will help inform clinical decisions, improve documentation, and facilitate assessment of interventions intended to improve function, use, and satisfaction among Service Members and Veterans with limb loss. The risks to the study participants are minimal as the study will only involve responding to survey questions and completion of established clinical tests routinely used in assessing physical performance.

Projected Timeline: The full scope of the study will require 4 years to complete. However, we anticipate that by year 2, the survey to measure attention will be freely available for use in clinical practice and research.

Benefit to Service Members and Veterans: We are committed to improving Service Members' and Veterans' rehabilitation and health care through ongoing research focusing on individuals with limb loss. The clinical trial we will conduct and finalized measure we produce are consistent with the Orthotics and Prosthetics Outcomes Research Program Prosthesis Strategic Goals to optimize patient-specific technology prescription and support standardized assessment of patient outcomes related to prosthetics. The clinical trial results could help address the gap in understanding about the benefits of different prosthetic control strategies that leaves Department of Defense and Department of Veterans Affairs providers unsure how to best serve the individual needs of our Service members and Veterans with upper limb loss. Furthermore, an assessment of attention to the prosthesis has specific relevance to Service Members and Veterans with limb loss because gaining a more complete understanding of prosthesis users' function will help inform the rehabilitation services working to maximize activity level, and an ability to maintain a high quality of life.

Proposal Title:	Standardized Multidomain Assessment of Rehabilitation Treatment for Prosthetics and Orthotics (SMART-P&O)
Log Number:	OP220022
Current PI Name:	Sarah Chang
Award Number:	HT9425-23-1-0090
Current Contracting Organization:	Orthocare Innovations, LLC
Current Performing Organization:	Orthocare Innovations, LLC
Web Approval Date:	02-08-2023

This proposal develops an innovative approach for assessing multiple domains of patient outcomes such as function and performance, community integration, pain, achievement of goals, and satisfaction that has not previously been implemented in clinical prosthetic/orthotic care. The proposed work provides a standardized approach to quantitatively assessing outcomes in the clinic, at home, and in the community.

The Orthotics and Prosthetics Outcomes Research Program Strategic Goal addressed is:

• Support standardized assessment of patient outcomes related to prosthetics and orthotics.

• “This mechanism supports impactful orthotics and prosthetics outcomes research, including the development and employment of new approaches and tools for measuring outcomes.”

The objective of this study is to develop and employ a new software tool, called SMART-P&O, that supports standardized assessment of patient-specific outcomes across multiple domains of prosthesis/orthosis function and performance, community integration, pain, achievement of goals, and satisfaction. Patient outcomes are assessed using outcome measures, which are instruments or methods that evaluate the impact of a treatment and typically result in a quantitative score. It is important to consider the outcomes most important to each patient. SMART-P&O does not require a standard set of the same outcome measures to be completed across all patients, but rather, standardizes how outcome measures are completed so that they are completed the same way each time. Use of outcome measures in the prosthetics and orthotics field is inconsistent and has barriers due to time burden. SMART-P&O provides the automation needed to overcome the time barriers and facilitate assessments becoming standard practice.

SMART-P&O is a software platform in the form of a mobile application that will be used to collect data on multiple domains of patient outcomes and automatically analyze and graph the data to assist clinicians in interpreting the results. Patients can rank the outcomes domains most important to them, so this receives more weight in the calculations. Advancements in smartphone and tablet technology enable the use of sensors and surveys in combination with a mobile application to collect, analyze, and report each patient’s outcomes over time and when at home outside of the clinic.

Specific Aim 1: Conduct stakeholder roundtable meetings.

Specific Aim 2: Develop the software to automatically calculate and report outcome measurement results.

Specific Aim 3: Develop mobile app software platform and user interface.

Specific Aim 4: Perform testing of outcomes measurement tool software platform.

Developing SMART-P&O will assist providers in holistically evaluating multiple domains of prosthesis /orthosis user function and performance, community integration, pain, achievement of goals, and satisfaction. This project focuses on developing the software tool and receiving feedback from stakeholders to improve ease of use and clinical relevancy.

This project will result in a sensitive and easy-to-use mobile application that assesses patient outcomes in multiple domains in a standardized approach and automatically analyzes outcome measurements. Prosthesis /Orthosis users will be able to engage with providers in their clinical care with this mobile application and better understand their own rehabilitation progress or regress. By empowering patients and providers with quantitative evidence, we can support rehabilitation progress that restores the highest levels of performance and quality of life for individuals who use prosthetic or orthotic devices. Making it easier to assess patient outcomes and clearly communicating the results can help providers proactively and holistically determine actionable treatment steps that allow patients to lead fuller and more active lives.

Proposal Title:	Identifying the Optimal Patient-Specific Dynamic Ankle-Foot Orthosis Bending Stiffness in an Evidence-Based Manner That Can Be Implemented by Clinical Providers
Log Number:	OP220026
Current PI Name:	Elisa Arch
Award Number:	HT9425-23-1-0106
Current Contracting Organization:	Delaware, University of
Current Performing Organization:	Delaware, University of
Web Approval Date:	01-31-2023

Spring-like ankle braces are a special type of ankle brace that can partially replicate, and replace, the function of ankle muscles that has been lost due to injury or disease. In the past decade, the rate of development and clinical prescription of these spring-like ankle braces has dramatically increased. Initially, development and prescription of these spring-like ankle braces was targeted for high-functioning individuals, such as Wounded Warriors with limb salvage. In recent years, however, the prescription of these ankle braces for other patient populations, including individuals who have suffered a stroke, has begun to substantially increase as the field recognizes the efficacy of these ankle braces for a range of populations.

Stroke is the leading cause of disability in the United States and one of the leading causes of disability in Veterans. Ankle muscle weakness is a common impairment in individuals who have sustained a stroke and often results in reduced walking ability. This reduced walking ability has debilitating long-term health consequences. Current rehabilitation strategies do not sufficiently improve the factors that limit walking ability after a stroke, suggesting a need for new and innovative approaches, such as new approaches for prescribing the optimal ankle brace for each patient.

To optimize a patient's outcomes, such as an individual's walking ability, the characteristics of these spring-link ankle braces likely need to be matched to each individual patient's needs. However, despite increasing prescription of these spring-like ankle braces, little information exists to guide providers as to how to optimally select the ankle brace characteristics to meet each patient's needs. Currently, the prescription process for ankle braces requires trial and error, often over numerous clinical visits, which results in highly variable and often suboptimal patient outcomes. Thus, the overall objective of this study is to identify the influence of different levels of assistance provided by a spring-like ankle brace on walking function and mobility for individuals who have suffered a stroke and provide evidence to guide providers in identifying this optimal level of assistance for each patient.

This study will take place over a 3-year period and include 40 individuals who have sustained a stroke. During the study, participants will have measurements (outcomes) taken in the laboratory while they walk while wearing a spring-like ankle brace that provides five different levels of assistance. These measurements will include how well they walk, how quickly they walk, their mobility and balance, and their satisfaction with the ankle brace. We expect that participants will walk and function differently with different levels of assistance provided by the ankle brace, and we expect that they will walk and function the best with one of the ankle braces. Finally, we expect to be able to identify a candidate set of outcome measures that are related to how the participants function in the ankle brace with varying level of assistance.

The risks associated with this study are small, such as experiencing mild skin irritation from the ankle brace or a minor fall during the walking tests. These risks are no more than an individual would incur during their

typical activities of daily living, and precautions will be taken to minimize the risks, including adjusting fit of the ankle brace if the participant experiences discomfort or skin irritation and having the participants wear a safety harness during the walking tests in the laboratory. Moreover, the potential benefits of this study outweigh the risks. This study will provide evidence to guide orthotists (those who prescribe ankle braces) as to how to optimally select the level of assistance that the spring-like ankle brace provides for any given patient. Improving the prescription of ankle braces, as will be done through this study, will help individuals who have suffered a stroke walk better. In turn, improved walking ability will enable these individuals to be more physically active and participate in the activities in society that they want to participate in. Enabling these individuals to be more physically active will improve their overall health, such as by decreasing their risk of having a second stroke and decreasing their risk for developing other diseases such as diabetes.

Approximately 6,000 Veterans are hospitalized in Department of Veterans Affairs (VA) facilities with a stroke each year, and stroke costs the Veterans Health Administration more than $250 million annually, with that number rising when accounting for the health consequences of stroke. Thus, this study will not only improve treatment and outcomes but also lead to reductions in health care costs for one of the largest Veteran and civilian patient populations. Ultimately, it is anticipated that this study will positively transform how orthotists prescribe ankle braces for individuals post-stroke.

Proposal Title:	The Effectiveness of Frontal Plane Adaptability in a Novel Foot Prosthesis for People with Above-Knee Amputations, Bilateral Amputations, or Limited Mobility
Log Number:	OP220033
Current PI Name:	Murray Maitland
Award Number:	HT9425-23-1-0314
Current Contracting Organization:	Washington, University of
Current Performing Organization:	Washington, University of
Web Approval Date:	04-02-2023

The proposed study evaluates whether an innovative prosthetic foot with side-to-side adaptability improves movement and performance for people with above-knee amputations, bilateral amputations, or unilateral below-knee amputations who have lower levels of mobility. The research design includes community activities and mobility tests that are evaluated quantitatively, with standardized questionnaires, and with participant feedback.

People with amputations have problems with balance, pain, and mobility because a prosthesis does not have all the capabilities of a normal foot. A foot moves side-to-side, inward and outward, during most activities. Foot prostheses have been developed mostly for forward walking. Prostheses do not perform as well on uneven ground, cross-slopes, turning, side-step, or stairs because they lack side-to-side motion. Also, when the foot hits the ground, forces push on the body depending on the angle of the foot to the ground. Protheses that do not adapt cause larger forces at the residual limb resulting in pain and can cause people to stumble.

The META Arc (WillowWood, Ohio) includes an innovative linkage that provides spontaneous side-to-side adaptability to ground contact similar to an anatomical foot. The proposed research will see if this linkage helps people with above-knee amputations, bilateral amputations, and people with lower levels of mobility manage their everyday activities better. In our previous clinical trial for people with amputations and high levels of mobility, they went to the beach, hiking, farming, and other challenging activities while using the innovative prosthetic foot. They also found simpler activities like cooking, cleaning, and carrying things worked better.

The goal of this project is to see how this new prosthesis affects other people with amputations.

The results from the proposed study will impact people with amputations and unique mobility needs. Based on 2005 projections, over 1 million adults live with lower extremity amputation in the U.S. Approximately 22,000 people undergo an above-knee amputation each year. Approximately 12,000 people have bilateral amputations, although precise numbers are not available. The largest group of clients seen by prosthetists are people with transtibial amputations and a limited level of mobility (36%).

The META Arc is currently available, but the foot prosthesis is currently indicated for people with higher levels of mobility. Recent research suggests that people with lower levels of mobility would benefit by more advanced feet, but there have not been clinical trials of this type of foot. People at all mobility levels need to position their feet on the ground. Multiple directions of adaptability might benefit many more people than would currently receive this foot. The proposed study will find this out. Also, the current study might find out about changes in prosthetic feet that would benefit a particular group of people with amputations if they have specific needs.

Evidence that people can benefit from multidirectional adaptability in prosthetic feet might change the type of feet that people receive and the amount of reimbursement. There are other prosthetic feet with side-to-side adaptability, and the proposed research would imply that these feet might also benefit people with lower levels of mobility.

Understanding the effect of prosthetic foot control and function is especially important for Veterans and Service Members because of the number of people in the populations to be studied. For Service Members determined to be unfit to complete their service due to battlefield injury, lower limb amputations were among the most prevalent and disabling injuries and with the most significant impacts. Above-knee and bilateral lower extremity amputations have become more common as a consequence of combat-related injuries. Veterans with amputations often have mobility challenges. Ultimately, these injuries reduce military effectiveness and incur significant lifelong disability and health care costs. There are other prosthetic feet with side-to-side adaptability on the market, and although the proposed study will not test those feet specifically, results of the study will support the prescription of those feet as well.

Proposal Title:	Patient-Specific Requirements of Upper Limb Prosthetic Technology
Log Number:	OP220037
Current PI Name:	Emily Graczyk
Award Number:	HT9425-23-1-0114
Current Contracting Organization:	Cleveland VA Medical Research & Education Foundation
Current Performing Organization:	Cleveland VA Medical Research & Education Foundation
Web Approval Date:	01-31-2023

Rationale: Upper limb prostheses can improve functional independence, enhance community integration, and improve quality of life. However, the process of matching each patient to the right prosthesis remains challenging, and suboptimal matching can result in device abandonment and poorer clinical outcomes. There are many prosthetic options for people with upper limb amputation (ULA), and navigating these options can be difficult for these persons, their families, and their healthcare providers. It is also not well understood how prosthetic features interact with patient preferences, goals, and other psychological or social factors related to prosthesis use. In order to improve the function and quality of life of Veterans/Warfighters with ULA, we need to more fully understand the patient-specific factors that contribute to prosthesis acceptance and rejection and develop strategies to optimize prosthesis prescription.

Objectives of the Study: The primary goal of the proposed study is to understand how the needs, expectations, and health care experiences of Veterans/Warfighters with ULA impact what they think and feel about their prosthesis and how and when they use a prosthesis. We will examine experiences with prosthesis education, selection, and training, as well as how psychological and social issues impact prosthesis use. We will interview people who use prostheses as well as people who have abandoned prostheses to learn about their experiences, opinions, and viewpoints on prosthesis use and their amputation rehabilitation process. We aim to build a conceptual framework explaining user attitudes toward different prosthetic devices and how these are influenced by experiences with rehabilitation care, social interactions, expectations about functional capability, and other important aspects of the prosthesis use experience. The secondary goal of the proposed project is to develop a tool to help clinicians and patients work together to select the prosthesis that will best meet the patient's needs. We will talk with groups of people with ULA and groups of rehabilitation clinicians through focus groups to create this tool so that it will have maximum impact on clinical practice.

Potential Impacts of the Research:

Who will be helped – This research will help people with ULA who currently use or are interested in using prostheses. ULA is a significant issue for the health and well-being of both the general population and the military. Approximately 2.2 million people in America are living with the loss of a limb, and about 66,000 of these persons have ULA. As of 2015, there were 1,645 American Service Members with one or more major traumatic limb amputations from recent military operations. Approximately 300 of these Service Members lost an upper limb. There are approximately 3,000 individuals with ULA served by the Department of Veterans Affairs (VA).

Clinical applications – Veterans/Warfighters with ULA will share their thoughts and views on currently available prostheses and rehabilitation processes to help rehabilitation researchers and clinicians better understand the complex experience of upper limb prosthesis selection, training, and use. The conceptual framework developed in this study will enable researchers to accelerate future discoveries and technology advancements. This framework, along with our clinical decision tool, will help clinicians recommend prosthetic devices and personalized treatments to improve patient outcomes. Study findings may ultimately influence ULA rehabilitation standard of care.

Projected timeline – During the study period, we will construct a conceptual framework that explains prosthesis acceptance and develop a prototype clinical decision tool to aid patient-prosthesis matching. The study findings can be implemented immediately in the clinic through training for clinical personnel, education materials for patients and their families, and clinical practice recommendations for upper limb amputation rehabilitation. In future clinical trials, the prototype decision tool will be refined and deployed to VA clinics.

Benefit for Veterans/Service Members – The outcomes of this proposal will promote personalized rehabilitation care for Veterans/Warfighters with ULA and facilitate prescription of prosthetic devices that better address individual needs. This will result in higher prosthesis satisfaction, increase quality of life, decrease the physical and psychological consequences of limb loss, and reduce the risk of prosthesis abandonment. Study findings may also lead to improvements in rehabilitation practice that will impact long-term clinical outcomes for future persons with ULA. Improved rates of prosthesis adoption may decrease the overall health care cost of limb loss and potentially enable more Service Members and Veterans to return to work or duty following ULA.

Proposal Title:	Assessment of the Control and Utility of Multigrip Prosthetic Hands
Log Number:	OP220046
Current PI Name:	Deanna Gates
Award Number:	HT9425-23-1-0097
Current Contracting Organization:	Michigan, University of
Current Performing Organization:	Michigan, University of
Web Approval Date:	01-20-2023

While the use of an upper limb prosthesis is positively correlated with increased health-related quality of life, employment rates, and ability to accomplish activities of daily living, over a quarter of people with upper limb absence choose not to wear one, citing a lack of perceived comfort and function. Numerous approaches have been developed to address the lack of prosthetic function, including the addition of moveable fingers enabling multiple functional grips, such as pinching or pointing, compared to the standard open and close. These “multi-grip” prostheses have been on the market since 2007, and there are now numerous commercially available options. Users also have several different commercially available options for control mechanisms that enable them to change their grip. Unfortunately, no standardized outcome measures currently exist that quantify the ability to change grips. Measuring the efficacy and benefit of these devices through outcome measures is vital to ensure optimal device selection, track rehabilitative progress, and inform health care policy and device prescription. This is particularly urgent given the continued effort and investment towards identifying ways to improve the reliability and intuitiveness of grip selection through approaches that often involve surgical interventions. To address these important needs, this study will develop a novel functional assessment that specifically quantifies how well users can change hand grip while completing an everyday task of brewing a cup of coffee. Aim 1 will determine if the Coffee Task is valid and responsive to changes in training with a prosthesis and different approaches to controlling grip switching. This aim will use healthy individuals controlling a prosthetic emulator. Aim 2 will determine the feasibility and reliability of the Coffee Task in upper limb prosthesis users who use multi-grip prostheses in their daily lives. This assessment will play an integral role in determining the level of functional benefits users can expect from different commercial multi-grip prostheses and their controllers. Outcomes from this newly developed assessment can be used to better inform prosthesis users, prosthetists who prescribe these devices, and insurance companies that determine coverage.

The proposed work is intended to benefit Service Members, Veterans, and/or other beneficiaries with upper limb loss. There were approximately 41,000 people in the U.S. living with major upper limb loss in 2005. There have also been an estimated 1,645 major limb amputations as a result of the U.S. military missions in Afghanistan, Iraq, and Syria since 2001. While any amputation can have a devastating effect on an individual’s quality of life, upper limb loss has been found to be considerably more life-altering than lower limb loss. The majority of individuals with traumatic upper limb loss are in their prime of life and otherwise healthy. Therefore, providing intuitive and reliable multi-grip prostheses has a potential to significantly improve the quality of life of individuals with upper limb loss and encourage successful reintegration into society. According to a recent national survey of Veterans, 11% of those who use a prosthesis are currently using a multi-grip prosthesis. While the benefits of these devices have been found in a few recent case studies, more research is needed to determine how easily and accurately the users can change the hand grips, and thus enhance function. Research is also needed to determine which hand designs and control approaches might provide the greatest benefit. To do this effectively, it is necessary that the field have a standard, valid assessment to use for comparison. The proposed work fills that important gap. Following the completion of the proposed study, researchers and clinicians will have access to a feasible and reliable standardized assessment that is able to capture changes in prosthesis users’ skill level in grip manipulation and relative benefits of different commercial prostheses and their control approaches.

Proposal Title:	Comparison of Upper Limb Virtual Outcome Measures and Control Accuracy to Physical Outcome Measures with a Prosthetic Device
Log Number:	OP220048
Current PI Name:	Laura Miller
Award Number:	HT9425-23-1-0162
Current Contracting Organization:	Shirley Ryan AbilityLab
Current Performing Organization:	Shirley Ryan AbilityLab
Web Approval Date:	03-15-2023

Objectives and Rationale: An estimated 8% of U.S. citizens live with an upper limb absence. Upper limb absence drastically reduces quality of life, negatively impacting everyday activities such as dressing, eating, personal hygiene, and social interactions. Most upper limb absence is caused by trauma and afflicts younger people that wish to return to work and continue an active lifestyle in their community. Thus, there is a great need to create upper limb prostheses that are easy to control, highly functional, and personalized to the user. However, methods to thoroughly test upper limb prosthesis control and function require that the components be purchased and the device be fit to the individual. This requires a lengthy time and financial commitment. Tools that can screen users' suitability to use a device are currently limited, and there is a lack of validated methods to predict and assess function for use by clinicians and researchers.

The objective of this study is to fill this gap in knowledge by comparing clinical performance measures in an immersive virtual reality (VR) environment to performance with a physical upper limb prosthesis. We anticipate that upper limb performance in VR and upper limb performance with a physical prosthesis will be correlated with each other. This correlation between VR and physical prosthesis performance will provide necessary data to allow clinicians to use upper limb VR performance measurements to more accurately predict function and control with an upper limb physical prosthetic device, thus better personalizing upper limb prostheses for users. This study will also provide necessary data to support the use of a VR environment by researchers when testing new prosthesis control strategies before building a device.

Who It Will Help and How: There is a large population of U.S. citizens, including Veterans and military Service Members, who live with limb absence and would benefit from improved prosthetic training and control methods. Although this study focuses on upper limb prosthetics, it would provide a platform of VR tools to evaluate new control methods and training protocols that could easily be translated to a wide variety of upper and lower limb prosthetics. Therefore, by using predictive VR training platforms, our study will help clinicians prescribe the most optimal prosthetic option for their patients.

Potential Clinical Applications, Benefits, and Risks: In our proposed study to compare upper limb performance in VR to performance with a physical prosthesis, we expect to address the following: (1) evaluate the relationship between accuracy and performance in VR, (2) evaluate the impact of prosthetic weight on performance in VR, and (3) compare performance in VR to performance with a physical prosthetic device. These will address how accurately VR can predict the control of a physical upper limb prosthesis. The risks of this study are equivalent to the usual risks of upper limb prosthetic training and testing. Further, clinical staff will be present for tests and training, minimizing these risks throughout the study.

Project Timeline: Participants in the study will be enrolled for up to 6 months. In our proposed 4-year timeline, we expect to enroll 28 participants, with 23 expected to fully complete the study. Upon completion, we will have obtained specific data on if VR outcomes accurately predict physical upper limb prosthetic control. The data obtained in this study will be used to move towards a controlled clinical trial for further

evaluation. We plan to communicate results in peer-reviewed journal publications, at relevant conferences and symposiums, and on our websites and social media platforms.

How the proposed research will benefit Service Members, Veterans, civilians, and caregivers: Many U.S. citizens who have lost a limb, including an upper limb, are Service Members or Veterans. As of June 2015, military operations in Iraq and Afghanistan had resulted in over 1,600 Service Members sustaining major limb absences, approximately 14% of which involved loss of the upper limb. Additionally, in 2016, 16% of Veterans who received amputation care at Department of Veterans Affairs (VA) facilities had an upper limb absence at the wrist level or higher. Difficulty in grasping and holding objects impedes leisure activities as diverse as reading or playing sports and, crucially, it may prevent a return to employment or active duty, which affects financial security, and a sense of identity and purpose. Thus, upper limb prosthetics need to be easy to control, especially for young civilians and Service Members who desire to continue an active lifestyle. This study will provide valuable information to allow for improved control and training methods for upper limb prosthetics. The correlation between VR performance measures and physical performance will allow clinicians and researchers to better predict device control before fitting an individual for a physical device. This can be helpful to choose what type of device best matches the person’s needs and abilities.

Proposal Title:	Investigating the Utilization Effects of Powered Wearable Orthotics in Improving Upper Extremity Function and ADL in Persons with SCI
Log Number:	OP220058
Current PI Name:	Ghaith Androwis
Award Number:	HT9425-23-1-0189
Current Contracting Organization:	Kessler Foundation
Current Performing Organization:	Kessler Foundation
Web Approval Date:	03-08-2023

Spinal cord injury (SCI) is a medically complex and life-disrupting condition. Each year in the United States, it is estimated that 17,700 new traumatic SCI cases are reported, including many active Service men and women, and Veterans. About half of those, the injury involves some part of the arm and hand, representing significant disability and dependence for those patients and their families. When arms/hands are impaired, patient's quality of life and level of independence are reduced. The proposed randomized control trial (RCT) investigation will evaluate the long-term effects of the upper extremities (UE)-MPWO (MyoPro) in ameliorating wrist/hand/UE movement capability and increasing activities of daily living (ADL) and quality of life in people with iSCI. This MyoPro orthosis can assist elbow, wrist, and hand function with built-in motors that are activated by patients' will represented by the residue voluntary muscle activities detected by built-in sensors.

Results from this research will establish the first guidelines of a myoelectric powered orthosis (MyoPro) for persons with SCI, for specifically improving function, activity of daily living, and quality of life. The impact of the data generated from this clinical trial investigation should advance application of new orthotic and prosthetic technologies to treating disabilities as a result of injuries or diseases such as SCI and promoting home and community uses of the technologies to improve daily function and independence. The study would also advance scientific knowledge regarding neural changes occurred in the nervous system by application of the technology. The learned knowledge from this investigation will further justify the utilization of such an orthotic technology for individuals with SCI.

Beyond the common therapeutic benefits of upper extremity motor function rehabilitation and assistance for daily living provided with utilization the MyoPro orthotic device, there may be additional benefits including improvements in quality of life and activities of daily living due to recovered function by using the device. The clinical trial investigation described in this application would provide clinicians and therapists with an initial, but stronger basis for integrating such an orthosis into regimens for managing upper extremity impairments in persons with SCI. This would represent a significant improvement to the existing paradigms of treating hand/arm disabilities in persons with SCI. The benefits, for the patients and society (including the VA community), of utilizing such an orthotic device during daily activities at home and in the community far outweigh the minimal risks associated with this U.S. Food and Drug Administration (FDA)-approved orthosis, particularly as those minimal risks have been minimized by using sound research methodologies.

Proposal Title:	Low-Dose Short-Term Ketorolac to Reduce Opioid Use and Pain Scores on Orthopaedic Polytrauma Patients
Log Number:	OR220015
Current PI Name:	Arun Aneja
Award Number:	HT9425-23-1-0413
Current Contracting Organization:	Kentucky, University of
Current Performing Organization:	Kentucky, University of
Web Approval Date:	09-03-2023

Post-traumatic pain (PTP) often occurs immediately following musculoskeletal injury and can last throughout the early recovery phase. In polytrauma patients, or patients who have experienced injuries to multiple body parts and organ systems, this pain is often severe and can be difficult to manage. Opioids remain the primary treatment for PTP but, unfortunately, are associated with a number of complications, such as long-term use, addiction, dependence, and the potential risk for overdose. The overuse of opioids and the potential for abuse have led to the current opioid epidemic and the need for alternative non-addictive PTP medications that can adequately manage patients' pain. The authors previously showed that the use of a low-dose nonsteroidal anti-inflammatory (NSAID), ketorolac, can reduce short and mid-term opioid use and pain. The proposed study looks to test whether this same medication can also reduce the rate of chronic opioid use.

Additionally, this study will look to address whether inpatient NSAID treatment improves the functional response to pain. Therefore, the proposed study addresses the fiscal year 2022 PRORP Focus Area "Retention Strategies, with an emphasis on rehabilitation and return to duty within one year of injury."

Patients from two busy trauma hospitals who have sustained polytraumatic orthopaedic injuries will be recruited over a three-and-a-half-year period to participate in this study. The last half-year will be used to interpret and gather the remaining data. Half of the patients will be randomized to receive ketorolac (treatment group), and the other half (control group) will receive the same volume and frequency of treatment without ketorolac. The endpoints of interest will include inpatient 24-hour morphine dose equivalence scores; short- and long-term opioid use; and multiple patient-reported outcome scores that measure pain, ability to return to work, and ability to return to activities of daily living. Even if the ketorolac treatment does not decrease chronic opioid use or improve patient reported outcomes, a mediation analysis will be performed and can help understand the reasons why the intervention did not have the intended effects. The authors hypothesize that the treatment group will have decreased short- and long-term opioid use with improved pain control and better ability to return to activities of daily living and work.

Military and civilian personnel who sustain polytrauma can have significant PTP that limits prompt rehabilitation and further delays one's return to work. Patients can become heavily reliant on opioid medication, with a higher likelihood of developing chronic PTP. Defining other pain management modalities that can better treat PTP, decrease opioid dependence, and hasten recovery time is critical. This study has the potential to address the challenging issue of PTP, while minimizing acute and chronic opioid intake. It may also improve patients' ability to return to duty and shorten recovery time following their polytraumatic injuries. Therefore, this study has the potential to change the overall pain management landscape, allowing for shorter hospital length of stay, mitigating delays in rehabilitation, decreasing opioid dependence, and improving our knowledge as we strive to help patients return to duty.

Proposal Title:	Recovery of Tendon Injury by Targeting Its Root Cause and Enhancing Tendon Stem Cells
Log Number:	OR220017
Current PI Name:	James Wang
Award Number:	HT9425-23-1-0617
Current Contracting Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Current Performing Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Web Approval Date:	09-03-2023

Tendons are soft tissues that connect bone to muscle and as such have functions essential for ease of movement. Tendons are highly prone to injury by repetitive use or high force loads placed on them. Tendinopathy is a term for such injuries that exhibit painful conditions occurring consistently in and around tendon tissue with reduced flexibility and motion. Military personnel who undergo strenuous physical training and activity suffer from tendinopathy and most of them are at high risk for tendinopathy, thus reducing their duty time, and in some cases leading to disability and discharge. Overuse injuries, from military-related training and overuse, lead to nearly 70% of musculoskeletal (tendon, ligament, bones, muscles) injuries (MSIs). Between 2011 to 2016, approximately 70% of medical disability discharges in the Army were due to MSIs, and from 2010 to 2015 90% of disability discharges due to MSIs occurred within the Soldiers' first year of service. In 2018, the direct patient care costs for combat-related MSIs in the Army was $434 million. The Achilles tendon is the largest tendon in the body but it is highly prone to injuries. Military Service Members with a history of Achilles tendon injury are three times more likely to develop subsequent tendinopathy. Moreover, a huge number (in excess of 18.2 million) of Veterans are over 65 years of age, raising the frequency of age-induced tendinopathy among them, considering the strenuous activity while at service, the chronic nature of the disease, and old age. Achilles tendinopathy is also common in athletic and occupational fronts due to tendon overuse, and also within the aging population due to a general declining function. Tendinopathy management is a huge socio-economic burden to both the military and general population that affects overall welfare and ability to work.

Unfortunately, current treatment options such as RICE (rest, ice, compression, elevation) only offer temporary pain relief. Commonly prescribed pain medications and corticosteroid injections negatively affect the healing and repair of injured tendons. Moreover, such treatments pose risks that range from common side effects (nausea, vomiting, diarrhea, dizziness) to more serious effects such as stomach ulcers, bleeding, kidney, and liver failure. Therefore, there is an urgent need to develop a safe and effective treatment /prevention plan that treats the root cause of tendinopathy for military personnel to preserve the fighting force and enhance warfighting readiness and for the general population. We aim to develop a safe and effective therapy to prevent the development of Achilles tendinopathy and to treat existing Achilles tendinopathy in high-risk military personnel that can be translated to the high-risk general population as well.

However, before this can be accomplished a preclinical study is necessary to assess our therapeutic approach in an animal model. We have developed a tendon overuse/tendinopathy animal model that simulates tendon overuse in humans. Using this model, we identified the root cause of tendinopathy as high mobility group box1 (HMGB1) that results in the development of tendinopathy. We developed strategies to inhibit HMGB1 using two well-known inhibitors and U.S. Food and Drug Administration-assessed compounds, glycyrrhizin (GL) and metformin (Met), that act in separate ways for HMGB1 inhibition. Our studies demonstrated that injection of GL and Met in our mouse model inhibits tendinopathy and hence pain. Furthermore, we have successfully formulated GL, Met, and their combination, GM, as topical lotions to prevent the negative actions of HMGB1. We have also shown that all three lotions penetrate the skin and reach the tendon in high

enough concentrations to inhibit HMGB1 in mice. The focus of our study will be to assess GM lotion efficacy on tendon pain and inflammation, as well as to assess safety and potential side effects. Additionally, synergistic effects of GM lotion with moderate exercise in the form of moderate treadmill running (MTR), which is known to enhance tissue wound healing, will be investigated in this study.

The GM lotion alone and its combination with MTR will serve as therapeutics that can be easily administered in a nonclinical setting to enhance military readiness as well as to preserve the fighting force to the highest form of function. Overall, this study will be beneficial to military Service Members, Veterans, athletes, and the general population in managing tendinopathy, both prevention and treatment, in a safe and highly economic manner.

Proposal Title:	Injectable Myoblast Scaffold to Regenerate Chronically Denervated Muscle
Log Number:	OR220018
Current PI Name:	Sami Tuffaha
Award Number:	HT9425-23-1-0476
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	09-03-2023

Background: Peripheral nerve injuries cause lifelong disability, chronic pain, and reduced quality of life. They occur in 1%-3% of extremity traumas in the general United States population and are even more common among military Service Members, comprising 8% of all combat casualties. Long-term functional recovery is typically poor, and most injured Soldiers do not return to full military duty. These functional impairments are often due to a condition termed chronic denervation-induced muscle atrophy, which is a process of progressive muscle degeneration that takes place while a muscle is disconnected from its nerve supply. There are currently no treatments to restore muscle function in this common situation.

Objective: Our goal is to develop the first targeted therapy for chronic denervation-induced muscle atrophy. In this proposed treatment, muscle progenitor cells from a patient's own unaffected muscle (i.e., autologous myoblasts) will be injected into atrophic muscle along with the bioactive factors, insulin-like growth factor 1 (IGF-1) and agrin. The objective of this proposal is to evaluate the effectiveness of our therapy in restoring muscle strength in a translational small-animal model. We will achieve this objective through accomplishing two independent specific aims: (1) optimize survival and reinnervation of autologous myoblasts within injured muscle and (2) evaluate post-treatment functional recovery in muscle that has been chronically weakened by a period of denervation.

Rationale: Our novel therapy involves the targeted delivery of four components directly into affected muscle via a single injection: cultured autologous myoblasts, IGF-1 nanoparticles, agrin nanoparticles, and a biodegradable nanofiber hydrogel composite (NHC) carrier. We hypothesize that the introduced myoblasts will develop into mature muscle cells that will serve to increase muscle strength. We further hypothesize (1) that the NHC will improve survival of introduced myoblasts, (2) that IGF-1 will stimulate myoblast proliferation and nerve fiber ingrowth, and (3) that agrin will facilitate the connection of these myoblasts to the native nerve supply. Our translational small-animal model will allow us to examine myoblast proliferation and maturation within atrophic muscle and to link how these cellular changes correspond to muscle strength.

Applications: This proposal addresses the following FY22 PRORP Applied Research Award Focus Area: composite tissue regeneration (muscle and nerve). Despite advances that have been made in nerve reconstruction, some degree of denervation-induced muscle atrophy is often unavoidable. Our proposed approach is logical and could be readily applied to clinical practice. We envision that this injectable therapy would be administered in clinic under ultrasound guidance to patients who have reached a stable but reduced functional plateau years after nerve injury.

Timeline: These studies will achieve a clinically relevant outcome upon their conclusion in 3 years. Isolation and proliferation of autologous myoblasts, sustained local release of IGF-1 and agrin using biodegradable nanoparticles, and the mechanistic effects of IGF-1 and agrin on muscle development have already been established. Therefore, in our proposed translational research, we will study the unique application of these technologies toward improving long-term functional recovery after traumatic orthopaedic injuries.

Autologous myoblast transplantation has been studied in several pediatric and adult clinical trials over the past 20 years. The NHC is also currently being investigated in a clinical trial, and biodegradable nanoparticles are well-developed technologies that have been used in U.S. Food and Drug Administration-approved formulations. Following successful completion of the studies in this proposal, we aim to evaluate the long-term safety of our novel therapy in a large-animal model before beginning clinical trial enrollment in approximately 6-7 years.

Impact: Extremity trauma is now the most common cause of permanent disability among wounded Service Members, and Soldiers with nerve injuries experience disproportionate disability relative to other battlefield orthopaedic traumas. Just 9% of Service Members who sustained nerve injuries in Iraq and Afghanistan returned to full military duty, while 73% were restricted to sedentary work or deemed unfit for service. The incidence of nerve injuries in this population is increasing due to improvements in body armor, trauma care, and evacuation that have made previously fatal injuries survivable. Unlike many therapeutic approaches that seek to prevent denervation-induced atrophy from occurring by accelerating nerve regeneration, our proposed approach aims to regenerate muscle that has already atrophied to a more functional state. Therefore, this unique therapy may benefit military Service Members and Veterans who are living with chronic functional impairments years after nerve injury.

Proposal Title:	Improving Mobility and Function Following Transfemoral Amputation: A Novel Approach to Reverse Volumetric Muscle Loss
Log Number:	OR220028
Current PI Name:	Lindsay Slater
Award Number:	HT9425-23-1-0474
Current Contracting Organization:	Illinois, University of, at Chicago
Current Performing Organization:	Illinois, University of, at Chicago
Web Approval Date:	09-13-2023

Lower limb amputations (LLA) are twice as common as upper-extremity amputations. After LLA, the long-term consequences are daunting, with most individuals with LLA experiencing severe joint pain in their back, hips, and knee(s). The existing pain can make it difficult for individuals with LLA to remain active and return to work, which can lead to a multitude of concerns. Lack of activity and community engagement is associated with psychological distress (e.g., depression) and worsened health (e.g., higher blood pressure). These health risks are present for individuals with LLA who have the most advanced prosthesis, suggesting that prosthetic design, fit, and use are not the only important factors in outcomes following LLA.

The goal of this study is to use an easy and safe treatment, ischemic preconditioning (IC), to improve function in individuals with LLA so that these individuals can lead longer, healthier lives. IC is a treatment that includes the use of an inflated blood pressure cuff on the leg to stop blood flow to the lower extremity (down through the toes) for five minutes followed by five minutes of normal blood flow while the cuff is deflated. This cycle is repeated five times for a total of 50 minutes. In our pilot study of three individuals with LLA, we found that seven sessions of IC improved strength, muscle volumes, and walking ability. Therefore, this study aligns with the FY22 PRORP Clinical Translational Research Award focus area of Tissue Regeneration Therapeutics because it specifically targets volumetric muscle loss after LLA. This study also aligns with the FY22 PRORP CTRA goal of Retention Strategies, specifically Return to Duty. Improving function after LLA could potentially increase return-to-duty rates and quality of life for Service Members and Veterans with LLA.

The goal of the proposed clinical trial is to improve care and quality of life for individuals with LLA, which would help Service Members, Veterans, and other civilians with LLA. In the current standard-of-care model outlined by the Department of Defense/Department of Veterans Affairs, the only opportunity to focus on the intact limb is to increase range of motion following amputation of other leg. We are proposing an innovative shift to the clinical care model after LLA to focus on strengthening the intact limb to improve overall function. Greater strength, muscle volume, and blood flow in the intact limb would lead to better functional performance and activities of daily living, which are the next two progressions of clinical care after rehabilitation intervention. This would potentially impact everyone involved in the clinical care of individuals with LLA. In order to better understand how this intervention could fit into the rehabilitation model, we are proposing an aim to measure perceptions and feedback of IC from physiatrists and patients. Understanding how these critical groups view the barriers and benefits of IC will provide more information about translating this clinical trial to larger clinical care.

Proposal Title:	Neurally Integrated Lower Limb Prosthesis for Home and Community Use
Log Number:	OR220032
Current PI Name:	Hamid Charkhkar
Award Number:	HT9425-23-1-0623
Current Contracting Organization:	Cleveland VA Medical Research & Education Foundation
Current Performing Organization:	Cleveland VA Medical Research & Education Foundation
Web Approval Date:	09-15-2023

Individuals living with lower limb loss face many functional and psychosocial challenges related to their amputations. Compared to able-bodied individuals, lower limb amputees experience increased fall rates, lower balance confidence, higher ambulatory energy expenditure, and overuse of their intact limb. These deficits are partly attributed to lack of appropriate sensory input from their missing limb, which further limits their ability to quickly adjust motor responses based on prosthetic foot-floor interactions. These issues are of higher importance for Warfighters with limb loss who intend to regain maximum function and return to active duty. Most notably, fall incidence is higher among younger amputees compared to older ones, possibly due to participation in riskier behaviors or performance of a wider range of daily activities.

In addition to the obvious physical and functional effects of amputation, limb loss can also have noticeable psychological consequences. Relative to national norms, Veterans with lower limb loss have reduced levels of perceived ability, poorer satisfaction with social role participation, poor body image, and reduced balance confidence. These physical and cognitive challenges can lead to activity avoidance and reduced social participation among Warfighters with limb loss, which can have a subsequent negative effect on quality of life at personal and professional levels, including their ability to return to active duty. Therefore, development of novel prosthetic devices to enhance whole-person performance is necessary.

In our prior and ongoing studies funded by the Defense Advanced Research Projects Agency, Department of Defense (DOD), and Department of Veterans Affairs, we developed and are deploying a neuroprosthesis that restores plantar sensation to individuals with lower limb loss. This is achieved by electrically activating the remaining nerves in the residual limb via an implanted neural interface. Through extensive laboratory tests, we demonstrated that our technology successfully elicits sensations perceived instantaneously as if arising from the missing limb. Furthermore, these elicited sensations have shown to significantly improve standing balance stability and ambulation. In this proposed 4-year project, we aim to take the next logical step to understand the real-world benefits of this technology by investigating its effects when used freely at home and in the community during self-selected daily activities.

We will enroll a total of six participants with lower limb loss. Each will receive two multi-contact, non-penetrating nerve cuff electrodes implanted around the remaining peripheral nerves located in the back of the thigh of the residual limb. A safe range of electrical currents will be delivered to the nerve cuffs to stimulate the nerve and elicit sensations as if they were arising from the missing foot. These sensations correspond to the location that pressure is applied to an instrumented prosthetic foot, with sensation intensity varying based on the magnitude of the pressure applied. Our team has already completed the technical development of a wearable, lightweight, self-contained version of this system (i.e., neuroprosthesis) suitable for use outside the laboratory. By conducting a 15-month-long home use trial, we will establish if the neuroprosthesis can provide reliable operation at home, in the community, and in rugged outdoor environments. Furthermore, we will determine its effects on functional and subjective outcomes during mobility-related activities of daily

living. Additionally, we will incorporate effective fall prevention training developed in previous DoD-funded efforts so recipients can rapidly adapt their motor responses to the neuroprosthesis, compensate for destabilizing perturbations, and regain pre-amputation balance confidence and gait mechanics.

This study will generate the required scientific evidence necessary for widespread dissemination of this valuable new assistive technology to Service Members, Veterans, and civilians living with lower limb loss.

Moreover, this will improve function, shrink the performance gap between Warfighters with and without lower limb loss, and facilitate return to duty and meaningful work. Therefore, this work is impactful and directly aligned with the FY22 PRORP CTRA Focus Area of Prostheses and Orthoses.

This work addresses a limitation in current technology and will facilitate translation of neurally-integrated lower limb prosthetic devices into the real world. During conflicts in Iraq and Afghanistan, more than 76% of amputations among U.S. Service Members occurred in the lower limb. Yet, no existing lower limb prosthetic device is truly integrated with the intact nervous system to provide real time feedback of sensory information vital to maintaining balance, navigating obstacles, preventing falls, or negotiating unfamiliar environments without visual input or conscious attention. By integrating somatosensation of the missing limb through our nerve-based approach, we will implement the first functional and fully implantable lower limb neuroprosthetic system suitable for use outside the laboratory.

Proposal Title:	Optimizing Transhumeral Osseointegration Prosthesis Control
Log Number:	OR220035
Current PI Name:	Jacqueline Hebert
Award Number:	HT9425-23-1-0398
Current Contracting Organization:	Alberta, University of
Current Performing Organization:	Alberta, University of
Web Approval Date:	09-03-2023

Our research will address the PRORP Clinical Translational Research Award Focus Area: "Prostheses and Orthoses: development of high-performance prosthetic devices designed to enhance whole person performance in patients with limb amputation." We will optimize outcomes from osseointegration after above-elbow (transhumeral) amputation by investigating a custom "press-fit" osseointegration implant and developing a new control strategy to drive the prosthesis movements.

Limb amputation has been an unfortunate hallmark of military conflict. Many Service Members have suffered traumatic arm amputations. Loss of an arm leads to severe limitations in function. Even when provided with an appropriate prosthetic limb, people with arm amputation rely on the remaining arm to perform activities of daily living far more than using the prosthesis. When the arm amputation includes loss of the elbow joint, function becomes even more limited. Many patients choose not to wear a prosthesis because it is difficult to control the prosthetic joints.

Furthermore, the prosthesis is traditionally attached to the remaining limb through a hard socket that squeezes the soft tissues and then is strapped across the chest or to the other shoulder. This setup restricts the ability to move the shoulder to position the prosthetic arm to accomplish tasks.

Newer surgical techniques insert a titanium rod into the remaining bone with a metal attachment that comes through the skin and allows a direct connection of the prosthesis to the bone. This technique is called osseointegration and is becoming more available in the United States and worldwide. There is evidence that osseointegration for the lower limb (above the knee) amputation is beneficial. However, upper limb osseointegration is less studied. Osseointegration could dramatically impact Service Members with above-the-elbow amputation by removing the need for a socket, providing secure attachment and suspension of the prosthesis directly to the bone, and providing better control and sensation of where the prosthetic limb is in space.

Our research will address the main barriers to advancing transhumeral osseointegration to clinical care. First, we will apply new machine-learning strategies to improve the ability to use surface muscle signals to control the prosthesis. Current upper limb prostheses suffer from not being reliable to control when the arm is moved in different positions -- for example, reaching to grasp a cup off a shelf. The lack of reliable control is a frustration for many prosthesis users, that we will solve.

Secondly, we will develop and deploy virtual reality training tools for people to train at home during their recovery after surgery. These tools will make rehabilitation more accessible and convenient for those that have difficulty accessing the clinic, and improve the transition to using the prosthesis.

Lastly, we will provide detailed evidence on outcomes from the osseointegration and our new control algorithm in a clinical trial.

We hypothesize that our intervention will improve active prosthesis usage in daily life, reduce abnormal movement compensations and improve function when using the prosthesis. These factors will ultimately improve satisfaction and quality of life for those with arm amputation.

This work will have an immediate impact by providing new control strategies for upper limb prostheses that can be used for persons with limb loss, even if still using a socket prosthesis. We will also provide crucial evidence on the efficacy and safety of the "press fit" osseointegrated implants for the upper limb, which have not yet been reported. In the long term, this evidence will improve the options available for prosthetic arm restoration and reduce long-term complications for persons with traumatic arm amputation.

Proposal Title:	Osseosurface Electronics to Monitor Rehabilitation and Accelerate Return to Duty Following Surgical Treatment of Long Bone Fractures
Log Number:	OR220049
Current PI Name:	David Margolis
Award Number:	HT9425-23-1-0233
Current Contracting Organization:	Arizona, University of, Tucson
Current Performing Organization:	Arizona, University of, Tucson
Web Approval Date:	08-31-2023

Focus Areas: Despite recent advances in fracture treatment, we are currently unable to accurately measure fracture healing in patients. Radiographs (X-rays) are used to monitor healing, but only demonstrate healing after it has occurred, cannot be performed daily due to the need for specialized equipment, and expose patients to radiation with long-term health consequences. Healing is unpredictable, and the inability to measure real-time fracture healing delays rehabilitation and return to duty in most patients, which is limited by the slowest healers. Our group is developing technology that accurately measures fracture healing in real-time. Continuous objective measurements of fracture healing will allow clinicians to accelerate rehabilitation allow early return to duty. The current proposal focuses on Retention Strategies to Facilitate Return to Duty, Enable Decision Support Tools, Diagnostic Capabilities, and Avoiding Reinjury. The proposal will test novel implantable wireless, battery-free electronics that permanently bond to bone, and provide multimodal sensing capabilities. The implants will be tested in a large animal fracture model as a tool to measure fracture healing and monitor rehabilitation. The primary goal of this technology is to provide a point-of-care technology that continuously monitors fracture healing and bone health throughout the entire treatment process from the time of surgery, through healing, rehabilitation, and following re-deployment to the battlefield. The technology is being developed in conjunction with wearables incorporated into standard military uniforms that allow for continuous power and readout of the implantable devices without the need for additional specialized equipment. The implantable sensors will also be used to monitor bone regeneration in large bone defects that typically lead to amputation. The defects will be treated using 3D-printed biomimetic scaffolds and stem cells isolated from a patient's own fat tissue.

Potential Research and Clinical Applications: The goal of current fracture management is skeletal stabilization to allow patient rehabilitation through mobilization that accelerates soft tissue recovery during bone healing. We have no method to measure fracture healing in real-time, which limits rehabilitation and return-to-duty protocols to the slowest healers. The immediate clinical benefit of our technology is to enable real-time continuous objective measurements of bone healing and allow clinicians to individualize and maximize rehabilitation protocols, accelerate return to duty, and ensure the best outcome for each patient. The technology will also allow for continued monitoring following redeployment in the battlefield to minimize the risk of reinjury. While the current proposal focuses on fracture healing and rehabilitation, there are numerous additional clinical and research applications of our implantable electronics. These include a means of early detection of infection, a tool to provide neuromuscular feedback in composite tissue regeneration, and a tool to monitor long-term bone health and treatment in metabolic bone diseases such as osteoporosis.

Projected Time to Achieve a Clinically Relevant Outcome: The electronic components of our implant are produced using materials that meet United States Pharmacopeia (USP) class VI and/or International Standards Organization (ISO) 10993-5 standards for implantable materials. While the final product would need to demonstrate safety and efficacy prior to U.S. Food and Drug Administration (FDA) approval, exclusive use of thoroughly tested non-toxic implantable components will minimize the cost and effort needed to demonstrate safety and efficacy. The electronics are powered and read using radiofrequency

waves approved under FDA guidelines. The experiments completed in this proposal will prepare this technology for a clinical trial, which will potentially directly benefit service personnel immediately following completion of the study.

Short- and Long-Term Impact on Patient Care Following Traumatic Orthopaedic Injuries: Musculoskeletal trauma is involved in over 60% of traumatic combat injuries, resulting in over 65% of medical non-deployability and medical discharges. Military personnel sustaining significant extremity injuries have poor functional outcomes with over one-third no longer able to remain on active duty, maintain employment, or attend school. In addition to the military, civilians frequently experience long bone fractures and can sustain severe extremity injuries with critical size bone defects from severe trauma such as injuries sustained in motor vehicle collisions and from bone resections secondary to cancer treatment. The short-term impact of our technology on patient care is that it allows real-time continuous measurement of fracture healing to allow individualization of rehabilitation protocols and accelerate return to duty. This is expected to minimize the long-term disability and high cost associated with traumatic orthopaedic injuries. Additional sensing capabilities will allow early detection of infections and long-term monitoring of bone health.

Proposal Title:	Prevention of Implant-Skin/Bone Interface Infection in Osseointegrated Prostheses with Photodynamic Therapy
Log Number:	OR220062
Current PI Name:	Ida Gitajn
Award Number:	HT9425-23-1-0800
Current Contracting Organization:	Dartmouth-Hitchcock Medical Center
Current Performing Organization:	Dartmouth-Hitchcock Medical Center
Web Approval Date:	09-28-2023

Injuries to the arms and legs sustained in battle commonly involve broken bones with open wounds (open fracture). Improvements in body armor and rescue strategies have increased the number of survivors of combat-related injuries. An unintended consequence of improved survival is an increase the number of wounded Warriors with lower extremity amputation, often at high level (like through the upper thigh) and/or in both legs. Combat-related trauma in Iraq and Afghanistan alone has resulted in over 1,600 major limb amputation through 2015, and more than one fifth of these Service Members have lost more than one limb. The negative functional consequences of this are profound. Traditional socket-based prostheses are associated with functionally limiting problems such as pain, skin ulcers, infections and swelling with inconsistent prosthesis fit, among others. Osseointegrated prostheses, in which a metal implant is anchored directly to residual bone, are functionally superior. Anchoring a prosthetic device to bone is a much closer to the physiology and mechanics of a non-amputated limb resulting in superior outcomes in terms of physical function, skin-related complications, and chronic pain and has the added benefit of vibratory sensation. However, an important barrier to widespread adoption of OI prostheses is infection at the implant-skin/bone interface.

This proposal is the next step towards developing an at-home treatment method to prevent infection associated with OI prostheses using a light-sensitive FDA-approved medication that is applied topically (or directly on to the skin and metal) followed by targeted light application (called photodynamic therapy or PDT). Our group has clearly shown that PDT can even penetrate and eradicate bacteria protected within communities called biofilm, which is frequently present on metallic implants that are exposed to the environment. This is an animal study in a rabbit contaminated osseointegrated prosthesis model designed to evaluate the efficacy of PDT at reducing the bioburden (amount of bacteria present) and preventing infection and to optimize the dose of PDT to optimize reduction in bacteria and minimize injury to adjacent skin. At the completion of this study, we will be prepared to translate PDT into human patients with demonstrated efficacy and an optimal dose of the topical medication and targeted light.

This study will have a substantial military benefit and clinical impact on the care and outcome of patients with leg or foot amputations. Complications, chronic pain and functional limitations are common using currently available socket-based prostheses. Critical to regaining normal lifestyles and returning to active duty is having a highly functional prosthetic device which can be achieved more frequently and reliably using bone-anchored or osseointegrated prostheses.

This study will fill a critical gap in our knowledge base and will serve as preparation for an early phase clinical trial in human patients with osseointegrated prostheses. We believe that this work will pave the way for widespread adoption of functionally superior OI prostheses and transform the lives of patients with amputation that are a result of both battlefield injuries and/or civilian causes.

Proposal Title:	Optimizing Muscle Function in Composite Tissue Injuries with Segmental Bone Defects
Log Number:	OR220094
Current PI Name:	Roman Natoli
Award Number:	HT9425-23-1-0309
Current Contracting Organization:	Indiana University
Current Performing Organization:	Indiana University
Web Approval Date:	09-03-2023

The purpose of this research is to determine the best way(s) to treat bone injuries in badly injured extremities to prevent further injury to the surrounding muscle. Our proposal involves using our pig composite tissue injury (CTI) model and directly aligns with the Focus Area Composite Tissue Regeneration: Techniques aimed at improving outcomes following high-energy extremity trauma, with a focus on improving wound healing, neuromuscular recovery following composite tissue loss and segmental bone loss". Composite tissue injury (CTI) is injury to all of the tissues in a limb, including the bone, muscle, nerves, and skin, all of which are at high risk to have poor long-term function that is primarily due to pain and weakness. We have developed a pig CTI model purposefully designed for these studies that can generate evidence that can be rapidly advanced for investigation in human trials.

Traditionally, the majority of focus in CTI extremity trauma is to get the bone to heal without infection, as poor bone healing is a clear cause of pain and residual weakness. Researchers and clinicians have determined that the viability of the muscle that surrounds the bone plays an important role in how the bone heals. In our recent work, we have conversely determined that the injury to the underlying bone plays an important role in how the overlying muscle functions and heals. Specifically, we determined that untreated large bone defects, called segmental bone defects (SBDs), cause injury and weakness to the adjacent muscle even when the muscle was uninjured.

In this study we will determine how two current clinical methods used at the time of injury to treat an SBD affect adjacent muscle function and composition. In our previous work, we showed that an SBD caused the adjacent muscle to fill with scar tissue that led to loss of 50% of its strength three months after injury. The loss of strength was no different in pigs that had an isolated SBD compared to pigs that had an identical SBD and a direct injury to the muscle. Based on this, we concluded that the SBD was the most important cause of injury and weakness in the adjacent muscle, even more so than the direct muscle injury. Therefore, we designed this study to determine the best way to treat the SBD to minimize collateral muscle injury. We will use our pig model that includes removing a 25-mm (roughly one inch) segment of bone from the middle of the tibia (the shin bone) that is fixed with standardized orthopaedic surgical methods using plates and screws. This model leads to significant injury in the surrounding muscle.

In this study, we will create the same SBD. In the first group of pigs, the SBD will be filled with what is done currently in the majority of injuries in humans. The SBD will be filled with a solid spacer made from standard bone cement (the same bone cement we use to secure joint replacements) filled with antibiotics. In a second group of pigs, the SBD will be filled with a bone-morphogenetic protein (BMP-2) which is a protein that speeds up bone healing. Typically, in clinical cases, therapies that accelerate bone healing are withheld until all of the underlying skin and muscle are healed, but it is possible that this may not be the best way to prevent muscle injury. In our initial studies, BMP-2 rapidly accelerated bone healing in the SBD. So, here we will test if immediate treatment to accelerate bone healing is better for the adjacent muscle. In the third group of pigs, the SBD will be untreated to replicate the conditions from our previous study. Strength will be measured monthly for 3 months using a custom pig testing apparatus that measures the peak amount

of torque the muscle adjacent to the SBD can produce. The pigs will be euthanized at 3 months, and we will remove the muscle adjacent to the SBD and do testing to determine concentrations of healthy muscle proteins, proteins that show that the nerves in the muscle are working well, and scar tissue which is detrimental to muscle function.

Ultimately, we are working toward identifying the best methods to treat CTI injuries. Our work will be impactful for both warfighters and civilians who sustain severe extremity trauma. There has been much less focus on treating muscle in these injuries compared to treating bone, but yet pain and weakness are the hallmark features of poor function in long-term studies even when the bone heals. Here we propose a novel investigation that will help determine the best way to prevent muscle weakness after injury. The findings from this study will likely be immediately available to test in humans as we are investigating the current accepted therapy against another therapy that is available and approved by the U.S. Food and Drug Administration. It is also remarkably relevant for Warfighters, as CTI in the extremities are, by far, the most common injury sustained by our Soldiers.

Proposal Title:	Primary Subtalar Arthrodesis for Calcaneal Fractures to Optimize Performance: A Randomized Clinical Trial
Log Number:	OR220101
Current PI Name:	Joseph Hsu
Award Number:	HT9425-23-1-0505
Current Contracting Organization:	Wake Forest University Health Sciences
Current Performing Organization:	Wake Forest University Health Sciences
Web Approval Date:	09-03-2023

Management of severe injuries to the heel (displaced intra-articular calcaneus fractures) continues to be a major challenge for orthopedic surgeons. Previous studies have demonstrated poor outcomes, and results show that patients experience long-term pain and decreased quality of life postoperatively. Poor outcomes are driven by pain, in particular, which is linked to post-traumatic subtalar arthritis. The proposed study addresses the FY22 PRORP Clinical Trial Award Focus Area of Retention: Return to Duty.

The purpose of this study is to examine patient outcomes following calcaneus fractures treated with two different surgical procedures -- Open Reduction and Internal Fixation (ORIF) + Primary Subtalar Arthrodesis (PSTA) and ORIF alone -- to determine if one procedure gets patients back to work or active duty faster. Both ORIF and ORIF+PTSA are standard and commonly performed procedures to treat calcaneus fractures at any trauma center. Typically, ORIF is performed; if patients develop painful post-traumatic subtalar arthritis, then they receive subtalar arthrodesis. Previous studies have demonstrated potential benefit of using subtalar arthrodesis initially (PSTA); however, a rigorous randomized controlled trial is needed to provide high quality evidence.

We propose a randomized clinical trial to compare two treatments for patients with displaced intra-articular calcaneus fractures: (1) ORIF + PSTA and (2) ORIF alone. The study will be conducted at civilian and military medical centers and will enroll patients undergoing operative treatment for displaced intra-articular calcaneus fractures.

Shortening the recovery period by performing an immediate subtalar arthrodesis (ORIF+PSTA) will likely facilitate returning a greater proportion of service members to duty compared to ORIF and sequential arthrodesis for those high number who will develop symptomatic post-traumatic arthritis. Avoidance of even minor complications can have an impact on timely return to duty in high demand occupations like the United States military. Minor complications or increasing recovery time by even a few weeks can have a negative impact on retention on active duty. We know that orthopaedic injuries and the sequalae of their reconstruction are the number one drivers of disability and loss to duty among wounded Service Members.

Proposal Title:	Glutamine-Targeted Therapies to Prevent Traumatic Heterotopic Ossification
Log Number:	OR220107
Current PI Name:	Courtney Karner
Award Number:	HT9425-23-1-0519
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	09-03-2023

FY22 PRORP Focus Area to be addressed: The proposed project directly relates to the FY22 PRORP Focus Area of Retention Strategies, including “Return to Duty: Treatment strategies that can be utilized along the continuum of care and enable return to duty within 1 year of injury” and Composite Tissue Regeneration including “techniques aimed at improving outcomes following high-energy extremity trauma, with a focus on improving wound healing.” The proposal is an applied research collaboration between a basic scientist and a clinician scientist whose laboratories were established to improve the outcomes of combat casualties and civilians after musculoskeletal trauma. This proposal specifically seeks to further determine the role of the amino acid glutamine in extremity trauma, with an end-goal of validating a translational approach to prevent aberrant extremity wound repair as seen in heterotopic ossification (HO).

With dramatic improvements in survival from combat-related blast injuries due to tourniquet use, we have witnessed a concomitant increase in patients with debilitating injuries that drastically diminish quality of life. Of the nearly 15,000 battle injuries suffered in Operations Iraqi Freedom/New Dawn and Enduring Freedom, over 50% of those injuries were extremity injuries. Of these wounded Soldiers with extremity injuries, over 60% of them will go on to develop HO. HO also causes significant disability in hundreds of thousands of civilians and Veterans with joint arthroplasties, amputations, and orthopedic injuries. For example, over 80% of patients with fractures and revision joint replacements will develop HO. As a result of forming bone outside of the skeleton, HO causes severe chronic pain, open wounds, and limited range of motion.

Current treatment strategies address HO after its development with surgical excision. However, surgery is unable to restore range-of-motion, which has often been chronically limited due to HO, cannot address chronic pain, and causes prolonged wounds with poor healing. After excision, patients often develop recurrence within the original tissue bed, which necessitates re-excision, or continues to cause the original signs and symptoms. Though several prophylactic medications have been previously trialed, all have negative side effects, and all fail to target the causative signaling mediators that lead to HO. Identifying a cohesive and timed strategy to prevent acute HO would greatly improve outcomes following high-energy extremity trauma as well as enhance return to duty. We believe glutamine metabolism in the causative initiator and can be targeted early through diet or drugs to prevent traumatic HO.

Potential clinical applications, benefits, and risks: This proposal is designed to be translatable and simulates the real-world trauma and management that patients may expect to receive. First, we use clinically relevant models of trauma-induced HO, which are broadly applicable to combat-wounded military personnel and to civilians with significant trauma. Secondly, we utilize dietary modifications and a near clinical pharmacotherapy strategy targeting glutamine transport in the bone formation pathway; this method is highly translatable, cost-effective, and easily implemented. Thirdly, this proposal addresses duration of treatment by selecting a short time period of treatment to minimize cost, improve adherence, and avoid adverse consequences. The combination of these techniques makes this proposal an important preclinical study that lends itself to establishing key data necessary to push forward definitive clinical trials.

Projected timeline and expected patient-related outcomes: In this proposal, we plan to rapidly deploy our optimized nutrition protocol and pharmacologic treatment interventions. In the first 12 months we will validate our dietary strategy to prevent HO in our proven extremity trauma models. In months 12-36, we will demonstrate the ability to pharmacologically target glutamine uptake to prevent HO.

Short- and/or long-term impact on patient care and/or restoration of function: This proposed research will significantly improve current occupational therapy and pharmacologic treatment strategies available to all patients who are at risk of developing HO. Through this proposal, we will improve our understanding of the role of glutamine on HO formation. This proposal will lead to a novel, targeted therapy and a dietary Standard Practice Guideline to prevent HO.

Proposal Title:	AIRFrac: Artificial Intelligence Radiographic Point-of-Care Decision-Making Aid for Prehospital Fracture and Dislocation in Military and Civilian Populations
Log Number:	OR220110
Current PI Name:	Chun-Nan Hsu
Award Number:	HT9425-23-1-0320
Current Contracting Organization:	California, University of, San Diego
Current Performing Organization:	California, University of, San Diego
Web Approval Date:	09-03-2023

Fractures and dislocations are the most common orthopedic trauma injuries among Service Members in both combat and non-combat situations. Effective treatment relies on imaging diagnosis, which requires a well-trained, board- certified radiologist to read and interpret the images. However, studies have found that as workload of radiologists increases over the years, many fractures were missed in the civilian emergency department in the night and when trainees were involved in the initial interpretation. Overlooked fractures left untreated may severely aggravate, resulting in long-term consequences such as osteoarthritis (joint narrowing) or even disability.

In response, automated fracture detectors have been developed by applying Artificial Intelligence (AI) technologies. Several AI fracture detection systems have cleared U.S. Food and Drug Administration (FDA) approval for clinical use. Wide adoption and improvement in diagnosis quality are yet to be seen, but they demonstrate the potential of the AI in assisting diagnosis of fractures.

Such AI systems may potentially be deployed in military health care to alleviate the workload of well-trained medical personnel. Moreover, they can be deployed in the battlefield near the point-of-injury to provide rapid diagnoses of fractures and prioritize which injured Warfighters need immediate attention and which are cleared with fractures, performed without the presence of experienced, well-trained medical personnel. The potential benefit is particularly eminent in mass casualty events.

The project aims at addressing the fiscal year 2022 PRORP Applied Research Award Focus Area "Retention Strategies - Battlefield Care: Strategies that can be utilized at or near the point of injury to allow an injured Service Member to remain on the battlefield or on mission without the need for evacuation" by developing an effective AI detector of orthopedic trauma injuries. The existing AI fracture detectors, however, may not be ready for deployment in the battlefield for many reasons. First, it is not clear if their systems work well if the images are acquired with portable X-ray machines available in the forward field medical aid stations. Their systems were tested on fixed X-ray machines in a hospital. Portable X-ray machines may not have the same image quality as those fixed machines. Second, their systems may require high-end computers to run and may not work with a tablet used by military medics. Also, none of them was evaluated at localizing where fractures present but only at the case level – whether this case has a fracture or no fracture, which is not sufficient to provide an actionable treatment recommendation. Lastly, none of them considers dislocations, subluxations, and other orthopedic trauma injuries. These are important considerations for an AI fracture detector to benefit the point of injury care in deployed environments.

The proposed project will develop AIRFrac, a novel AI software system for fracture and dislocation detection. AIRFrac will also detect dislocations because an automated AI detector will be the most useful for assisting screening of patients with mild and moderate injuries. Confirmation of the presence of dislocations

in addition to fractures will be beneficial for these patients and our preliminary result with dislocation detection was encouraging. AIRFrac will be trained by deep machine learning algorithms with a large data set of fracture and dislocation cases sampled from the trauma center of UC San Diego Health and eight VA medical centers in the western states. The cases will match the intended population of Service Members. AIRFrac will be developed on top of previous research of the study team on AI for radiology supported by DOD. Preliminary results with foot and ankle showed that our idea can achieve a fracture detection rate comparable to the state-of-the-art AI systems. Fractures and dislocations of 17 body parts will be considered.

The project will also develop a functional prototype system that integrates the AIRFrac software system with a portable X-ray machine to demonstrate the feasibility of deploying the system in the battlefield. This prototype system will include an app running on a tablet computer connecting wirelessly with a portable X-ray. The app will guide one or more radiographers of varying skill levels to scan a body site of injury. After all different required angles of the injury site views are properly taken, the app will show on the screen the acquired radiographs with bounding boxes in different colors indicating estimated certainty scores of any fractures and dislocations detected by AIRFrac. A reader study will be conducted to let radiologists with different levels of experience to test use the prototype. Correction rates of diagnoses and time spent to complete a scan and diagnosis will be measured to prove that the prototype works and is ready for a large-scale field trial in the next phase of the development. The long-term goal of the project is to integrate AIRFrac into military standard-of-care.

Proposal Title:	Regenerative Peripheral Nerve Interfaces to Enhance Function and Sensation in People with Transfemoral Amputation
Log Number:	OR220120
Current PI Name:	Deanna Gates
Award Number:	HT9425-23-1-0678
Current Contracting Organization:	Michigan, University of
Current Performing Organization:	Michigan, University of
Web Approval Date:	09-18-2023

Individuals with an above-knee lower-limb amputation are known to walk more slowly, expend more energy, have a greater risk of falling, and have reduced quality of life compared to individuals without amputation and those with below-knee amputation. One of the driving factors behind these deficits is the lack of active function provided by above-knee prostheses with prosthetic knees and ankles. While many prosthetic devices have been developed for functional restoration after major lower-extremity amputation, there remains no stable interface to facilitate reliable, long-term volitional control of an advanced robotic limb capable moving multiple joints. Moreover, there is no existing interface that provides useful sensory feedback that, in turn, enhances the functional capabilities of the prosthesis.

To achieve both greater signal specificity and long-term signal stability, we have developed a biologic interface known as the Regenerative Peripheral Nerve Interface (RPNI). An RPNI consists of a peripheral nerve that is implanted into a free muscle graft that would otherwise go unused in the residual limb. As the nerve grows, it reinnervates the free muscle graft, which undergoes a predictable sequence of revascularization and regeneration. The RPNI leverages these biological processes to provide three essential benefits to people with amputation: intuitive motor control, sensory feedback, and mitigation of post-amputation pain. This proposal will determine the extent to which the RPNIs enable generation of high-fidelity motor control signals for an advanced lower-limb prosthesis with active knee and ankle motion. We will evaluate the amplitude, movement specificity, and stability of signals derived from the lower-limb RPNIs over 1 year. We will use these signals to control a powered knee-ankle prosthesis while participants perform cyclic tasks (e.g., walking) and unpredictable tasks (e.g., sudden stops). We expect that this control approach will enable users to feel more confident and stable with decreased cognitive effort associated with their movements. Finally, we will determine whether stimulation of lower-extremity RPNIs provides meaningful sensory feedback that can enhance stability while the user is standing and walking with their prescribed prosthesis. We expect these findings will motivate future clinical trials using RPNIs for control and sensation simultaneously.

Proposal Title:	Osseointegrated Limb Implant Microbiome Population Adaptation Study (OLIMPAS)
Log Number:	OR220135
Current PI Name:	Ean Saberski
Award Number:	HT9425-23-2-0009
Current Contracting Organization:	Henry M. Jackson Foundation
Current Performing Organization:	Walter Reed National Military Medical Center
Web Approval Date:	09-03-2023

This work addresses the FY22 PRORP Clinical Translational Research Award Focus Area Osseointegration: Identification of Best Practices to Address Infection. The world, including our own skin, is dominated by hundreds of small organisms we call the microbiome community. Previous work has shown that these microbes are important in the progression of many diseases like autoimmune disorders, digestive disease, respiratory disease, urogenital disease, and even cancer. Importantly, recent studies have shown that these microbes also play a crucial role in the rate a wound can heal. However, little work has been done in understanding the role of these microbes when it comes to healing after a major surgery like osseointegration (OI). Here, we tease apart the role microbes play in wound healing after OI surgery to better serve the military community and other as a whole.

Our research area has three concrete aims: Aim 1: Define the natural changes in the microbial community resulting from osseointegration surgery, Aim 2: Identify the microbial species associated with improved or worsening wound healing, and Aim 3: Define the characteristics of soft tissue stability that effect microbe community homeostasis.

To complete our aims, we will acquire genetic sequence of all the microbes around the surgical site, before and after surgery. Using the sequences, we can determine which microbial species are present and how many individuals of each species live in the wound. Then we will compare how species change through time for all patients. Additionally, we will use state-of-the-art modeling approaches to determine which species are responsible for wound healing rates. First, we will design different hypothesis to predict which species may be responsible for wound healing rates. Using this modeling approach, we will be able to identify which species are responsible for improved wound healing and which species are responsible for worsened wound healing. Lastly, we will determine if excess skin around the surgical site may contribute to the microbial species found in the wound. Because extra skin can cause higher humidity and temperature, such as in your armpit, this environment might change which species can be present. We will first determine if the species differ between sites where there taught skin versus sites with extra skin around the surgical site. Then, we will determine if the different species present at different skin types could also be playing a role in how quickly a wound heals. This work will help us to better treat infection after OI surgery and can help us understand how the wound healing process works to serve the military community and limb loss community as a whole.

Proposal Title:	Translation of Soft sEMG Electrode Limb Suspension System and Volitional Control of Robotic Prostheses for Clinical Impact
Log Number:	OR220138
Current PI Name:	Matthew Wernke
Award Number:	HT9425-23-1-0847
Current Contracting Organization:	Ohio Willow Wood Company, The
Current Performing Organization:	Ohio Willow Wood Company, The
Web Approval Date:	09-28-2023

The current state of art for controlling prosthetic knee components is to utilize onboard sensors to measure movement of the user and make decisions for how the knee should behave to best assist the user. While this paradigm works well for many situations, there are also many limitations. The controller of the knee needs data to analyze before a decision of how the knee should perform can be completed. This can result in poor transitions between different types of movement, for example, the controller will need some new data to indicate that the user has transitioned from walking on flat ground to going up stairs. While many prosthetic knees use the movement of the hip to flex and extend the prosthetic knee, some prosthetic knees have powered flexion and extension. While powered knee systems can lessen the need for the patient's hip to drive knee motion, the current controllers in these knees do not allow the user to take full advantage of the knee's power. Since these knees can flex and extend without motion of the user, it is possible for them to activate when the user is seated or stationary. However, a new control input is required for users to take advantage of these capabilities of powered knee systems. Patient muscle contractions are a promising solution for improving the control and enabling direct patient activation of the prosthetic knee. Previously, the Department of Defense and other federal agencies have invested considerable resources toward understanding the use of muscle contractions to control prosthetic limbs. The proposed project is a culmination of this investment with the goal of translating this previous work into new commercial products that directly benefit military Service Members.

The proposed project addresses the FY22 PRORP CTRA focus area of Prostheses and Orthoses, specifically the development and clinical translation of innovative prosthetic devices targeted to enhance whole person performance of transfemoral amputees. The outputs of this work will be a production-like version of a powered knee with muscle contraction enhanced control schemes, a new device for acquiring skin surface muscle signals in a weight bearing environment, and research outcomes from a multicenter study.

Immediately following this award, transfemoral prosthesis patients are expected to benefit from new technologies that enable them to more directly interact with the prosthesis. This includes a new prosthetic liner that can non-invasively detect surface muscle contractions and transfer that signal to a new powered knee system. It is expected that this combination of technologies will create more stability, better control, and greater synergy between user and prosthesis. Patients with this technology will also be able to activate their prosthesis when seated or stationary allowing them to utilize their device in situations they previously were not able to due to technology limitations. The research outcomes from this work will also help with the planning of future clinical trials. The new technologies from this award will also enable future technology advancements for other assistive devices such as prosthetic hips, prosthetic ankles, lower extremity orthotics, and exoskeletons. Many of these technologies have similar control challenges, which could be improved through the addition of muscle contraction input.

Improvements in protective armor and medical practices have increased combat injury survival rates. Thus, the number of service personnel surviving with amputations has increased with current estimates exceeding 1,700 surviving amputees, many of whom have sustained more than one limb amputation. While a primary

goal of many military personnel having an amputation is to lead normal, productive lifestyles, an important secondary goal is the potential to return to active-duty status and continue their military service. Critical to regaining normal lifestyles and even returning to active duty is to have a fully functional prosthesis that can replicate the motion and responsiveness of the human limb and does not hinder the performance of a prosthesis user. The results of this research will advance prosthetic control technology and create commercial products that positively impact prosthesis users. These results will also extend beyond military clinics and help civilians by guiding clinical care through objective measurement and action to better treat a person with limb loss.

Proposal Title:	Transtibial Osseointegration Surgery Study (TOSS)
Log Number:	OR220143
Current PI Name:	Benjamin Potter
Award Number:	HT9425-23-2-0012
Current Contracting Organization:	Henry M. Jackson Foundation
Current Performing Organization:	Walter Reed National Military Medical Center
Web Approval Date:	09-13-2023

This work addresses the FY22 PRORP Clinical Translational Research Award area of Osseointegration. Osseointegration is a relatively novel procedure derived from dentistry. In the 1950's, Osseointegration (OI) was first used to anchor teeth directly to the skeleton via a metal fixture. In the 1990's, OI was developed for long bones, like the femur and humerus, in a two-stage procedure. During the first stage (Stage I), a titanium rod is inserted into the long bone and allowed to anchor to the bone over a period of 3 months. During the second stage (Stage II), a titanium abutment is introduced into the internal fixture and secured under tension with a titanium alloy screw. Stage II is also when soft tissue rearrangement is performed, when needed. Much work has been done in studying OI procedures on the femur (transfemoral) and even the humerus (transhumeral), however, much less work has been done on the tibia (transtibial) for persons with limb loss. In order to better serve the military community and Service Members that may undergo a transtibial OI procedure, we have designed a research plan that focuses on filling the gaps of knowledge in transtibial procedures with a goal of advancing the U.S. Food and Drug Administration approval of OI for persons living with transtibial amputations.

We have five hypotheses we will address: Hypothesis 1: Persons that have undergone a transtibial OI procedure will have similar rates of complications as other OI procedures; Hypothesis 2: Persons that have undergone a transtibial OI procedure will have an improved quality of life and other important functional measures compared to before they had their OI procedure; Hypothesis 3: Persons that have undergone a transtibial OI procedure will have improved physical function compared to before they underwent the OI procedure; Hypothesis 4: Persons that have undergone a transtibial OI procedure will have a change in bone quality that mirrors their outcome measures; and Hypothesis 5: Persons that have undergone a transtibial OI procedure will have comparable results as those persons that underwent a transfemoral OI procedure.

In addition to our hypotheses, we have five specific aims we will reach by the end of the study: Aim 1: We will quantify skin complications and infections for transtibial participants; Aim 2: Determine whether transtibial participants are improving in their outcome measures; Aim 3: Determine whether transtibial participants are improving in their mobility and physical function; Aim 4: Determine if change in the residual bone quality mirror the changes in the outcome measures; and finally, Aim 5: Compare the outcomes of transtibial participants to transfemoral participants. We will achieve these aims and address our hypotheses by following 30 Service Members or beneficiaries that have the need for a transtibial OI procedure. We will first collect information before the procedure (baseline) to later compare their outcomes to follow-up visits at specific time points. We will also perform biomechanical tests, like walking and getting up from a chair, to determine if the procedure allows Service Members to achieve improved physical function. We also collect bone quality data using radiographs, computed tomography scans, dual-energy X-ray absorptiometry, and other tools to quantify the bone quality throughout their time in the study. This work will allow us to better understand the transtibial OI procedure and will allow us to better serve both the military amputee community and the limb loss community as a whole.

Proposal Title:	Incisional Negative Pressure Wound Therapy to Reduce Infection and Complications in High-Risk Fractures: A Multicenter Randomized Controlled Trial
Log Number:	OR220155
Current PI Name:	Ida Gitajn
Award Number:	HT9425-23-1-0357
Current Contracting Organization:	Dartmouth-Hitchcock Medical Center
Current Performing Organization:	Dartmouth-Hitchcock Medical Center
Web Approval Date:	09-03-2023

This application is a direct response to the fiscal year 2022 Focus Area "Retention Strategies Return to Duty". Injuries to the arms and legs sustained in battle commonly involve broken bones with severe soft tissue injury and/or open wounds (open fracture). Improvements in body armor and rescue strategies have increased the number of survivors of combat-related injuries. An unintended consequence of improved survival is an increase the number of wounded warriors' severe injuries that are at high risk for infectious and /or wound-healing complications. The negative functional consequences of these complications are profound, resulting in delayed rate of recovery and inferior outcomes in terms of pain, physical function, health care expenditure, and cost to society (including increased disability and reduction in ability to return to active duty or other work activities).

Incisional Negative Pressure Wound Therapy (NPWT) is a closed, sealed wound management system that applies negative pressure (suction) at the closed wound surface. This provides a sealed environment that prevents contamination from the external environment, helps improve blood flow to the healing wound, helps manage drainage from the wound, and reduces tension on the closed incision. This wound dressing can be easily deployed in a field-forward manner.

Based on the current literature, incisional NPWT appears to be effective at reducing complications in patients who have injuries at high risk for complication. However, there remain gaps in the literature, which contribute to practice variation. Based on these gaps, a well-designed multicenter clinical trial focusing on patients with who are at high risk for complication is urgently needed.

This proposal represents the next step toward demonstrating efficacy, paving the way for wider adoption with a reduction in complications, improved outcomes, and earlier return to duty and/or work. In this multicenter randomized clinical trial including 352 patients with high-risk injuries, we will compare the incidence of complications within 12 months in patients treated with incisional NPWT versus those treated with standard-of-care wound dressing. We will compare both health care costs and societal costs between these two groups. Lastly, among patients who develop infection, we will compare the infection microbiome between patients treated with incisional NPWT and those treated with standard-of-care wound dressing. At the completion of this trial, we will have demonstrated efficacy of incisional NPWT in a high-risk patient population. We will also demonstrate benefit with regard to both societal and health care costs and will better understand an important component of the mechanism underlying the effect of NPWT. We anticipate that this trial will have an enormous impact on clinical practice, providing both military and civilian surgeons with critical information around efficacy and indications/best practices.

Following the completion of this trial, it is anticipated to have an immediate substantial military benefit and clinical impact on the care and outcome of patients with severe lower-extremity fractures. Critical to

regaining normal lifestyle and returning to active duty is having a highly functional limb, which can be achieved more frequently and reliably when complications are prevented. We believe that this work will pave the way for clearer clinical indications and widespread adoption of this field-forward, easy-to-implement intervention.

Proposal Title:	Improving Wound Healing and Recovery by Blocking Excessive Scar Formation in Peripheral Nerves, Muscles, and Joints After Composite Injuries to Extremities
Log Number:	OR220161
Current PI Name:	Andrzej Fertala
Award Number:	HT9425-23-1-0638
Current Contracting Organization:	Thomas Jefferson University
Current Performing Organization:	Thomas Jefferson University
Web Approval Date:	09-17-2023

Objectives and Rationale: While scarring from injuries is normal and helps the body rebuild, excessive scarring can cause significant physical and psychological burdens and delayed return to military duties after traumatic limb injuries. Soldiers are uniquely prone to suffer from complex limb injuries due to the risks associated with their military activities. About half of all combat injuries to the musculoskeletal system involve the limbs. One of the significant complications of these injuries is post-traumatic scarring, which severely limits the regeneration of damaged limb tissues, including ligaments, tendons, joint capsules, muscles, and nerves. Thus, the overarching long-term goal of this study is to help wounded Soldiers recover from injuries to their limbs by applying a therapeutic antibody (referred to as ACA) that prevents excessive scarring of injured tissues.

Although surgery and lengthy physical therapies can reduce unwanted scarring, these interventions have poor outcomes and are not fully effective. Furthermore, no currently available medications have been proven to reduce excessive scar buildup effectively and safely. However, we are exploring using the antibody-based system to reduce scarring through antibody-based therapeutics, the fastest-growing class of modern drugs due to their high effectiveness, specificity, and safety.

Responding to the need to regain limb function, we developed ACA to block the formation of excessive fibrotic scars responsible for poor functional recovery. We have already demonstrated the effectiveness and safety of this antibody in a relevant animal model of limb injury. Here, we will move our antibody-based therapeutic closer to human trials by providing bases for its use in regenerating composite tissue injuries to the joints, muscles, and peripheral nerves.

Alignment with FY22 PRORP Strategic Goals: This proposal is in response to the Peer Reviewed Orthopaedic Research Program: Applied Research Award (Funding Opportunity Number: W81XWH-22-PRORP-ARA). The proposed study aligns with the Focus Area "Composite Tissue Regeneration." By targeting excessive scarring, a central barrier to the regeneration of musculoskeletal and neural tissues, with an advanced humanized therapeutic antibody, our proposal aligns with this Focus Area precisely.

Benefit for Service Members: The leading patient group that will benefit from this study is Service Members who have sustained trauma to their extremities. Since post-traumatic scarring is a leading cause of poor limb function recovery, applying our anti-scarring antibody is the right approach to reducing the excessive scarring problem. Thus, our research study will have an immediate positive impact on the healing of the limbs. It will also positively affect functional recovery and return to military service in the long term.

Consequently, faster, more predictable recovery will allow injured Soldiers to resume their military activities and help to maintain unit readiness. Moreover, returning to military or civil work activities will positively

impact the well-being of Soldiers' Families and help reduce the burden imposed on caregivers. Since the consequences of excessive post-traumatic scarring overlap in military and civilian populations, the proposed therapy will have a broad positive impact on many patients.

Far-Reaching Clinical Applications: Although our main target here is to reduce excessive post-traumatic scarring in extremities, the potential clinical applications of our technology in military personnel are far-reaching. They include reducing burn scars, limiting abdominal adhesions, blocking ocular scarring, and others. Since clinically used therapeutic antibodies are generally safe, we expect no significant risks associated with our approach. Furthermore, our research will positively impact broad research activities to heal wounded tissues better. By demonstrating ACA's effectiveness in reducing excessive scarring in multiple tissues, our research will expand potential anti-scarring targets beyond those few we focus on here.

Timeline for Patient-Related Outcomes. Our studies on applying the ACA to reduce unwanted scarring follow a logical trajectory that includes the following milestones: (i) identifying a clinical problem; (ii) defining a therapeutic target; (iii) developing a therapeutic agent; (iv) testing a selected agent in simple experimental models of disease; (v) testing a selected agent in relevant animal models; (vi) developing relevant biomarkers; (vii) checking concentration-dependent efficacy and potential side effects; (viii) establishing methods for large-scale production and delivery into target sites; (ix) performing preclinical tests in large-animal models; and (x) moving toward clinical studies with human patients. While we have already reached many of these milestones, the research proposed here will broaden the potential use of our technology. Apart from the direct study outcomes, we expect this proposal's success will help attract needed commercial partners and move the proposed technology into clinical trials within a few years.

Proposal Title:	Assessing an SRC Kinase Recruitment Inhibitor for Therapeutic Management of Knee Arthrofibrosis
Log Number:	OR220166
Current PI Name:	Denis Evseenko
Award Number:	HT9425-23-1-0718
Current Contracting Organization:	University of Southern California
Current Performing Organization:	University of Southern California
Web Approval Date:	09-26-2023

Focus Areas: Retention Strategies and Return to Duty

Orthopedic extremity trauma is a major problem for military Service Members, resulting in limited duty days for Service Members and long-term functional disability and substantial medical cost. The most common reported complication of the complex knee injury is arthrofibrosis (arthron, from Greek, meaning articulation, joint).

Arthrofibrosis is an inflammatory condition that leads to the production of excessive scar tissue in or around major joints. These joints can include the knee, shoulder, ankle, wrist, and hip. Arthrofibrosis can be the result of surgery or the initial injury to the joint. The knee is the most commonly injured joint, and due to its complex anatomy, restoration of its function after complex injury represents a significant challenge. In active military members, joint injury has severe forms including multiligamentous complex knee injuries and knee dislocations caused by high-energy trauma. Return to duty after treatment of a complex knee injury in the military population is challenging, with nearly half of the injured Soldiers eventually undergoing medical discharge for their injury. Currently, no efficient treatment of arthrofibrosis is available to prevent this debilitating condition.

The excess scar tissue limits range of motion, functionality, and can be painful and debilitating. Specialized care is required in the treatment of arthrofibrosis, and only a handful of orthopedic surgeons in the world have a significant amount of experience treating this condition.

The rate of arthrofibrosis oftentimes triples when patients have undergone previous surgeries for multiple ligamentous injuries that require early immobilization. A number of medical, social, and genetic risk factors for developing arthrofibrosis have been identified: Patients with autoimmune diseases (e.g., lupus), patients who need total joint replacement as a result of severe and/or early onset of osteoarthritis, patients who are prone to post-operative infections or have bleeding disorders (e.g., hemophilia, platelet disorders).

Arthroscopic lysis of scar tissue and manipulation under anesthesia are the most commonly performed treatments for arthrofibrosis. However, there are no guarantees of improved function or decreased pain following these approaches. In addition, both treatments damage the surrounding tissue, which may induce an inflammatory response that may cause further deposition of scar tissue, and the pharmacologic treatment for arthrofibrosis is currently limited. Administration of different anti-inflammatory drugs and biologics has been unsuccessful and there has been no specific pharmacological therapy able to prevent or cure arthrofibrosis. Thus, preventive treatment of knee arthrofibrosis after major knee injury is an unmet medical need, especially in military medicine.

The proposed project is designed to advance a safe, noninvasive treatment for prevention and treatment of arthrofibrosis in high-risk groups after injury by developing a novel, highly selective, and potent small-molecule SRi-1 designed to disrupt a recruitment and signaling of a critical protein required for transferring

scar-promoting signals within the specialized cells. This project will be conducted by a multidisciplinary team of surgeons, scientists, and bioengineers. Proposed studies include production and testing of slow drug-release formulations of SRi-1 using clinically approved biomaterials. The drug will be tested in a highly reproducible rodent model of posttraumatic arthrofibrosis recently developed by the research team.

The development of a novel small molecule will serve as an excellent therapeutic strategy that will prevent or halt the progression of arthrofibrosis by preventing the rapid formation of scar tissue, without adversely affecting the overall wound healing process. This therapy will be especially valuable in patients from high-risk groups of injured military personnel.

Proposal Title:	A New Therapeutic Target to Prevent Pancreatic Cancer Metastasis
Log Number:	PA220024
Current PI Name:	Yuan Chen
Award Number:	HT9425-23-1-0559
Current Contracting Organization:	California, University of, San Diego
Current Performing Organization:	California, University of, San Diego
Web Approval Date:	09-03-2023

Scientific Objective and Rationale: Pancreatic ductal adenocarcinoma is the most common type of pancreatic cancer and the third leading cause of cancer death in the United States, with a 5-year survival rate of only about 10%. Even for patients for whom the cancer has not spread to other parts of the body (metastasis), tumor removal followed by chemotherapy and radiation therapy typically does not result in a clean bill of health. Indeed, 3-year survival rates following tumor removal have improved only modestly in recent years. Moreover, clinical studies that have tried various combinations of chemotherapy in patients with metastasis have improved their survival by only a few months. Furthermore, immunotherapy with checkpoint inhibitors, which has been quite effective in treating several other tumor types, has not yet shown benefit to patients suffering pancreatic cancer. Clearly, a new type of treatment for pancreatic ductal adenocarcinoma is needed to improve the well-being of those affected by it.

Against this backdrop, the proposed project will investigate a new immunotherapy that is involved in not only controlling the antitumor immune response in the body, but also maintaining the self-renewal of cancer stem cells that seed tumor regrowth at either the original or new tumor sites in the body, which is responsible for recurrence and metastasis of the cancer -- especially in the case of pancreatic cancer. We will carry out our investigation using patient-derived organoids (a miniaturized and simplified version of an organ produced to study disease and treatment) and a mouse model of the disease, which will generate information necessary to bring this new immunotherapy to the clinic to help improve the outcomes of patients facing pancreatic cancer metastasis.

The proposed project addresses this FY22 PCARP Focus Area: Understanding the events that promote pancreatic cancer metastasis

Potential Impact of the Proposed Project on Pancreatic Cancer Research and/or Patient Care: We will carry out proposed project with cutting edge techniques. In particular, we will perform cross-species (human-mouse) transcriptomic analysis at the level of the single cell. This single-cell insight will help us determine the most effective and least toxic therapeutic combination to test in the mouse model. Determining this therapeutic combination will allow other researchers in the field of pancreatic cancer research to further develop this new immunotherapy and quickly deliver it to the clinic to better treat patients with pancreatic cancer.

Potential Impact of the Proposed Project on Pancreatic Cancer Patients and Their Families and/or Caregivers: Currently, being diagnosed with pancreatic cancer essentially is a death sentence. In addition to the low survival rate, the standard treatments for pancreatic cancer still feature mostly chemotherapy drugs developed decades ago. Indeed, the toxicity of these drugs is so high that patients typically require caregivers or family members to help them with their daily routine. Moreover, these toxic therapies extend the lives of patients by only a few months. Knowing this, many pancreatic cancer patients choose not to receive chemotherapy. Meanwhile, immunotherapy has revolutionized the treatment of other cancers, resulting in long-term survival not previously seen with chemotherapy, likely due to the development of immunological memory against the tumors.

To address these shortcomings, the proposed project will investigate a new immunotherapy for treating pancreatic cancer. In addition, because this new immunotherapy involves a first-in-class small molecule that was developed by Takeda Pharmaceuticals and exhibited excellent safety in Phase I trials, it will enable the results of the proposed project to easily and quickly be delivered to the clinic to improve the outcomes of pancreatic cancer patients. Overall, representing a team that spans the continuum from laboratory to clinic, the proposed project will provide the foundation for a new immunotherapy for pancreatic cancer that will eliminate the side effects of current treatments and greatly improve the outcomes and well-being of pancreatic cancer patients and their families and/or caregivers.

Proposal Title:	A New Therapeutic Target to Prevent Pancreatic Cancer Metastasis
Log Number:	PA220024P1
Current PI Name:	Andrew Lowy
Award Number:	HT9425-23-1-0560
Current Contracting Organization:	California, University of, San Diego
Current Performing Organization:	California, University of, San Diego
Web Approval Date:	09-03-2023

Scientific Objective and Rationale: Pancreatic ductal adenocarcinoma is the most common type of pancreatic cancer and the third leading cause of cancer death in the United States, with a 5-year survival rate of only about 10%. Even for patients for whom the cancer has not spread to other parts of the body (metastasis), tumor removal followed by chemotherapy and radiation therapy typically does not result in a clean bill of health. Indeed, 3-year survival rates following tumor removal have improved only modestly in recent years. Moreover, clinical studies that have tried various combinations of chemotherapy in patients with metastasis have improved their survival by only a few months. Furthermore, immunotherapy with checkpoint inhibitors, which has been quite effective in treating several other tumor types, has not yet shown benefit to patients suffering pancreatic cancer. Clearly, a new type of treatment for pancreatic ductal adenocarcinoma is needed to improve the well-being of those affected by it.

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Proposal Title:	Targeting Organelle pH Homeostasis in Pancreatic Cancer
Log Number:	PA220031
Current PI Name:	Cosimo Commisso
Award Number:	HT9425-23-1-0795
Current Contracting Organization:	Sanford Burnham Prebys Medical Discovery Institute, La Jolla
Current Performing Organization:	Sanford Burnham Prebys Medical Discovery Institute, La Jolla
Web Approval Date:	10-02-2023

Fiscal Year 2022 Pancreatic Cancer Research Program (FY22 PCARP) Focus Areas to be Addressed in the Proposed Research: (i) Our work will impact the understanding of the relationship between oncogenic signaling and the tumor microenvironment that drives therapeutic response, and (ii) our work will impact new drug development targeted toward cancer sensitivity and resistance mechanisms.

Innovative Aspects of the Proposed Research Project: Pancreatic cancer cells are exquisitely sensitive to changes in their internal pH. If a cancer cell becomes too acidic or too basic, this disrupts almost every molecular pathway involved in tumor growth, leading to cancer cell death. We recently discovered that pancreatic cancer cells regulate their internal pH through a protein called NHE7. This protein acts to maintain pH balance and, importantly, it only functions in cancer cells and not in normal cells. This specificity to tumors means that NHE7 represents a protein that we can try to stop in cancer cells while leaving the normal cells of the body unharmed. To find new drugs that might block pH balance in pancreatic cancer, we used a drug discovery approach. We took advantage of the state-of-the-art molecular testing capabilities that we have in-house and checked thousands of drugs for an effect on the pH of the pancreatic cancer cells. We identified a drug called IMD-0354, which blocks a molecular switch that is critical to pancreatic cancer progression. We show in this application that IMD-0354 disrupts pH balance in pancreatic tumor cells and causes cell death specifically in the cancer cells, leaving normal cells unaffected. We are excited about this new discovery because it means that we might be able to develop IMD-0354 or a similar drug as a therapeutic approach for pancreatic cancer.

Impact of the Proposed Research Project on the Field of Pancreatic Cancer Research: We have two specific goals for this application that will impact the field of pancreatic cancer research. The first goal is to understand how IMD-0354 disrupts pH balance in pancreatic cancer cells. To fully examine whether IMD-0354 can be used as a therapy in pancreatic cancer, we need to have a better understanding of how it works from a molecular perspective. To do this, we will use genetic approaches in cells from pancreatic cancer patients and test how IMD-0354 leads to an acidic internal cellular pH that specifically kills the cancer cells. Our second goal is to find out whether IMD-0354 can stop tumor growth in an animal model of pancreatic cancer. To test this, we will surgically transfer cancer cells directly into the pancreases of mice and then treat the animals with IMD-0354. We will measure tumor growth, as well as the lifespan of the mice. Using these animal models, we will also test whether IMD-0354 treatment can improve chemotherapy or immunotherapy.

Impact of the Proposed Research Project on Pancreatic Cancer Patients: While IMD-0354 and similar drugs have been studied in sicknesses like non-alcoholic fatty liver disease, they have never been tested in pancreatic cancer. Our findings show for the first time that IMD-0354 can kill pancreatic cancer cells, leaving normal cells healthy. We are very encouraged by these results and plan to find out whether IMD-0354 can be used to control the growth of pancreatic tumors. If successful, our work could set the stage for the clinical usage of IMD-0354 or similar drugs in pancreatic cancer. This has the potential to have immediate impact for patients since IMD-0354 and related molecules have been used in the clinical setting for other diseases, such as eye degeneration. This means that it would be relatively straightforward to test whether repurposing these drugs for pancreatic cancer has any benefit to clinical outcomes for patients.

Proposal Title:	Leveraging the Tumor Microenvironment in Primary and Metastatic Pancreatic Cancer
Log Number:	PA220063
Current PI Name:	Jordan Winter
Award Number:	HT9425-23-1-0656
Current Contracting Organization:	Case Western Reserve University
Current Performing Organization:	Case Western Reserve University
Web Approval Date:	09-26-2023

This application addresses the FY22 Pancreatic Cancer Research Program (PCARP) Focus Area "Understanding the relationship between oncogenic signaling and the tumor microenvironment that drives drug resistance and therapeutic response." Close to 50,000 people die each year from pancreatic ductal adenocarcinoma (PDAC) in the United States, making it the third most lethal cancer in the country. When PDAC cells spread to other parts of the body (known as metastatic PDAC, ~90% are found in the liver), existing chemotherapies do not work and 97% of patients die within 5 years after diagnosis. The overall goal of this project is to develop new therapies for treating patients with metastatic PDAC.

PDAC has limited access to nutrients and oxygen due to dense and fibrotic tissues surrounding the tumor. As a result, PDAC cells alter their metabolism to adapt and survive under a harsh environment. The Initiating PI Winter recently discovered that blocking a key metabolic enzyme isocitrate dehydrogenase 1 (IDH1) with an inhibitor can block metabolic adaptation. Treating PDAC with the inhibitor dramatically decreased cancer cell growth, and PDAC tumors shrunk in mice. As a result, mice with PDAC survived much longer than those that did not receive the inhibitor. Prior clinical studies reveal that metastatic PDAC in the liver prefers another type of metabolism and may be less susceptible to blocking this enzyme, requiring a different treatment approach. Thus, it is important to fully understand how PDAC cells behave both within the pancreas and at metastatic sites. The liver has a unique environment composed of many endothelial cells (ECs), which normally build blood vessels. The Young Investigator Partnering PI Wang found that liver ECs secret factors that communicate with cancer cells in the liver and accelerate cancer growth. These factors also alter cancer metabolism. Liver ECs do so by activating a surface protein HER3 (human epidermal growth factor receptor 3, also known as ErbB3) in PDAC cells, which in turn activates another molecule (AKT) to reprogram PDAC metabolism. Blocking HER3 decreased PDAC cell growth, and switched cancer metabolism back to a "primary tumor-like" biology that is maximally susceptible to an IDH1 inhibitor.

Our proposed studies will help us to understand how liver ECs help PDAC cells to grow and change cancer metabolism, especially in metastatic sites of spread. Moreover, we will leverage these insights to combine two strategies that should cooperate to effectively treat both primary and metastatic PDAC. We will employ state-of-the-art techniques to determine the effects of liver ECs and HER3/IDH1 inhibition on PDAC cell metabolism and survival, and will identify the pathways that drive those effects. We will also determine if combining an anti-HER3 therapy (seribantumab) and an anti-IDH1 (ivosidenib) agent will lead to stronger anti-cancer effects in cell culture conditions. Additionally, we will employ a mouse model that closely replicates PDAC spread in human, to study the effect of anti-HER3 therapy (seribantumab) and anti-IDH1 therapy (ivosidenib), alone or in combination, on both primary and metastatic disease.

Impact: Findings from this project will help us to better understand the differences in metabolism between primary and metastatic PDAC, and the key factors regulating these activities. This project will also help us to understand how the liver microenvironment affects the metabolism of metastatic deposits. These pathways can be targeted with drugs that block IDH1 (ivosidenib) and HER3 (seribantumab), and both are safe in patients. Therefore, if successful, this work can be rapidly translated into clinical trials to test a completely

new strategy to treat this lethal disease. More importantly, we anticipate that these studies will identify a drug combination that is especially effective against metastatic PDAC.

Innovation: This project will determine the antitumor effects of combining two therapies that target separate metabolic pathways and have never been tested in combination prior to this work. These studies are entirely based on the independent studies of the mentor (PI Winter) and mentee (PI Wang) and were selected because of their separate effects on PDAC metabolism. Additionally, the study is unique because it seeks to understand how the most prevalent non-tumor element, specifically ECs, impacts tumor growth and can be targeted through this therapeutic approach (anti-HER3 therapy).

Proposal Title:	Leveraging the Tumor Microenvironment in Primary and Metastatic Pancreatic Cancer
Log Number:	PA220063P1
Current PI Name:	Rui Wang
Award Number:	HT9425-23-1-0657
Current Contracting Organization:	Case Western Reserve University
Current Performing Organization:	Case Western Reserve University
Web Approval Date:	09-26-2023

This application addresses the FY22 Pancreatic Cancer Research Program (PCARP) Focus Area "Understanding the relationship between oncogenic signaling and the tumor microenvironment that drives drug resistance and therapeutic response." Close to 50,000 people die each year from pancreatic ductal adenocarcinoma (PDAC) in the United States, making it the third most lethal cancer in the country. When PDAC cells spread to other parts of the body (known as metastatic PDAC, ~90% are found in the liver), existing chemotherapies do not work and 97% of patients die within 5 years after diagnosis. The overall goal of this project is to develop new therapies for treating patients with metastatic PDAC.

PDAC has limited access to nutrients and oxygen due to dense and fibrotic tissues surrounding the tumor. As a result, PDAC cells alter their metabolism to adapt and survive under a harsh environment. The Initiating PI Winter recently discovered that blocking a key metabolic enzyme isocitrate dehydrogenase 1 (IDH1) with an inhibitor can block metabolic adaptation. Treating PDAC with the inhibitor dramatically decreased cancer cell growth, and PDAC tumors shrunk in mice. As a result, mice with PDAC survived much longer than those that did not receive the inhibitor. Prior clinical studies reveal that metastatic PDAC in the liver prefers another type of metabolism and may be less susceptible to blocking this enzyme, requiring a different treatment approach. Thus, it is important to fully understand how PDAC cells behave both within the pancreas and at metastatic sites. The liver has a unique environment composed of many endothelial cells (ECs), which normally build blood vessels. The Young Investigator Partnering PI Wang found that liver ECs secret factors that communicate with cancer cells in the liver and accelerate cancer growth. These factors also alter cancer metabolism. Liver ECs do so by activating a surface protein HER3 (human epidermal growth factor receptor 3, also known as ErbB3) in PDAC cells, which in turn activates another molecule (AKT) to reprogram PDAC metabolism. Blocking HER3 decreased PDAC cell growth, and switched cancer metabolism back to a "primary tumor-like" biology that is maximally susceptible to an IDH1 inhibitor.

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Impact: Findings from this project will help us to better understand the differences in metabolism between primary and metastatic PDAC, and the key factors regulating these activities. This project will also help us to understand how the liver microenvironment affects the metabolism of metastatic deposits. These pathways can be targeted with drugs that block IDH1 (ivosidenib) and HER3 (seribantumab), and both are safe in patients. Therefore, if successful, this work can be rapidly translated into clinical trials to test a completely

new strategy to treat this lethal disease. More importantly, we anticipate that these studies will identify a drug combination that is especially effective against metastatic PDAC.

Innovation: This project will determine the antitumor effects of combining two therapies that target separate metabolic pathways and have never been tested in combination prior to this work. These studies are entirely based on the independent studies of the mentor (PI Winter) and mentee (PI Wang) and were selected because of their separate effects on PDAC metabolism. Additionally, the study is unique because it seeks to understand how the most prevalent non-tumor element, specifically ECs, impacts tumor growth and can be targeted through this therapeutic approach (anti-HER3 therapy).

Proposal Title:	CTGF Targeting as a Novel Mechanism to Impede Pancreatic Cancer Cachexia
Log Number:	PA220091
Current PI Name:	Sarah Judge
Award Number:	HT9425-23-1-0437
Current Contracting Organization:	Florida, University of
Current Performing Organization:	Florida, University of
Web Approval Date:	09-03-2023

Cancer-associated cachexia is defined as the involuntary loss of body and skeletal muscle mass (with or without fat loss), which cannot be completely reversed through nutritional support. Cachexia is a serious complication of cancer that reduces physical function, impairs patients' ability to tolerate cancer therapies, and is estimated to be responsible for greater than 20% of all cancer-related deaths. In fact, nearly half of world-wide cancer-related deaths (approximately 8.2 million) can be attributed to cancers that induce cachexia, including pancreatic, colorectal, and esophageal cancer. Among these cancers, pancreatic cancer has the highest prevalence, affecting 63% of newly diagnosed pancreatic ductal adenocarcinoma (PDAC) patients, and which increases to up to 80% as the disease progresses. This is particularly relevant to military service members, who have a 40%-90% increased risk of pancreatic cancer that may be related to increased smoking and prevalence of diabetes, and for Vietnam Veterans, exposure to agent orange. Moreover, advancing age is also an important risk factor for pancreatic cancer, with the median age of onset 72 years. Since approximately 1.5 million Vietnam Veterans were born between 1946 and 1950, pancreatic cancer is expected to be a significant health concern facing our Veteran population now and in the coming years. Thus, identifying therapies that preserve muscle mass during the progression of pancreatic cancer has tremendous potential to increase the quality of life and prolong the survival of a significant majority of veterans affected by this devastating disease.

The current proposal addresses the fiscal year 2022 PCARP Focus Area "Understanding the relationship between metabolic disruptions in pancreatic cancer and their systemic effects, including diabetes and cachexia". Indeed, our studies will investigate CTGF produced locally within PDAC tumors, and in skeletal muscle in response to the tumor, as key events that promote distinct features of cachexia.

Proposal Title:	Defining the Impact of Kras Mutational Heterogeneity on the Tumor Microenvironment in Pancreatic Cancer
Log Number:	PA220094
Current PI Name:	Lukas Dow
Award Number:	HT9425-23-1-0728
Current Contracting Organization:	Joan and Sanford I Weill Medical College of Cornell University
Current Performing Organization:	Joan and Sanford I Weill Medical College of Cornell University
Web Approval Date:	09-15-2023

Background: Pancreatic cancer is a deadly disease with very few effective treatment options. In fact, of those patients with stage IV pancreatic ductal adenocarcinoma (PDA), less than 11% survive more than 5 years. Immunotherapy is an exciting new therapy for cancer patients, but it has limited efficacy in PDA. Understanding why PDA tumors are resistant to immunotherapy will increase sensitivity to current treatments, improving patient outcomes.

Tumors are not just cancer cells, but include many other cell types, including immune cells. A tumor's DNA makeup can profoundly influence the number and types of immune cells in the tumor microenvironment, but this association has not been well-defined in PDA. KRAS gene mutations are present in over 90% of pancreatic tumors, with the vast majority occurring as mutations in a single amino acid of the protein -- glycine 12. We have shown that different amino acid substitutions in KRAS glycine 12 can change cellular signaling and alter tumor development and progression. For example, we revealed that mice with a KrasG12D mutation in the pancreas develop widespread "early-PDA" lesions called PanINs at 12 weeks of age, while mice carrying a KrasG12R mutation in the pancreas show very little change from normal. In PDA patients, a tumor's KRAS mutation type is associated with varying clinical outcomes. For example, patients with KRASG12D tumor mutations have poorer prognoses than patients with WT KRAS or KRASG12R mutations.

Several research studies have demonstrated that KrasG12D mutations in tumor cells can remodel the immune microenvironment, yet the effect of other cancer-associated Kras mutations common in human PDA (G12V, G12C, G12R) have not been explored. Our poor understanding of the different Kras mutations limits our ability to design and implement appropriate treatments. Building on our previous work demonstrating that cells with specific Kras mutations exhibit different behaviors, we hypothesize that tumors with specific Kras mutations will display distinct immune microenvironments. Our proposal will determine how specific Kras mutations modulate the tumor microenvironment and promote evasion of immune-based therapies.

This application will address the following PCARP Focus Areas:

1. Understanding the relationship between oncogenic signaling and the tumor microenvironment that drives drug resistance and therapeutic response

2. New drug development targeted toward cancer sensitivity and resistance mechanisms including immune mechanisms of resistance

Innovation: We do not understand how tumor DNA genotype shapes the surrounding immune cells in PDA, what signaling pathways underlie these changes, or how we can design personalized immune-based combination therapy. We are leveraging our combined expertise in tumor immunology, preclinical models, oncogenic Kras signaling, and CRISPR-based gene editing to address a poorly understood but important question. We will use cutting-edge CRISPR-based editing technologies in 3D tumor organoid models,

combined with state-of-the-art transcriptomic profiling technologies to search for drug targets that render PDA tumors susceptible to therapy. Our proposed project is conceptually novel through a focus on understanding the molecular and immunologic differences driven by prevalent, yet distinct KRAS variants. This work may help explain why patients with KRASG12D mutant tumors have poorer prognoses than patients with KRASG12R, or KRASG12V mutant tumors, in addition to guiding the design of personalized therapies appropriate to patients based on genotype.

Impact: KRAS mutational status (e.g., G12D, G12R, G12V) is associated with different clinical outcomes in PDA patients. Although tumor genetic sequencing (which includes KRAS) is currently recommended for all stage IV pancreatic cancer patients, KRAS mutation status is not currently used as a predictive biomarker for therapy selection. Our short-term goal is to delineate immune profile differences between distinct Kras mutant tumors using preclinical models of PDA. Our medium-term outcome is to rationally design novel immunotherapy drug combinations based on an integrated analysis of the patient's tumor genetic makeup and immune profile. The long-term goal of this proposal is to improve patient outcomes by designing precision immunotherapy regimens that can be tested in clinical trials. This proposal will have a major impact on pancreatic cancer research by providing key proof-of-concept data revealing the heterogeneity of the tumor microenvironment within pancreatic cancer while highlighting the importance of distinct KRAS mutations beyond the well-defined KrasG12D mutation.

Proposal Title:	Defining the Impact of Kras Mutational Heterogeneity on the Tumor Microenvironment in Pancreatic Cancer
Log Number:	PA220094P1
Current PI Name:	Despina Siolas
Award Number:	HT9425-23-1-0731
Current Contracting Organization:	Joan and Sanford I Weill Medical College of Cornell University
Current Performing Organization:	Joan and Sanford I Weill Medical College of Cornell University
Web Approval Date:	09-15-2023

Background: Pancreatic cancer is a deadly disease with very few effective treatment options. In fact, of those patients with stage IV pancreatic ductal adenocarcinoma (PDA), less than 11% survive more than 5 years. Immunotherapy is an exciting new therapy for cancer patients, but it has limited efficacy in PDA. Understanding why PDA tumors are resistant to immunotherapy will increase sensitivity to current treatments, improving patient outcomes.

Tumors are not just cancer cells, but include many other cell types, including immune cells. A tumor's DNA makeup can profoundly influence the number and types of immune cells in the tumor microenvironment, but this association has not been well-defined in PDA. KRAS gene mutations are present in over 90% of pancreatic tumors, with the vast majority occurring as mutations in a single amino acid of the protein -- glycine 12. We have shown that different amino acid substitutions in KRAS glycine 12 can change cellular signaling and alter tumor development and progression. For example, we revealed that mice with a KrasG12D mutation in the pancreas develop widespread "early-PDA" lesions called PanINs at 12 weeks of age, while mice carrying a KrasG12R mutation in the pancreas show very little change from normal. In PDA patients, a tumor's KRAS mutation type is associated with varying clinical outcomes. For example, patients with KRASG12D tumor mutations have poorer prognoses than patients with WT KRAS or KRASG12R mutations.

Several research studies have demonstrated that KrasG12D mutations in tumor cells can remodel the immune microenvironment, yet the effect of other cancer-associated Kras mutations common in human PDA (G12V, G12C, G12R) have not been explored. Our poor understanding of the different Kras mutations limits our ability to design and implement appropriate treatments. Building on our previous work demonstrating that cells with specific Kras mutations exhibit different behaviors, we hypothesize that tumors with specific Kras mutations will display distinct immune microenvironments. Our proposal will determine how specific Kras mutations modulate the tumor microenvironment and promote evasion of immune-based therapies.

This application will address the following PCARP Focus Areas:

1. Understanding the relationship between oncogenic signaling and the tumor microenvironment that drives drug resistance and therapeutic response

2. New drug development targeted toward cancer sensitivity and resistance mechanisms including immune mechanisms of resistance

Innovation: We do not understand how tumor DNA genotype shapes the surrounding immune cells in PDA, what signaling pathways underlie these changes, or how we can design personalized immune-based combination therapy. We are leveraging our combined expertise in tumor immunology, preclinical models, oncogenic Kras signaling, and CRISPR-based gene editing to address a poorly understood but important question. We will use cutting-edge CRISPR-based editing technologies in 3D tumor organoid models,

combined with state-of-the-art transcriptomic profiling technologies to search for drug targets that render PDA tumors susceptible to therapy. Our proposed project is conceptually novel through a focus on understanding the molecular and immunologic differences driven by prevalent, yet distinct KRAS variants. This work may help explain why patients with KRASG12D mutant tumors have poorer prognoses than patients with KRASG12R, or KRASG12V mutant tumors, in addition to guiding the design of personalized therapies appropriate to patients based on genotype.

Impact: KRAS mutational status (e.g., G12D, G12R, G12V) is associated with different clinical outcomes in PDA patients. Although tumor genetic sequencing (which includes KRAS) is currently recommended for all stage IV pancreatic cancer patients, KRAS mutation status is not currently used as a predictive biomarker for therapy selection. Our short-term goal is to delineate immune profile differences between distinct Kras mutant tumors using preclinical models of PDA. Our medium-term outcome is to rationally design novel immunotherapy drug combinations based on an integrated analysis of the patient's tumor genetic makeup and immune profile. The long-term goal of this proposal is to improve patient outcomes by designing precision immunotherapy regimens that can be tested in clinical trials. This proposal will have a major impact on pancreatic cancer research by providing key proof-of-concept data revealing the heterogeneity of the tumor microenvironment within pancreatic cancer while highlighting the importance of distinct KRAS mutations beyond the well-defined KrasG12D mutation.

Proposal Title:	Targeting Nuclear Necroptosis Pathway in Pancreatic Cancer Microenvironment
Log Number:	PA220130
Current PI Name:	Igor Astsaturov
Award Number:	HT9425-23-1-0941
Current Contracting Organization:	Institute for Cancer Research
Current Performing Organization:	Institute for Cancer Research
Web Approval Date:	09-29-2023

Scientific Objective and Rationale: Pancreatic cancer in 2022 remains one of the most devastating cancers irrevocably affecting the lives of nearly 50,000 Americans including Service Members, Veterans, and their Families. Unfortunately, the poor survival of PDAC patients, in whom the number of diagnosed narrowly exceeds the number of deaths, is the result of limited treatment options. Pancreatic cancer patients typically do not benefit from immunotherapy, which has brought major improvements in longevity, quality of life, and cures in lung, colon, melanoma, and other major cancers. This failure of the current immunotherapy approaches is determined by what is called a "cold" or immune suppressive microenvironment in the pancreatic tumors, which prevents immune cells from entering the tumor tissue, and deactivates even the few immune cells that are present in the vicinity of cancer cells.

Research Idea/Hypothesis: We designed an approach to overcome this challenging barrier of immunotherapy resistance in pancreatic cancer by imitating changes similar to those in severe viral infections. When there is an ongoing viral infection, such as flu virus, the immune cells recognize the viral genetic material because its nucleic acids are twisted in the opposite direction than in humans (called Z-nucleic acids). A specialized protein called Z-binding protein 1, or ZBP1, is the critical sensor for such viruses. Once ZBP1 recognizes and binds Z-DNA, it triggers an all-out cascade leading to formation of holes in the membranes of cells, leading to a form of cell death called nuclear necroptosis (published in Cell (2020) and Nature (2022) by the Balachandran lab). We will target with a drug a specific subset of fibroblastic cells in the pancreatic cancer tissue microenvironment, called inflammatory fibroblasts, or iCAFs, which happen to express high level of ZBP1, and will push these iCAFs to execute nuclear necroptosis.

What we ultimately aim to do next is to stimulate the immune system to kill pancreatic cancer cells. Remarkably, we found a drug called CBL0137 that can twist the cellular DNA in pancreatic tumors in the Z-form and to activate necroptosis. We hypothesized that nuclear necroptosis in iCAFs would send the "danger signals" to the immune cells and reinvigorate an immune attack on pancreatic cancer. Toward this objective, we will test why ZBP1 is expressed in pancreatic tissues because this will help us define the biomarkers for future patients' selection (Aim 1). Secondly, we would like to test the efficacy of CBL0137 alone and in combination with immunotherapy drugs against the preclinical models of pancreatic cancer in mice (Aim 2).

Relevance to Intent-Partnership: The proposed studies are the result of an ongoing and sustained collaboration between Dr. Siddharth Balachandran, who is an expert in nuclear necroptosis, and Dr. Igor Astsaturov, a physician-scientist with expertise in pancreatic cancer. The Balachandran lab will deploy unique research tools to study the effects of CBL0137 on nuclear necroptosis triggered by ZBP1. Dr. Astsaturov is a physician-scientist and a co-Director of the Greenberg Pancreatic Cancer Institute at Fox Chase. His laboratory and the clinical team will work synergistically with the Balachandran lab to deploy the necroptosis inducer, CBL0137, as a transformative immunotherapy tool in PDAC. Dr. Astsaturov's lab will contribute their expertise in pancreatic cancer preclinical models and analyses of PDAC TME using the cutting-edge single-cell genomics and spatial transcriptomics methods to define a subset of PDAC tumors, which will be highly vulnerable to nuclear necroptosis induction with CBL0137.

Impact: We will make major advancements in the field of pancreatic cancer immunotherapy by: (1) nominating a subset of fibroblastic cells called inflammatory fibroblasts (iCAFs) as a new vulnerability target for immunotherapeutic exploitation through their high expression of ZBP1; (2) developing CBL0137 as a small-molecule inducer of Z-DNA formation and potent activator of nuclear necroptosis; and (3) by converting cold pancreatic cancer TME to hot via ZBP1-initiated necroptosis.

FY22 PCARP Overarching Challenge: Therapeutics -- advance immunotherapy. We will directly test the ability of nuclear necroptosis to synergize with ICB in clinically relevant models of localized and metastatic PDAC. The proposed validation and preclinical testing of necroptosis activation as a therapeutic concept will lead to a rapid translation of the findings to pancreatic cancer patients. Our clinical team (Olszanski /Astsaturov) is poised to add a pancreatic cancer cohort to the currently open phase 1 clinical trial of CBL0137 in combination with ICB and to translate the findings from this project to the clinic. With completion of the proposed studies, we will acquire all the necessary expertise and supportive mechanistic data to lead toward a transformative reversal of the PDAC immune resistance mechanism.

Proposal Title:	Targeting Nuclear Necroptosis Pathway in Pancreatic Cancer Microenvironment
Log Number:	PA220130P1
Current PI Name:	Siddharth Balachandran
Award Number:	HT9425-23-1-0942
Current Contracting Organization:	Institute for Cancer Research
Current Performing Organization:	Institute for Cancer Research
Web Approval Date:	09-29-2023

Scientific Objective and Rationale: Pancreatic cancer in 2022 remains one of the most devastating cancers irrevocably affecting the lives of nearly 50,000 Americans including Service Members, Veterans, and their Families. Unfortunately, the poor survival of PDAC patients, in whom the number of diagnosed narrowly exceeds the number of deaths, is the result of limited treatment options. Pancreatic cancer patients typically do not benefit from immunotherapy, which has brought major improvements in longevity, quality of life, and cures in lung, colon, melanoma, and other major cancers. This failure of the current immunotherapy approaches is determined by what is called a "cold" or immune suppressive microenvironment in the pancreatic tumors, which prevents immune cells from entering the tumor tissue, and deactivates even the few immune cells that are present in the vicinity of cancer cells.

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Proposal Title:	Netrin G1 Ligand: A New Immunomodulatory Target and Early Biomarker in Pancreatic Cancer
Log Number:	PA220131
Current PI Name:	Edna Cukierman
Award Number:	HT9425-23-1-0584
Current Contracting Organization:	Institute for Cancer Research
Current Performing Organization:	Institute for Cancer Research
Web Approval Date:	09-26-2023

Background: Pancreatic ductal adenocarcinoma (PDAC), soon to be the second deadliest cancer, has a 5-year survival of only 11%. Some of the reasons for this outcome are the lack of biomarkers for early detection and the presence of a unique microenvironment, marked by the expansion of cancer-associated fibroblasts, fibrotic stroma and immunosuppression. Therefore, uncovering new targets for detection and treatment, together with the better understanding of this unique microenvironment is critical. In preliminary data for this study, we identified the synaptic protein NGL-1 in fibroblasts and immune cells of pre-cancerous and PDAC lesions. NGL-1 germline knockout mice (KO) orthotopically allografted with PDAC cells presented limited tumor burden and immunosuppressive cytokines, with increased presence of CD8+ T cells, compared to wild type (WT) mice. Mechanistically, NGL-1 KO hosts downregulated key pro-tumor signaling pathways, displaying a signature that would support T cell activity. Results indicate that NGL-1 is important for both the functionality of pro-tumor stromal cells and the inhibition of antitumor cells such as CD8+ T cells, highlighting NGL-1 as a novel tumor microenvironment target that can be modulated in different cells, resulting in an antitumor effect.

Hypotheses: We hypothesize that (1) NGL-1 is an immunomodulatory protein that controls immune cell function to favor tumor development; (2) NGL-1 is an early biomarker in PDAC, being detected in precursor lesions and indicative of a tumor supportive pre-cancerous stroma; (3) NGL-1 is a potential new therapeutic target in PDAC.

Specific Aims: Aim 1: Determine the immunomodulatory functions of NGL-1 in T cells; Aim 2: Explore the roles of NGL-1 expression in pre-cancerous tissue and its potential value as an early PDAC biomarker; Aim 3: Determine the therapeutic potential of targeting NGL-1 in PDAC.

Our proposal will address the following FY22 PCARP Focus Areas: (1) Early detection research for pancreatic cancer, (2) Understanding precursors, origins, and early progression of pancreatic cancer, (3) New drug development targeting immune mechanisms of resistance.

Study Design: In Aim 1, we will perform ex-vivo tests with T cells isolated from NGL-1 WT and KO murine tumors, comparing their ability to kill PDAC cells, to proliferate and to secrete pro- and antitumor cytokines, as a measurement of their functionality. We will perform the same experiments in naive WT and KO T cells, to explore the intrinsic roles of NGL-1 in T cell function. These results will be important in the context of PDAC, as well as other diseases. In Aim 2, we will explore the roles of NGL-1 in precursor lesions and in patients at genetic risk, to confirm the value of NGL-1 as an early biomarker in PDAC. First, using a chemically induced model of pancreatitis in WT and KO mice, we will determine if NGL-1 plays a role in exacerbating pre-cancerous inflammatory tissue. Translationally, we will assess NGL-1 expression in human normal pancreas tissue and in tissue with precursor lesions, as well as in patients bearing germline mutations that predispose to cancer development, correlating its expression with of other stromal pro-tumor markers, lesion grade and clinical annotations. In Aim 3, we will dissect the contributions of major immune cell populations for the pro-tumor effects of NGL-1, by functionally depleting these during tumor

development in orthotopic allografts in WT and KO mice. To understand PDAC progression in relation to NGL-1, we will cross the NGL-1 KO mice with KPC mice (KrasLSL-G12D/+; p53LSL-R172H/+; Pdx-Cre /+) and compare with the WT KPC mice, assessing tumor burden and the pro-tumor stromal signature. Finally, we will inquire if targeting NGL-1 globally (in all cell compartments, using the new KPC/NGL-1 KO mice) could promote the effect of chemo/immunotherapies.

Impact: PDAC is a challenging disease, projected to become the second leading cause of cancer-related deaths by 2026. The lack of early biomarkers and the poor understanding of its unique microenvironment contribute to this abysmal prediction. Importantly, PDAC risk increases with ionizing exposure, smoking, diabetes, and other factors often associated with chronic pancreatitis and inflammation. These are all relevant factors that are overrepresented in military populations and their families. Therefore, uncovering new targets for detection and treatment is critical. Our preliminary data suggest that NGL-1 could be a potential new target in PDAC, a cancer that is refractory to different therapeutic approaches, including immunotherapies. The successful completion of our study will (1) define a new molecular target that simultaneously controls tumor, fibroblastic, and immune cell function, providing mechanisms behind its activity in antitumor CD8+ T cells; (2) define a potential early PDAC biomarker, providing an opportunity for early detection and intervention; and (3) overcome key roadblocks for immunotherapy in PDAC. We believe that these discoveries will lead to better outcomes for patients and their families, having a particularly profound impact on military personnel health.

Innovation: This proposal is innovative because it addresses multiple aspects of PDAC. Different from most reported targets, NGL-1 is a global target in PDAC (stroma, immune and epithelial cells) that leads to tumor progression and inversely correlates with patient survival. We will also have an opportunity to better understand how the establishment of the pro-PDAC stroma and the acquisition of immunosuppression occurs, with the goal to modulate these aspects by targeting NGL-1, and therefore facilitating the action of other therapies. Finally, and importantly, we will not only investigate NGL-1 in PDAC tissue, but also in precursor lesions and in patients with familial pre-dispositions to PDAC. These combined perspectives could solidify NGL-1 as new early biomarker in PDAC. These are innovative approaches for such devasting and incurable disease.

Proposal Title:	Netrin G1 Ligand: A New Immunomodulatory Target and Early Biomarker in Pancreatic Cancer
Log Number:	PA220131P1
Current PI Name:	Debora Vendramini Costa
Award Number:	HT9425-23-1-0585
Current Contracting Organization:	Henry Ford Health System
Current Performing Organization:	Henry Ford Health System
Web Approval Date:	09-26-2023

Background: Pancreatic ductal adenocarcinoma (PDAC), soon to be the second deadliest cancer, has a 5-year survival of only 11%. Some reasons for this outcome are the lack of biomarkers for early detection and the presence of a unique microenvironment, marked by the expansion of cancer-associated fibroblasts, fibrotic stroma, and immunosuppression. Therefore, uncovering new targets for detection and treatment, together with the better understanding of this unique microenvironment, is critical. In preliminary data for this study, we identified the synaptic protein NGL-1 in fibroblasts and immune cells of pre-cancerous and PDAC lesions. NGL-1 germline knockout mice (KO) orthotopically allografted with PDAC cells presented limited tumor burden and immunosuppressive cytokines, with increased presence of CD8+ T cells, compared to wild type (WT) mice. Mechanistically, NGL-1 KO hosts downregulated key pro-tumor signaling pathways, displaying a signature that would support T cell activity. Results indicate that NGL-1 is important for both the functionality of pro-tumor stromal cells and the inhibition of antitumor cells such as CD8+ T cells, highlighting NGL-1 as a novel tumor microenvironment target that can be modulated in different cells, resulting in an antitumor effect.

Hypotheses: We hypothesize that (1) NGL-1 is an immunomodulatory protein that controls immune cell function to favor tumor development; (2) NGL-1 is an early biomarker in PDAC, being detected in precursor lesions and indicative of a tumor supportive pre-cancerous stroma; (3) NGL-1 is a potential new therapeutic target in PDAC.

Specific Aims: Aim 1: Determine the immunomodulatory functions of NGL-1 in T cells; Aim 2: Explore the roles of NGL-1 expression in pre-cancerous tissue and its potential value as an early PDAC biomarker; Aim 3: Determine the therapeutic potential of targeting NGL-1 in PDAC.

Our proposal will address the following FY22 PCARP Focus Areas: (1) Early detection research for pancreatic cancer, (2) Understanding precursors, origins, and early progression of pancreatic cancer, (3) New drug development targeting immune mechanisms of resistance.

Study Design: In Aim 1, we will perform ex-vivo tests with T cells isolated from NGL-1 WT and KO murine tumors, comparing their ability to kill PDAC cells, to proliferate, and to secrete pro- and antitumor cytokines, as a measurement of their functionality. We will perform the same experiments in naive WT and KO T cells, to explore the intrinsic roles of NGL-1 in T cell function. These results will be important in the context of PDAC, as well as other diseases. In Aim 2, we will explore the roles of NGL-1 in precursor lesions and in patients at genetic risk, to confirm the value of NGL-1 as an early biomarker in PDAC. First, using a chemically induced model of pancreatitis in WT and KO mice, we will determine if NGL-1 plays a role in exacerbating pre-cancerous inflammatory tissue. Translationally, we will assess NGL-1 expression in human normal pancreas tissue and in tissue with precursor lesions, as well as in patients bearing germline mutations that predispose to cancer development, correlating its expression with other stromal pro-tumor markers, lesion grade, and clinical annotations. In Aim 3, we will dissect the contributions of major immune cell populations for the pro-tumor effects of NGL-1, by functionally depleting these during tumor

development in orthotopic allografts in WT and KO mice. To understand PDAC progression in relation to NGL-1, we will cross the NGL-1 KO mice with KPC mice (KrasLSL-G12D/+; p53LSL-R172H/+; Pdx-Cre /+) and compare with the WT KPC mice, assessing tumor burden and the pro-tumor stromal signature. Finally, we will inquire if targeting NGL-1 globally (in all cell compartments, using the new KPC/NGL-1 KO mice) could promote the effect of chemo/immunotherapies.

Impact: PDAC is a challenging disease, projected to become the second leading cause of cancer-related deaths by 2026. The lack of early biomarkers and the poor understanding of its unique microenvironment contribute to this abysmal prediction. Importantly, PDAC risk increases with ionizing exposure, smoking, diabetes, and other factors often associated with chronic pancreatitis and inflammation. These are all relevant factors that are overrepresented in military populations and their families. Therefore, uncovering new targets for detection and treatment is critical. Our preliminary data suggest that NGL-1 could be a potential new target in PDAC, a cancer that is refractory to different therapeutic approaches, including immunotherapies. The successful completion of our study will (1) define a new molecular target that simultaneously controls tumor, fibroblastic, and immune cell function, providing mechanisms behind its activity in antitumor CD8+ T cells; (2) define a potential early PDAC biomarker, providing an opportunity for early detection and intervention; and (3) overcome key roadblocks for immunotherapy in PDAC. We believe that these discoveries will lead to better outcomes for patients and their families, having a particularly profound impact on military personnel health.

Innovation: This proposal is innovative because it addresses multiple aspects of PDAC. Different from most reported targets, NGL-1 is a global target in PDAC (stroma, immune, and epithelial cells) that leads to tumor progression and inversely correlates with patient survival. We will also have an opportunity to better understand how the establishment of the pro-PDAC stroma and the acquisition of immunosuppression occurs, with the goal to modulate these aspects by targeting NGL-1 and therefore facilitating the action of other therapies. Finally, and importantly, we will not only investigate NGL-1 in PDAC tissue, but also in precursor lesions and in patients with familial pre-dispositions to PDAC. These combined perspectives could solidify NGL-1 as new early biomarker in PDAC. These are innovative approaches for such devasting and incurable disease.

Proposal Title:	Real-Time Metabolic Imaging to Interrogate Early Detection and Prevention of Pancreatic Cancer
Log Number:	PA220132
Current PI Name:	Pratip Bhattacharya
Award Number:	HT9425-23-1-0664
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	09-15-2023

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive malignancies with a dismal prognosis. Most patients present with unresectable disease and therapies are limited. It is therefore crucial to develop novel strategies for early detection and prevention.

Since pancreatic premalignant lesions preceding cancer development cannot be detected by conventional imaging modalities (magnetic resonance imaging, MRI, or computed tomography, CT), PDAC preventive strategies must be developed in parallel with novel biomarkers to monitor efficacy. We propose to use hyperpolarized 13C pyruvate-based metabolic MRI for early detection of pancreatic premalignant lesions, to follow their progression in the context of preventive agents that could be easily translated into patients. Hyperpolarized MRI offers unprecedented insights into cellular metabolism in real time by enhancing the signal of conventional MRI by greater than 10,000-fold. This methodology measures metabolic changes and has proven highly sensitive for detecting pancreatic premalignant lesions to monitor immunopreventive efficacy of antibiotics.

PDAC development is associated with an immunosuppressive microenvironment. Recently, studies have shown that gut bacteria associated with human and mouse PDAC is distinctive. Furthermore, PDAC-associated gut bacteria induces tumor immunosuppression and its ablation reverses it. We plan to repurpose antibiotics for PDAC prevention, including determination of best regimen and dose, and to study the mechanisms of immunomodulation in depth.

Like immunotherapies, in which antitumoral responses are usually delayed, immunopreventive interventions cannot be effectively tracked by measuring tumor incidence/size at one time point. Instead, highly sensitive methods that can assess premalignant lesions and tumor incidence in live animals at real time are needed. In this translational research proposal, we plan to establish the utility of this hyperpolarized metabolic imaging to track preventive agents in murine models of premalignant neoplasia (Aim I). We will then validate the detection of early-stage PDAC in patients recently diagnosed and further explore the detection of premalignant lesions in high-risk individuals with the ultimate goal of facilitating early diagnosis and prevention of PDAC (Aim II). The research proposal will address the following two FY22 PCARP Focus Areas of (1) Early detection research for pancreatic cancer, and (2) Understanding precursors, origins, and early progression of pancreatic cancer, and will address the above hypothesis. This is a collaborative translational research proposal between the laboratories of Pratip Bhattacharya, Ph.D. (Principal Investigator, PI) in the Department of Cancer Systems Imaging, MD Anderson Cancer Center (MDACC) with Florencia McAllister, M.D. (Partnering PI) in the Department of Clinical Cancer Prevention, MDACC. Establishing the utility of this technique to track preventive agents in murine models of premalignant neoplasia as well as the clinical use on patients with high-risk lesions would be a fundamental step in moving the findings into interventional trials on patients at high risk.

Proposal Title:	Real-Time Metabolic Imaging to Interrogate Early Detection and Prevention of Pancreatic Cancer
Log Number:	PA220132P1
Current PI Name:	Florencia McAllister
Award Number:	HT9425-23-1-0665
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	09-15-2023

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive malignancies with a dismal prognosis. Most patients present with unresectable disease and therapies are limited. It is therefore crucial to develop novel strategies for early detection and prevention.

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Proposal Title:	Remote Malnutrition Monitoring After Surgery for Pancreatic Cancer Patients
Log Number:	PA220138
Current PI Name:	Kea Turner
Award Number:	HT9425-23-1-0514
Current Contracting Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Current Performing Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Web Approval Date:	09-03-2023

About half of pancreatic cancer patients who receive surgery as part of their treatment are at risk for malnutrition. Malnutrition makes it challenging for pancreatic cancer patients to recover from surgery. Patients with malnutrition often feel worse after surgery, are more likely to come back to the hospital and be readmitted, and have reduced survival. Currently, there are no interventions designed to address malnutrition among pancreatic cancer patients after surgery. There is an urgent need to develop interventions that reduce malnutrition among pancreatic cancer patients after surgery.

After surgery, pancreatic cancer patients have trouble with getting proper nutrition. One surgery patient recalled, "I felt like I'd been hit by a bus. It was quite painful. It took a lot out of me. I lost 25 pounds in two and a half weeks." To get proper nutrition, patients must change how they eat, such as having smaller and more frequent meals and eating more protein. While patients get some help with their diet while they are in the hospital, patients often report not having enough help from their health care team after they leave hospital. As a result, many pancreatic cancer patients feel overwhelmed and uncertain about how to manage their nutrition after surgery.

To help pancreatic cancer patients manage nutrition after surgery, our team developed the Support through Remote Observation and Nutrition Guidance (STRONG) intervention. As part of the STRONG intervention, pancreatic cancer patients meet with a dietician, are screened for malnutrition, and receive counseling on how to manage nutrition after surgery. Patients also receive a smartphone app to help them track their nutrition and make sure they are eating enough. Patients without a smartphone are given a tablet so they can still participate. If a patient has a caregiver, the caregiver is trained on how to use the app to assist the patient with tracking their nutrition. We tested out the STRONG intervention in 19 patients in a pilot study. Most patients (80%) attended the dietician visits, used the app to track their nutrition, and were satisfied with the STRONG intervention. These findings suggest our pilot study of the STRONG intervention was successful.

The objective of the current proposal is to test out the STRONG intervention in more patients and see how it compares with the care that patients usually receive. First, we will translate the STRONG intervention into Spanish to reach our Cancer Center's growing population of Spanish-speaking patients. This will be important for making sure the STRONG intervention is available to Spanish-speaking patients in the future. Second, we will deliver the STRONG intervention to 40 pancreatic cancer patients and provide usual care to 40 pancreatic cancer patients (the care that patients would normally receive at our Cancer Center). We will use a process called randomization to assign patients to either the intervention group or the usual care group. Randomization means that participants have a 50-50 chance of being assigned to the intervention or control group. Randomization will help ensure that the patients assigned to the intervention and usual care groups are similar. We will collect data on patients' participation in the intervention, such as attendance at the dietician visits and use of the app, and their satisfaction with the intervention. We will also collect data on how well patients recover from surgery including whether they develop malnutrition, how well they feel,

whether they are readmitted to the hospital, and if they pass away. We will compare outcomes for patients in the intervention group and patients in the usual care group. We will monitor patients for 90 days after surgery, which is a common timeframe for seeing how patients do after surgery.

This study addresses the fiscal year 2022 PCARP Focus Area of "supportive care interventions, patient-reported outcomes, quality of life, and perspectives during treatment and survivorship" by pilot testing the first intervention aimed at reducing malnutrition and improving quality of life among pancreatic cancer patients who receive surgery as part of their treatment. The long-term goal of this line of research is to develop an intervention for reducing malnutrition among pancreatic cancer patients who receive surgery as a part of their treatment. This intervention could improve patient care by providing surgical patients with nutrition support after they are discharged from the hospital, a time when patients often report feeling overwhelmed and underprepared to manage their nutrition. This intervention could also directly benefit patients by reducing malnutrition and improving recovery from surgery. Further, if this intervention is successful, it could be expanded to other cancer patients who are at-risk for malnutrition after surgery, such as gastric or esophageal cancer patients.

Proposal Title:	Remote Malnutrition Monitoring After Surgery for Pancreatic Cancer Patients
Log Number:	PA220138P1
Current PI Name:	Pamela Hodul
Award Number:	HT9425-23-1-0515
Current Contracting Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Current Performing Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Web Approval Date:	09-03-2023

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whether they are readmitted to the hospital, and if they pass away. We will compare outcomes for patients in the intervention group and patients in the usual care group. We will monitor patients for 90 days after surgery, which is a common timeframe for seeing how patients do after surgery.

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Proposal Title:	APOBEC3A Drives Tumor Evolution and Intratumoral Heterogeneity in PDAC
Log Number:	PA220181
Current PI Name:	Sonja Woermann
Award Number:	HT9425-23-1-0855
Current Contracting Organization:	Other Lab
Current Performing Organization:	Other Lab
Web Approval Date:	10-02-2023

Pancreatic cancers are very aggressive and nearly always lethal. Pancreatic tumors have had very many changes to their DNA, leading to very complex genomes. They tend to spread to other body parts early on and typically do not respond well to therapy. Changes to the DNA occur in nearly all cancers and are important in cancer development. They also lead to drug resistance, and tumor spread to other parts of the body, which is the most important cause of death in pancreatic cancer patients. Large studies investigating the DNA of cancer have identified specific enzymes that can modify DNA, called APOBEC cytidine deaminases, as important players that lead to many changes in DNA and, therefore, they are likely very important in the development of multiple cancers, including pancreatic cancer. These APOBEC enzymes can create differences between different cells that make up the cancer, and these differences allow the most aggressive cancer cells to survive and grow, in a process similar to Darwin's evolution of species. This can increase the cancer's ability to spread to other parts of the body, as well as its ability to evade cancer treatments. A3A is one of those APOBEC enzymes and has been identified as a key player in these processes. We have recently found that, in addition to this, A3A also plays a role in making large-scale changes to the cancer's chromosomes, which further accelerates early pancreatic tumor lesions to develop into aggressive pancreatic cancers, and also makes the tumors more aggressive and able to spread to other parts of the body. However, to really understand these processes, we need to decipher the exact role A3A plays in creating differences between cancer cells, and exactly which chromosomal changes A3A makes and how this impacts the early and late stages of pancreatic cancer evolution and its spread to other parts of the body.

We hypothesize that A3A's role of making both small-scale changes to the cancer's DNA and large-scale chromosomal changes, leads to differences between cancer cells and allows more aggressive cancer cells to develop, playing a key role in pancreatic cancer aggressiveness and spreading to other parts of the body. If we could, in the future, develop medicines that could block A3A, that would be a very promising approach to help us prevent pancreatic cancers from developing, becoming aggressive, and spreading to other body parts.

The overarching goal of this proposal is to show that A3A can be used to predict how pancreatic cancers develop and progress to become aggressive and spread to other parts of the body. This would indicate that A3A would be a very promising target that medicines can be developed against, leading to better outcomes for pancreatic cancer patients.

This project addresses two fiscal year 2022 Pancreatic Cancer Research Program Focus Areas: (1) Understanding precursors, origins, and early progression of pancreatic cancer, and (2) Understanding the events that promote pancreatic cancer metastasis. We focus on understanding the mechanistic underpinnings of changes to the DNA in cancer cells and differences between cancer cells, both of which promote the early development and progression of pancreatic cancers ("precursors, origins, and early progression"), as well as the spread of pancreatic cancers to other parts of the body ("metastasis").

Our proposal is innovative on several fronts: (1) To the best of our knowledge, this will be the first study that will use engineered animal models of A3A, as well as its variant A3AE72A (which is unable to cause small changes to the DNA), to investigate the role of A3A in creating differences between cancer cells, in causing small-scale DNA changes and large-scale changes to the cancer's chromosomes, and in tumor development and in spreading to other parts of the body. (2) In addition, we believe this is the first time that small-scale and large-scale changes to the DNA caused by A3A will be studied as a major source of the development of pancreatic cancer and its ability to spread to other body parts.

We believe the proposed work will be impactful because it will provide new insights into the concepts and mechanisms of how A3A, as a critical player causing small-scale changes to the DNA and large-scale changes to the cancer's chromosomes, may drive differences between cancer cells and thereby pancreatic cancer development, the aggressiveness of the disease, and spread to other parts of the body. This will allow us to determine how A3A changes the profile and behavior of pancreatic cancers. Importantly, it will also allow us to evaluate how promising future medicines targeting A3A would be in preventing pancreatic cancer development, its progression to aggressive disease, and its spread to other parts of the body. If so, such future medicines could significantly improve the clinical outcome and overall survival of pancreatic cancer patients.

Proposal Title:	APOBEC3A Drives Tumor Evolution and Intratumoral Heterogeneity in PDAC
Log Number:	PA220181P1
Current PI Name:	Peter Van Loo
Award Number:	HT9425-23-1-0856
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	10-02-2023

Pancreatic cancers are very aggressive and nearly always lethal. Pancreatic tumors have had very many changes to their DNA, leading to very complex genomes. They tend to spread to other body parts early on and typically do not respond well to therapy. Changes to the DNA occur in nearly all cancers and are important in cancer development. They also lead to drug resistance, and tumor spread to other parts of the body, which is the most important cause of death in pancreatic cancer patients. Large studies investigating the DNA of cancer have identified specific enzymes that can modify DNA, called APOBEC cytidine deaminases, as important players that lead to many changes in DNA and, therefore, they are likely very important in the development of multiple cancers, including pancreatic cancer. These APOBEC enzymes can create differences between different cells that make up the cancer, and these differences allow the most aggressive cancer cells to survive and grow, in a process similar to Darwin's evolution of species. This can increase the cancer's ability to spread to other parts of the body, as well as its ability to evade cancer treatments. A3A is one of those APOBEC enzymes and has been identified as a key player in these processes. We have recently found that, in addition to this, A3A also plays a role in making large-scale changes to the cancer's chromosomes, which further accelerates early pancreatic tumor lesions to develop into aggressive pancreatic cancers, and also makes the tumors more aggressive and able to spread to other parts of the body. However, to really understand these processes, we need to decipher the exact role A3A plays in creating differences between cancer cells, and exactly which chromosomal changes A3A makes and how this impacts the early and late stages of pancreatic cancer evolution and its spread to other parts of the body.

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The overarching goal of this proposal is to show that A3A can be used to predict how pancreatic cancers develop and progress to become aggressive and spread to other parts of the body. This would indicate that A3A would be a very promising target that medicines can be developed against, leading to better outcomes for pancreatic cancer patients.

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We believe the proposed work will be impactful because it will provide new insights into the concepts and mechanisms of how A3A, as a critical player causing small-scale changes to the DNA and large-scale changes to the cancer's chromosomes, may drive differences between cancer cells and thereby pancreatic cancer development, the aggressiveness of the disease, and spread to other parts of the body. This will allow us to determine how A3A changes the profile and behavior of pancreatic cancers. Importantly, it will also allow us to evaluate how promising future medicines targeting A3A would be in preventing pancreatic cancer development, its progression to aggressive disease, and its spread to other parts of the body. If so, such future medicines could significantly improve the clinical outcome and overall survival of pancreatic cancer patients.

Proposal Title:	Pancreatic Cancer Risk Predicted from Electronic Health Records in U.S. Veterans Using Artificial Intelligence
Log Number:	PA220205
Current PI Name:	Chris Sander
Award Number:	HT9425-23-1-0463
Current Contracting Organization:	Harvard University, Boston
Current Performing Organization:	Harvard University, Boston
Web Approval Date:	09-03-2023

Fiscal Year 2022 (FY22) PCARP Focus Area(s) to be addressed in the proposed research: This proposal addresses the FY22 PCARP Focus Area "Early detection research for pancreatic cancer, including the prevalence in individuals with pre-diabetes and diabetes and/or those in underserved ethnic and minority communities". Pancreatic cancer is an aggressive disease that typically presents late with poor patient outcomes. For example, 70% of Veterans with pancreatic cancer are diagnosed at a late stage of disease. However, patients who present with early-stage disease can be treated successfully. A better understanding of the risk factors for pancreatic cancer and detection at early stages coupled with the design of surveillance and intervention programs can improve patient survival and reduce overall mortality from this aggressive malignancy. The goal of the proposed research is to develop machine learning/artificial intelligence based predictive tools based on trajectories of data routinely available in the electronic health record (HER) to predict pancreatic cancer risk. We will develop a model in the Veteran population using nationwide data from the Veterans Affairs (VA) health care system and externally validate it in a civilian cohort from the Mass General Brigham (MGB, Boston) system.

Innovative aspects of the proposed research project: This research is innovative in several respects. We propose to use the time sequence of clinical events, instead of only assessment at a single time point, in our artificial intelligence prediction model. In contrast, prior efforts to develop pancreatic risk prediction tools using patient records have used only the occurrence of disease codes, not the time sequence of disease states in a patient trajectory. We use a wide range of information from the EHR, including not only known risk factors but also less structured data that is routinely collected and could provide valuable clues under machine analysis. In addition, the proposed prediction methods predict not only whether cancer is likely to occur, but also provide risk assessment in incremental time intervals following the assessment, where time of assessment is defined as the day on which the risk prediction is performed based on the history of clinical records of the particular patient. This is important because the likely action resulting from a personalized positive prediction of cancer risk ideally should take into account the probability of the disease occurring within a shorter or longer time frame.

Impact that the proposed research project's results might have on the field of pancreatic cancer research and /or patient care, including the goal of diminishing the burden of pancreatic cancer: Early identification of high-risk pancreatic cancer patients is an urgent need in current clinical practice, since detecting pancreatic cancer at an early stage is crucial toward avoiding poor outcomes and diminishing the burden of pancreatic cancer. Given the low predictive value of imaging and blood test screening, our high-performance AI model will provide an alternative and cost-efficient tool for pancreatic cancer risk assessment facilitating early detection. With completion of these aims, (a) we will be poised to implement a prediction-surveillance program that can be used to identify Veterans and civilians who are at elevated risk for pancreatic cancer and should be enrolled in surveillance and/or interception programs for disease detection, therapy, and prevention; (b) we will have characterized interactions among clinical risk factors on pancreatic cancer risk; and (c) we will have produced a prototype that applies AI technology to patient trajectory data for disease risk prediction for pancreatic cancer and beyond.

Impact, in the short or long term, on individuals with pancreatic cancer, their families/caregivers, and/or the understanding of pancreatic cancer. The proposed research will have a major positive impact on individuals with pancreatic cancer as well as their families/caregivers because outcomes for patients diagnosed at early stage are much better than for patients diagnosed at late stage, and successful completion of the proposed research will facilitate earlier detection of this disease. The research will also have a positive impact on the understanding of pancreatic cancer by identifying new potential pancreatic cancer risk factors and shedding new light on how interactions of pancreatic cancer risk factors interact to influence pancreatic cancer risk.

Proposal Title:	Pancreatic Cancer Risk Predicted from Electronic Health Records in U.S. Veterans Using Artificial Intelligence
Log Number:	PA220205P1
Current PI Name:	Nathanael Fillmore
Award Number:	HT9425-23-1-0464
Current Contracting Organization:	Boston VA Research Institute, Inc. (BVARI)
Current Performing Organization:	Boston VA Research Institute, Inc. (BVARI)
Web Approval Date:	09-03-2023

Fiscal Year 2022 (FY22) PCARP Focus Area(s) to be addressed in the proposed research: This proposal addresses the FY22 PCARP Focus Area "Early detection research for pancreatic cancer, including the prevalence in individuals with pre-diabetes and diabetes and/or those in underserved ethnic and minority communities". Pancreatic cancer is an aggressive disease that typically presents late with poor patient outcomes. For example, 70% of Veterans with pancreatic cancer are diagnosed at a late stage of disease. However, patients who present with early-stage disease can be treated successfully. A better understanding of the risk factors for pancreatic cancer and detection at early stages coupled with the design of surveillance and intervention programs can improve patient survival and reduce overall mortality from this aggressive malignancy. The goal of the proposed research is to develop machine learning/artificial intelligence based predictive tools based on trajectories of data routinely available in the electronic health record (HER) to predict pancreatic cancer risk. We will develop a model in the Veteran population using nationwide data from the Veterans Affairs (VA) health care system and externally validate it in a civilian cohort from the Mass General Brigham (MGB, Boston) system.

Innovative aspects of the proposed research project: This research is innovative in several respects. We propose to use the time sequence of clinical events, instead of only assessment at a single time point, in our artificial intelligence prediction model. In contrast, prior efforts to develop pancreatic risk prediction tools using patient records have used only the occurrence of disease codes, not the time sequence of disease states in a patient trajectory. We use a wide range of information from the EHR, including not only known risk factors but also less structured data that is routinely collected and could provide valuable clues under machine analysis. In addition, the proposed prediction methods predict not only whether cancer is likely to occur, but also provide risk assessment in incremental time intervals following the assessment, where time of assessment is defined as the day on which the risk prediction is performed based on the history of clinical records of the particular patient. This is important because the likely action resulting from a personalized positive prediction of cancer risk ideally should take into account the probability of the disease occurring within a shorter or longer time frame.

Impact that the proposed research project's results might have on the field of pancreatic cancer research and /or patient care, including the goal of diminishing the burden of pancreatic cancer: Early identification of high-risk pancreatic cancer patients is an urgent need in current clinical practice, since detecting pancreatic cancer at an early stage is crucial toward avoiding poor outcomes and diminishing the burden of pancreatic cancer. Given the low predictive value of imaging and blood test screening, our high-performance AI model will provide an alternative and cost-efficient tool for pancreatic cancer risk assessment facilitating early detection. With completion of these aims, (a) we will be poised to implement a prediction-surveillance program that can be used to identify Veterans and civilians who are at elevated risk for pancreatic cancer and should be enrolled in surveillance and/or interception programs for disease detection, therapy, and prevention; (b) we will have characterized interactions among clinical risk factors on pancreatic cancer risk; and (c) we will have produced a prototype that applies AI technology to patient trajectory data for disease risk prediction for pancreatic cancer and beyond.

Impact, in the short or long term, on individuals with pancreatic cancer, their families/caregivers, and/or the understanding of pancreatic cancer. The proposed research will have a major positive impact on individuals with pancreatic cancer as well as their families/caregivers because outcomes for patients diagnosed at early stage are much better than for patients diagnosed at late stage, and successful completion of the proposed research will facilitate earlier detection of this disease. The research will also have a positive impact on the understanding of pancreatic cancer by identifying new potential pancreatic cancer risk factors and shedding new light on how interactions of pancreatic cancer risk factors interact to influence pancreatic cancer risk.

Proposal Title:	Novel Immunonanoengineering Strategy to Overcome Neutrophil-Mediated Stromal Inflammation and Therapy Resistance in Pancreatic Cancer
Log Number:	PA220217
Current PI Name:	Jashodeep Datta
Award Number:	HT9425-23-1-0699
Current Contracting Organization:	Miami, University of, Coral Gables
Current Performing Organization:	Miami, University of, Coral Gables
Web Approval Date:	09-15-2023

Pancreatic cancer is a lethal malignancy refractory to standard chemotherapy due to intrinsic and acquired therapeutic resistance. The key culprits associated with therapeutic resistance are (1) early and frequent infiltration of tumors by immunosuppressive innate immune cells, particularly neutrophilic myeloid-derived suppressor cells (MDSC); and (2) pro-tumorigenic signaling by cancer-associated fibroblasts (CAF), which dominate the non-tumor stroma and act as cellular antennae to transmit inflammatory cues that further beckon MDSCs to the tumor microenvironment. In dissecting the unifying mechanisms that underpin the relationship between MDSC-CAF communication and chemoresistance in pancreatic cancer, our data uncover inflammasome activation in MDSCs -- culminating with the release of IL-1-beta, a pro-inflammatory cytokine -- as a previously unrecognized regulator of inflammatory CAF development in the tumor microenvironment. Interestingly, dampening global inflammasome activation with a novel pharmacologic inhibitor in mouse models of pancreatic cancer underscored the crucial importance of restricting inflammasome inhibition specifically to the MDSC compartment, in order to spare immunostimulatory effects of inflammasome activation in antitumor dendritic cells and T cells.

Building on these observations, the overall mission of this proposal is twofold: (1) to mechanistically delineate the pathogenic role of MDSC-restricted inflammasomes in instigating stromal inflammation in the pancreatic tumor microenvironment; and (2) catalyze development of a bold nanoengineered immunotherapy to disrupt inflammasome activation solely in neutrophilic MDSCs to overcome chemoresistance in pancreatic cancer. The first aim -- addressing the PCARP Focus Area relating to oncogenic signaling in the tumor microenvironment -- will interrogate the effects of context-specific silencing of MDSC-derived inflammasome activation on CAF evolution using innovative preclinical mouse modeling and single-cell methodologies.

The second aim, utilizing a unique high-dimensional tissue imaging platform, will uncover whether precise spatial relationships between these inflammasome-enriched MDSC-CAF communities in human pancreatic tumors can predict poor chemotherapy responses in patients receiving treatment in our clinics. Moreover, we will investigate if the density of these MDSC-CAF neighborhoods is disproportionately concentrated in pancreatic tumors from Black patients, in an attempt to discover tissue-level molecular insights that explain racial disparities observed with chemotherapy responses in pancreatic cancers from Black patients. The latter will address another PCARP Focus Area related to underserved ethnic/minority communities.

The third aim will offer a conceptual breakthrough in the therapeutic landscape of pancreatic cancer by proposing a novel neutrophil-homing nanoparticle that can deliver inhibitors of inflammasome activation with incredible precision solely to neutrophilic MDSCs. Leveraging this technology, we will determine if this innovative strategy mitigates stromal inflammation but spares (and even augments) antitumor immunity to improve chemosensitivity in pancreatic cancer models. This will address another PCARP Focus Area related to new drug discovery targeting immune mechanisms of resistance.

Ultimately, successful completion of this proposal will not only advance our understanding of contextually sensitive signaling circuitry in the tumor microenvironment that underlies therapeutic resistance in pancreatic cancer, but also lay the groundwork for commercialization and human translation of our novel MDSC-directed immunonanotherapeutic approach to improve chemosensitivity in patients afflicted with this lethal disease. To demonstrate our commitment to human translation, we have assembled a collaborative team with an outstanding track record of bringing promising cancer nanotherapeutics to fruition. Given the urgent need for novel myeloid- directed immunotherapies, our research could have substantial impacts in diminishing the burden of pancreatic cancer for patients and their families/caregivers.

Proposal Title:	Investigating One-Carbon Metabolic Alterations to Target Pancreatic Cancer Cachexia
Log Number:	PA220269
Current PI Name:	Kamiya Mehla
Award Number:	HT9425-23-1-1001
Current Contracting Organization:	Oklahoma, University of, Health Sciences Center
Current Performing Organization:	Oklahoma, University of, Health Sciences Center
Web Approval Date:	10-02-2023

Pancreatic ductal adenocarcinoma (PDAC) -- the fiscal year 2022 Pancreatic Cancer Research Program (FY22 PCARP) Focus Area for this proposal -- is the third-leading cause of cancer-related deaths in the United States. Despite increased efforts for a couple of decades, the 5% survival rate for PDAC patients remains dismal. Up to 80% of advanced PDAC cases exhibit cancer cachexia. Cachectic patients display anorexia, attenuated adipose, and skeletal muscle mass, and hence less tolerance to chemotherapies and radiation treatments. Often, cancer patients that exhibit 30% loss of their body weight display up to 75% loss of skeletal muscle proteins, resulting in an irreversible functional impairment and significantly poor quality of life. Studies have shown that nutrition-based approaches alone have a limited efficacy in reversing the cachectic phenotype. The failure of these approaches is, at least in part, due to the fact that tumor cells also need common nutrients (such as essential amino acids and vitamins) for their unregulated growth. In fact, tumor cells vigorously devour circulating nutrients supplemented to these patients. Pancreatic tumors are under nutrient constraints and favor the metabolic pathways that support the tumor growth demands. Whether these metabolic adaptations by tumor cells have systemic consequences, such as cancer cachexia, remains to be explored. Thus, the main rationale of our project is driven by the gaps in our understanding of the systemic effects of tumor metabolism that drive cancer cachexia in pancreatic cancer patients.

The pancreatic tumor microenvironment is dense and presents significant nutritional constraints. Our data show that pancreatic tumor cells upregulate 1 carbon (1C) metabolism under glucose limitation in conditions mimicking the tumor microenvironment. The 1C pathway supports the production of building blocks needed for the growth of tumor cells and requires vitamins including vitamin B6 (helps in three steps of 1C cycle pathway) for the maintenance of the pathway. Of note, vitamin B6 (VB6), is also very important for the proper functioning of the immune system. Studies have shown depleted circulating VB6 levels in PDAC patients. In our preliminary data, we also observed decreased circulating VB6 levels in PDAC patients and tumor-bearing mice compared to non-cancer patients or the respective healthy mice controls. We noted increased pancreatic tumor cell growth upon VB6 addition to the culture medium and tumor cell growth dependency on VB6. More importantly, VB6 is also critical for muscle biology. An earlier study showed that deregulation of the transsulfuration pathway in muscle leads to muscle cell breakdown, also called muscle atrophy. VB6 is critically required for the transsulfuration pathway, and its absence leads to the buildup of byproducts in muscle, causing muscle damage. We thus hypothesize that, under nutritional constraints, the tumor cell metabolically adapts and upregulates the mechanisms that cause systemic imbalances of VB6 in the tumor-bearing host. This systemic (blood) depletion of VB6 triggers muscle damage and induces cancer cachexia in pancreatic cancer patients. Based on our data with myeloid-derived suppressor cells (MDSCs), we further hypothesize that VB6 supplementation can, in parallel, reprogram the immune cells in skeletal muscles to diminish muscle wasting. Thus, our study has a direct impact on the FY22 PCARP Focus Area "Understanding the relationship between metabolic disruptions in pancreatic cancer and their systemic effects, including diabetes and cachexia."

To date, only few studies have shown the impact of vitamins (vitamin D) on muscle biology. The role of VB6 in muscle weight loss and cancer cachexia has not been investigated so far. Hence, the proposed study

on understanding the critical role of VB6 limitation on muscle loss in pancreatic cancer patients is highly innovative. Given the exploratory nature of this award mechanism, the short-term impact of our study will close the gap on understanding the novel mechanisms through which tumor cell promotes muscle loss and function, and contribution of VB6 in restoring muscle strength and improving the quality of life in PDAC patients (Aim 1 and 2). Furthermore, we will investigate the correlative relationship of VB6 levels with trans sulfuration and MDSCs in human patient skeletal muscles and muscle wasting in human PDAC patients (Aim 3). This contrasts with the current clinical practice of using “nutritional alone” approaches in restoring muscle function in cancer patients. As targeting the tumor-intrinsic pathway (1C pathway, in our study) that exhaust systemic VB6 will make VB6 supplementation more successful and will yield greater clinical benefits. Given that restoration of VB6 levels in patients can be easily achieved through dietary interventions, data obtained through our work will have very high clinical and translational applicability (long term impact) in improving the muscle function and the quality of life for PDAC patients.

Relevant to FY22 PCARP Focus Area: While military personnel actively maintain healthy diet and fitness regimen, they are continuously exposed to the multiple environmental and occupational hazards such as radiations and chemical toxins. These exposures can contribute to the increased risk of developing pancreatic cancer. Also, an earlier study showed that military service accounted for 40% increased risk of developing pancreatic cancer in Texas veterans who died in 1998. Thus, there is an urgent need to study PDAC metabolism and how it impacts the quality of life by exacerbating muscle wasting in affected war veterans.

Proposal Title:	Targeting CD200/CD200R Signaling as a Novel Strategy for the Treatment of Therapy-Resistant Prostate Cancer
Log Number:	PC220004
Current PI Name:	Chengfei Liu
Award Number:	HT9425-23-1-0144
Current Contracting Organization:	California, University of, Davis
Current Performing Organization:	California, University of, Davis
Web Approval Date:	12-13-2022

In the United States, about 268,490 prostate cancer new cases and 34,500 deaths are expected in 2022. Prostate cancer is diagnosed more than any other type of cancer in men and is the second leading cause of cancer-related deaths in the same population. After initial diagnosis of prostate cancer, radical prostatectomy and radiation are used to treat the primary tumors. When cancer recurs, drugs are used to block androgen (such as testosterone) activity associated with the growth of cancerous tissues. For a long time, the treatment has been hampered by drug resistance-the tendency for drugs to stop working over an extended period of time. These androgen receptor signaling inhibitors (ARSI) include enzalutamide (XTANDI®), abiraterone acetate (ZYTIGA®) and apalutamide (ERLEADA™). Although all drugs are highly effective initially, patients quickly develop resistance to these drugs through mechanisms that are not completely understood. Therefore, there is an urgent need to identify resistant mechanisms to improve the treatment outcome of castration-resistant prostate cancer (CRPC).

Over the past decades, the new conceptual and technical advances in immunology have led novel discovery between the immune system and tumors, including prostate cancer. Literatures and our data showed that CRPC treated with enzalutamide may escape the immune surveillance through increasing a cell surface protein named programmed cell death ligand 1 (PD-L1), along with its receptor PD1, which are the widely used targets in immunotherapy for cancer treatment. Unfortunately, clinical trial using enzalutamide and PD-L1 inhibitor (atezolizumab) combination treatment failed to extend the overall survival in CRPC patient and the underlying mechanisms remain unknown. Emerging data from my group found that accompanying with the PD-L1 overexpression in enzalutamide resistant prostate cancer cells, another novel cell surface protein-CD200 was also significantly upregulated. Notably, CD200 expression was negatively regulated by androgen receptor signaling. CD200 and its receptor CD200R works together to suppress immune response in multiple diseases, including cancer. Our data further revealed that enzalutamide treatment not only promoted the CD200 expression in tumor cells but also increased its binding receptor-CD200R population in immunosuppressive cells infiltrating into tumors which may further suppress the immune response.

In this project, we hypothesize that the CD200/CD200R signaling activation evades the anti-tumor immunity and causes enzalutamide or anti-PD1/PD-L1 treatment failure in prostate cancer patients. To address the hypothesis, we will investigate the androgen receptor signaling in CD200 regulation and define the role of CD200/CD200R signaling in escaping anti-tumor immunity. We will uncover the underlying mechanisms of CD200/CD200R-directing immune evasion through studying its effects on myeloid-derived suppressor cells (MDSC) which plays an important role in controlling immune response and T cells in cancer patients. We will also find out if using drugs against both PD-L1 and androgen receptor activates CD200/CD200R signaling. Finally, we will determine the CD200/CD200R status in clinical specimens and blood samples from the prostate cancer patients and test the utility of anti-CD200 antibody treatment in combination with anti-PD1/PD-L1 or enzalutamide in animal models.

Our proposal will address the 2022 PCRP overarching challenge to “Define the biology of prostate cancer progression to lethal prostate cancer to reduce death” and “Develop treatments that improve outcomes for

men with lethal prostate cancer.” Identification and characterization of CD200/CD200R signaling involved in immune evasion related to ARSI and anti-PD-L1 treatment will improve current understanding on how patients failed to these treatments and enable us to design strategies for targeted therapies in lethal prostate cancer. This study will contribute to a more thorough understanding of drug resistance and immune surveillance breakthrough that is critical for the advancement of current prostate cancer treatment. Successful development of strategies to target CD200/CD200R signaling for prostate cancer treatment has the potential to significantly improve the treatment outcomes as with ARSI or anti-PD1/PD-L1. CD200 immune checkpoint inhibitor samalizumab is currently in clinical trial stage to treat other cancer types. The anticipated results of our proposed studies will provide strong rationale to initiate clinical trials to treat prostate cancer patients by developing strategies to block CD200/CD200R signaling within the next 5 years. Thus, our proposed studies will not only help us gain knowledge of prostate cancer disease progression and understand the mechanisms of treatment resistance, but also facilitate new therapy development to treat ARSI-resistant CRPC patients.

Proposal Title:	Antagonizing Glutamine Bioavailability Promotes Radiation Sensitization in Prostate Cancer
Log Number:	PC220006
Current PI Name:	Manish Thiruvalluvan
Award Number:	HT9425-23-1-0145
Current Contracting Organization:	Cedars-Sinai Medical Center
Current Performing Organization:	Cedars-Sinai Medical Center
Web Approval Date:	12-13-2022

Background and Research Goals: Prostate cancer is the most commonly diagnosed cancer in men in the western world. The standard treatments for localized prostate cancer are surgery and radiation, usually in combination with androgen targeted therapy. Our research group has demonstrated that androgen targeted therapy can cause stromal near epithelial cells to secrete nutrients in the form of the amino acid glutamine, which allows cancer cells to not only survive, but eventually develop into drug resistant prostate cancer. This advanced form of disease requires treatment with toxic chemotherapy drugs and usually leads to death of the patient within 2 years. Unfortunately, there has not been advent of any new drugs that is able to completely overcome or reverse this type of drug resistance of prostate cancer. So far, glutamine has only been studied in regards to androgen targeted therapy in prostate cancer, and we do not know how it contributes to resistance to radiation therapy. Further, even less is understood about how radiation affects the stromal cells. We hypothesized that depriving prostate cancer cells alone with their stroma will increase their sensitivity to radiation therapy.

Preliminary data obtained in our laboratory suggests that taking away glutamine from prostate cells in culture drastically reduces their ability to survive radiation. This may be because glutamine, in addition to being a nutrient, is a key signaling molecule in a cellular process called O-glycosylation, a type of protein modification that is carried out by the enzyme OGT. We believe glutamine through this way is able to alter the function of hundreds of proteins within the cell, some of which may be a key in radiation resistance. Thus, we believe limiting glutamine will impact both metabolic and DNA repair capabilities of cancer cells by altering protein O- glycosylation, leading to radiation sensitization. In this proposal, we first want to identify how glutamine supports the acquisition of radio-resistant features in prostate cancer progression. First, we will test whether removing OGT via gene editing as well as stopping its function with a clinically viable small molecule antagonist in PC epithelia in response to radiation and androgen therapy. In parallel, we will employ the use of sodium phenylbutyrate, an FDA-approved drug, to deplete glutamine supply in the blood. We will use mouse models with orthotopic grafts of prostate cancer cells to validate this hypothesis. Next, we want to determine how glutamine metabolism regulates the stromal cell activation in the support of PC radiation resistance. As mentioned before, stromal cells release glutamine in response to androgen therapy. We believe that O-glycosylation may also be involved in this process, especially in the context of radiation. Once again, we will remove OGT via gene editing and deplete glutamine with sodium phenylbutyrate in stromal cells and graft them orthotopically alongside cancer cells in mice. Next, we will check to see if these tumors are able to leave the initial graft site and travel to other sites in the body as metastasis is often a key factor in patient survival. We expect that the interruption of the glutamine metabolism will greatly diminish PC radio-resistance, tumor progression and eventual metastasis.

Impact: Our main objective with this study is to better define the biology of lethal prostate cancer to reduce death and develop treatments that improve outcomes for men with lethal prostate cancer. This proposal aims to target glutamine metabolism and identify if this is viable strategy to improve disease free survival in both local and advanced patients. There are two novel aspects of this study; first, we will determine how radiation influences stromal cells in the tumor to supporting epithelial cells in surviving radiation therapy, and second,

we will repurpose sodium phenylbutyrate, a well-tolerated FDA drug, for use in lethal prostate cancer clinical in combination androgen and radiation therapy for immediate clinical impact. This project will test these therapeutic combinations in clinically relevant model systems, if successful, could lead to near-term clinical application.

Personal Statement: My overarching career goal is to apply basic research findings that translate to therapeutic strategies to benefit prostate cancer patients. Going forward, my research training to become an independent prostate cancer investigator will be complemented in three specific ways: (1) foster collaborations to enable a multidisciplinary approach and enable clinical translation; (2) communicate my research at scientific meetings and publication; and (3) learn to develop successful grants. With this proposal, I expect to identify novel targets and companion biomarkers for patient selection in improving outcome of these therapies. Toward the end of the award period for this grant, I want to become an independent researcher in the field of prostate cancer and develop my own niche in glutamine metabolism and radiation sensitization. My mentor, Dr. Neil Bhowmick, is an expert in the field of prostate cancer. He is credited with demonstrating that prostatic stromal cells can, not only support, but mediate cancer initiation and therapy resistance. My co-mentor, Dr. Edwin Posadas, is the Medical Director for the Center for Uro-Oncology Research Excellence. His insight will be key to translating key findings and tailoring experiments for maximal clinical impact. With their combined expertise and guidance, I am confident in achieving the aims set out in this proposal.

Proposal Title:	Extracellular Vesicles-Mediated Delivery of CRISPR Machinery for Inhibiting Prostate Cancer
Log Number:	PC220015
Current PI Name:	Houjian Cai
Award Number:	HT9425-23-1-0086
Current Contracting Organization:	Georgia, University of
Current Performing Organization:	Georgia, University of
Web Approval Date:	11-03-2022

Prostate cancer is the second leading cause of cancer-related deaths for men. There are about 1.4 million new cases of prostate cancer globally. About 3.2 million people are living with prostate cancer in the U.S. The current treatment primarily relies on using chemical compounds to inhibit the activity of a major oncogene, called androgen receptor (AR) activity, thereby inhibiting prostate cancer progression. Anti-androgen or AR signaling agents, such as the second-generation of AR inhibitors enzalutamide and abiraterone, have been developed for prostate cancer treatment. However, multiple molecular mechanisms have emerged to maintain AR signaling leading to drug resistance. As a result, the proliferation of a majority of prostate cancer cells is still maintained through AR signaling by responding to either a very low amount of androgen or by constitutively active AR mutants. These patients no longer benefit from current androgen deprivation therapy and are usually treated with chemotherapy (which has many side effects and often a low survival rate). Currently, 10-20% of the prostate cancer population will develop disease that is castration-resistant within 5 years. The median survival of patients with castration-resistant prostate cancer (CRPC) is about 9-30 months. Progression to CRPC affects quality of life, and few therapeutic options are available for CRPC patients. There is an urgent need to provide additional therapeutic options for treatment of the CRPC.

CRISPR technology holds great promise in genome editing for gene therapy. However, reliable and efficient delivery of the CRISPR machinery in vivo remains the primary challenge to implementing this potentially transformative technology. The overall objective of this study is to develop a novel technology that will encapsulate the genomic editing CRISPR machinery into extracellular vesicles (EVs). EVs are biological nanoparticles that are secreted by almost all cell types. A large body of studies has shown that EVs mediate cell- to-cell communication by transmitting their encapsulated contents. Therefore, EVs are naturally produced by the body and serve as biological delivery vehicles. EVs can enter the circulatory system and release their contents to regulate the physiological state of recipient cells in a distant organ. In this study, the investigators will genetically modify the CRISPR/Cas9 protein. The modification will allow CRISPR machinery to be packed into EVs. Additionally, the investigators will engineer the membrane of EVs to target prostate cancer cells preferentially. This engineered delivery system will preferentially silence the androgen receptor gene in prostate cancer cells, thereby inhibiting the growth of prostate tumors.

In contrast to the current treatment strategy to inhibit AR activity with chemotherapy, the distinctive proposition of this technology is deleting the AR gene at the DNA level in prostate cancer cells, thereby inhibiting the growth of prostate tumors while minimizing unfavorable side effects associated with chemotherapy. This novel technology will eliminate numerous molecular mechanisms leading to castration-resistance in prostate cancer. Of note, if successful, the technology could also be used to silence other oncogenes in androgen receptor negative prostate cancer cells.

Proposal Title:	Exploiting Androgen Receptor's Allosteric Regulation to Develop Next-Gen Prostate Cancer Therapeutics
Log Number:	PC220022
Current PI Name:	Ujjwal Dahiya
Award Number:	HT9425-23-1-0802
Current Contracting Organization:	Cleveland Clinic Foundation
Current Performing Organization:	Cleveland Clinic Foundation
Web Approval Date:	10-02-2023

Prostate cancer causes the death of more than 34,000 men each year in the United States alone. These deaths occur because metastatic prostate cancer stops responding to the current treatment options. The cornerstone approach for current treatment is androgen deprivation therapy. As the name suggests, androgen derivation therapy relies on depriving prostate cancer cells of androgens. Androgens are male hormones that bind a protein called the androgen receptor, which activates the androgen receptor and causes prostate cancer cells to grow. Androgen deprivation therapy blocks binding of androgens to the androgen receptor, which stops prostate cancer cell growth. However, over time, therapy fails, prostate cancer continues to grow because the androgen receptor uses alternative routes to become activated, and almost all patients die of their disease.

We need new treatments that can block androgen receptor activity in prostate cancer that is no longer responsive to androgen deprivation therapy. Our laboratory is investigating how the activated androgen receptor controls prostate cancer cell growth, namely by turning on or off the expression of genes that control cancer growth. To control expression of these genes, the androgen receptor needs to partner with several other proteins that are known as coregulators. We have shown that this partnership is very gene-specific and that partnerships with different coregulators can turn on/off distinct sets of genes that are relevant for different aspects of prostate cancer progression. For instance, androgen receptor’s partnership with a coregulator known as WDR77 controls genes that are more relevant to metastatic prostate cancer that has returned after androgen deprivation; in contrast, its partnership with a coregulator known as STAT3 is less relevant. These results fit well with findings from other groups that show coregulator interactions induce distinct changes in the shape of the androgen receptor and then control distinct gene sets, a phenomenon scientifically known as allosteric regulation. This suggests that disrupting distinct interactions between androgen receptor and coregulators that contribute to aggressive prostate cancer growth (such as WDR77) may be especially worthwhile for development of new prostate cancer therapies.

Unfortunately, critical information needed to succeed in these efforts is missing. For treatments that disrupt androgen receptor-coregulator partnerships to work, we need to understand the three-dimensional organization of these partnerships and how this organization impacts the expression of genes that are controlled by these partnerships. That is exactly what my proposal is designed to do. I propose to use new state-of-the-art technologies to unravel the three-dimensional interaction sites between androgen receptor and WDR77 and, as a control, STAT3, and define the impact of these two androgen receptor partnerships on the genes that the androgen receptor controls. Using information from these studies, I will design tools to disrupt these partnerships and determine whether they prevent growth of prostate cancer cells cultured in the lab and in animal models.

My proposed study thus aligns well with FY22 PCRP overarching challenges to (1) define the biology behind prostate cancer progression to lethal stage and (2) develop treatment that improve outcomes for men with lethal prostate cancer. Results from my studies can ultimately slow progression of prostate cancer that kills men and may lead to an alternative and entirely novel therapy for advanced prostate cancer. If successful, my studies will lead to highly novel approaches to treat patients who (a) have locally advanced

prostate cancer that cannot be treated effectively by surgery or radiation; (b) develop prostate cancer recurrence after initial surgery or radiation; or (c) develop recurrence after androgen deprivation therapy. By the end of this award, I will have obtained proof-of-principle for therapies to prevent androgen receptor-coregulator partnerships and will have leads for drugs. Developing this approach further until it can be tested in patients will take another 5 years.

My career goal is to establish a laboratory working in the area of prostate cancer nanomedicine. I have been trained in nano-carrier based approaches for drug and small molecule delivery to cancer cells; this approach uses extremely small, almost atom-size, materials to deliver treatment. To mature into an independent researcher working in prostate cancer and nanomedicine, I need to learn new skills and to pursue and validate novel targets for therapeutic interventions, develop collaborations with clinicians and other prostate cancer researchers, and learn project and lab management skills. My proposed research plan will allow me to acquire these skills. My mentor, Dr. Hannelore Heemers, is very supportive and an established prostate cancer researcher and will oversee my professional development by helping me to set up new collaborations within and outside Cleveland Clinic and to administer this award. The Cleveland Clinic has invested heavily in prostate cancer research and has many experienced clinicians and researchers with whom I will interact during this award. My research environment is made even stronger as it is embedded in a National Cancer Institute-funded Cancer Center. At the end of this award, the training plan will leave me well-prepared to pursue an independent career in prostate cancer research.

Proposal Title:	A CLOUD (CRISPR-Mediated Loci-Specific Unbiased Discovery) Atlas of Regulatory Binding Proteins for Driver Genes in Prostate Cancer Bone Metastases
Log Number:	PC220043
Current PI Name:	Shang Su
Award Number:	HT9425-23-1-0015
Current Contracting Organization:	Toledo, University of, Health Science Campus
Current Performing Organization:	Toledo, University of, Health Science Campus
Web Approval Date:	11-03-2022

Prostate cancer (PCa) is the second most lethal cancer in U.S. men. One of the major reasons for PCa patient death is activation of the MYC gene. Unfortunately, we have not had any success yet in directly stopping MYC. Therefore, we are developing a tool to identify the proteins causing MYC activation so that scientists can develop effective methods to stop these proteins. The success of our proposal will address the overarching challenge to “Define the biology of prostate cancer progression to lethal prostate cancer to reduce death.” Our findings will help physicians and scientists understand the MYC activation better and develop drugs for PCa patients with MYC activation. The tool developed is also applicable in the studies of other genes and will open new avenues for discovery of key factors of PCa progression, metastasis, drug resistance, and tumor dormancy.

Proposal Title:	A Novel Strategy Targeting the Metabolic Vulnerability of CRPC
Log Number:	PC220058
Current PI Name:	Jian Wang
Award Number:	HT9425-23-1-0130
Current Contracting Organization:	Wayne State University
Current Performing Organization:	Wayne State University
Web Approval Date:	12-13-2022

A hallmark of aggressive prostate cancer is the excessive metabolism of sugar and lipids, which cancer cells use to generate cellular building blocks to support uncontrolled cell growth, enhance survival, and develop therapeutic resistance. Thus, a promising approach for treating prostate cancer targets the improper metabolism of cancer cells, thereby starving the cells of the building blocks needed to create and maintain tumors.

We have discovered a “molecular switch” that blocks the metabolism of sugar and lipids in prostate cancer cells. We have also found that this molecular switch is critical to contain the significant “molecular drivers” of the so- called castration-resistant prostate cancer, the deadly form of prostate cancer. Our work indicates that this switch is turned off in castration-resistant prostate cancer and that activation of the switch could block tumor growth. Importantly, we have discovered novel compounds that potently activate this molecular switch and thus might be developed into new anti-cancer medicines. Our research seeks a better understanding of how this molecular switch alters metabolism and contains the major molecular drivers of prostate cancer cells. Besides, we will test whether our novel chemical activators of the switch reduce or eliminate the ability of these cells to create tumors. Our chemical activator for this novel prostate cancer suppressor could be developed into new medicines to treat prostate cancer if we are successful.

Prostate cancer is currently the leading cause of cancer-related death among men in the United States. The recorded incidence of prostate cancer has been increasing in the past two decades. While current standard treatments work initially, tumors can become resistant to these therapies. Therefore, there is an urgent need to discover new medicines to cure therapeutic resistant recurrent prostate cancer. Our research explores a new pathway of prostate cancer metabolism and has the strong potential to identify a class of new anti-cancer medicine. We expect to complete our work in the next three years and achieve broad benefits in patient-related outcomes for patients with castration-resistant prostate cancer. Our proposed research directly addresses the FY22 PCRP Overarching Challenges: “Define the biology of prostate cancer progression to lethal prostate cancer to reduce death” and “Develop treatments that improve outcomes for men with lethal prostate cancer.”

Proposal Title:	Role of Tumor Suppressors in Prostate Cancer
Log Number:	PC220062
Current PI Name:	E. Shyam Reddy
Award Number:	HT9425-23-1-0294
Current Contracting Organization:	Morehouse School of Medicine, Atlanta
Current Performing Organization:	Morehouse School of Medicine, Atlanta
Web Approval Date:	11-03-2022

Micro-RNAs are small RNA molecules that are well conserved in animals and plants, suggesting that they have an important role in cell growth and cell death (their discoverers received the Nobel Prize). Nearly 2,500 micro-RNAs were discovered and the functions of many micro-RNAs were also revealed. Each micro-RNA binds to specific messenger RNA (which codes for proteins) and inhibits the expression of proteins. Mature micro-RNAs are originated from large RNA molecules by processing the large RNA in two steps. Researchers made a breakthrough finding that larger RNA molecules (from which mature micro-RNAs are made) actually code for very small proteins, miPEPs (published in the prestigious journal, Nature) in plant cells. These small proteins are called peptides. They have shown that these small peptides (miPEPs) control the expression of mature micro-RNAs, which control the expression of proteins of interest. In brief, these small peptides are master regulators that control the expression of cancer proteins.

Recently, it was shown that a micro-RNA (called miR-24) functions as a prostate cancer metastatic inhibitor /suppressor. They have shown that this micro-RNA (miR-24) inhibits the expression of prostate cancer metastasis-inducible genes. Based on the breakthrough finding described above, we hypothesized that a large RNA molecule (a precursor of micro-RNA miR-24) may code for small peptides (called miPEP-24) and that these peptides function as prostate cancer metastatic inhibitors/suppressors. Loss of expression of these peptides (seen in the case African Americans) leads to a selective advantage for the prostate cancer cells to metastasize in Black Americans compared to White Americans. To test this hypothesis, we have cloned the gene that codes for miPEP-24. We will test whether these peptides have prostate metastatic suppressor activity on a variety of prostate cancer cells and identify the mechanism by which it regulates prostate cancer metastasis.

Since loss of expression of these peptides in prostate cancer cells suggest that prostate cancer cells are undergoing metastasis, one can use this as a biomarker for the early detection of prostate cancer metastasis. Therefore, this will be first immediate outcome of this research project that will help millions of prostate cancer patients. Furthermore, these results will also provide novel biomarkers for the early detection of metastasis of other cancers, as the loss of miR-24 was shown to be responsible in other cancers. In future, pri-miRNA-encoded peptides signatures will be tested as novel clinical biomarkers for further subtyping of prostate cancer and their potential for predicting metastasis. In addition, these peptides can be easily synthesized and can be used as prostate cancer metastatic suppressors and to target prostate cancer metastatic cells. If our results are positive, it will be highly encouraging to study in future whether synthetic peptides (miPEP-24) can be used as therapeutic agents to target prostate cancer metastatic cells in clinical trials to treat prostate cancer metastatic patients. In fact, one can modify the synthetic peptides and test them for more efficient therapeutic agents. Thus, our research will revolutionize the early diagnosis and treatment of metastatic prostate cancer and also other cancers and diseases.

If our hypothesis is true, it will have a major impact in the fundamental biology that precursors of micro-RNA encoded peptides exist in mammalian cells (like in the case of plant cells), and this novel finding may open doors to new investigations such as biomarkers and therapy of all human cancers and diseases. This finding may be as important as the discovery of miRNA. Thus, we believe strongly that our research may change the landscape of the cancer field.

Proposal Title:	AR Proxisomes: Exploring Loci-Specific Role of AR and Its Coregulators in Castration Resistance
Log Number:	PC220069
Current PI Name:	Sushant Kachhap
Award Number:	HT9425-23-1-0098
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	11-03-2022

Androgen deprivation therapy (ADT) is a cornerstone of treatment for advanced prostate cancer. However, after an initial dramatic response, the therapy invariably fails as cancer cells become resistant to therapy and the disease progresses to a castration-resistant stage. It is unclear how this resistance is acquired; however, studies indicate that the androgen receptor that is targeted by ADT regains activity and androgen receptor signaling continues unabated driving cancer progression.

Androgen receptor functions as a transcription factor that binds to certain defined DNA sequences and affects expression of its target genes that drive tumor proliferation and invasion. The androgen receptor is aided by various chromatin regulatory proteins that help the receptor in executing its cancer-promoting function. Understanding the nature and identity of these chromatin co-regulators is essential, as it will not only provide insights into how prostate cancer becomes castration-resistant, but also help in targeting these regulators with drugs to prevent castration-resistance. Traditional approaches to study the role of chromatin regulator proteins in castration resistance are often non-physiological and rely on a limited number of reagents(antibodies) raised against known chromatin regulators. These approaches do not offer insights about novel chromatin regulators that may play a role in castration resistance. We intend to fill this gap by devising a strategy that is physiologically relevant, highly precise, and can identify novel chromatin regulators and the downstream genes that they regulate. This strategy would provide a high-resolution map of how chromatin co-regulatory protein cooperates with androgen receptor to bring about castration resistance, a feat that has never been achieved before.

The first aim in our proposal will devise a labeling strategy to identify known and unknown chromatin coregulators that drive castration resistance, and the second aim will utilize the strategy in human prostate cancer organoids, which are prostate tumors that can be grown in laboratory culture conditions. These studies will identify novel chromatin regulators and provide physiological relevant information about how AR aided by chromatin regulators drives castration resistance. We hope to provide an unprecedented map of how castration resistance is driven at a molecular level, offering novel molecular targets for overcoming castration resistance. We envisage that this endeavor will expand our knowledge about chromatin co-regulators of androgen signaling and provide novel targets for castration-resistant prostate cancer.

Proposal Title:	Exploiting a Novel Vulnerability in Prostate Cancer
Log Number:	PC220070
Current PI Name:	Bryce Paschal
Award Number:	HT9425-23-1-0063
Current Contracting Organization:	Virginia, University of
Current Performing Organization:	Virginia, University of
Web Approval Date:	11-03-2022

Current treatments for prostate cancer that are based on inhibition of androgen receptor (AR) signaling are generally not durable, owing to the ability of cancer cells to acquire molecular changes that promote drug resistance. Molecular changes in castrate-resistant prostate cancer (CRPC) include mutations and altered RNA splicing that give rise to therapy-resistant forms of AR. We have devised a novel strategy for inhibiting prostate cancer cells. The strategy uses a new drug that has yielded promising results in preclinical models of lung cancer. The strategy involves induced expression of the drug target, which is an enzyme with known biological functions. The drug-enzyme complex induces cell growth inhibition in multiple prostate cancer models through a mechanism referred to as a dominant-negative effect. The growth inhibition is observed in AR-positive, AR-negative, and AR-mutant prostate cancer models. Thus, the strategy has the potential to be effective in therapy-resistant prostate cancer. The strategy includes packaging both the inducer and the drug in nanoparticles (NPs), a tiny carrier that protects the drug from degradation. The NPs are also endowed with a prostate cancer cell- specific targeting signal. This will enable the NPs to bind to prostate cancer cells, deliver its payload of inducer and drug, and inhibit cell growth. The Exploration Hypothesis Development Award will support development and optimization of the NP strategy. The project addresses the PCRP Overarching Challenge of developing treatments that improve outcomes for men with lethal prostate cancer. This is a high-risk/high reward, translational project that represents a novel approach to prostate cancer treatment that can help eliminate death from prostate cancer, which will enhance the well-being of Service Members, Veterans, and all the men, women, and their families who are touched by the impact of the disease.

Proposal Title:	Deciphering the Role of mRNA Dynamics and Modifications for Prostate Cancer Progression
Log Number:	PC220077
Current PI Name:	Crystal Conn
Award Number:	HT9425-23-1-0122
Current Contracting Organization:	Pennsylvania, University of
Current Performing Organization:	Pennsylvania, University of
Web Approval Date:	11-03-2022

Prostate cancer remains the second leading cause of cancer-related deaths in men of the United States. Though ~80% of patients will remain cancer free after curative surgery, the remaining 20% account for the severity of the metastatic disease. This is partially because our current diagnostic options fail to classify non-aggressive from aggressive tumors starting from the primary tumor. This leads to potentially invasive treatment options with poor therapeutic response for advanced disease. The focus of this proposal is to identify and understand the biological mechanisms that lead to aggressive prostate development, therapy resistance, and progression through studying oncogenic adaptation pathways that reconstitute gene expression. One fundamental hallmark of cancer cells is their adaptation to cellular stress allowing for cell survival. By studying the “cancer stress response,” we can gain access to a tremendous window of opportunity for understanding this particular vulnerability of cancer cells, allowing us to create novel therapeutic strategies. Strikingly, our research suggests that there is a unique regulation of a subset of proteins that are required for this cancer cell adaption to stress. These novel targets are selectively expressed by an adaptive stress response rewiring our genetic code through mRNA translation during advanced cancer development. The mechanism of action for the regulation and basic biology of these epi-transcriptomic mechanisms are poorly understand in disease. This proposal will utilize several state-of-the-art technologies including deep sequencing and bioinformatic analysis to study this adaptation and the factors involved in the “cancer stress response” required for tumor progression. Thereby, our research will unravel the genetic and molecular mechanisms that underlie and maintain aggressive cancer cell survival under stress and contribute to the identification of novel targets that detect clinically relevant disease during early stages of prostate cancer. Furthermore, this research will provide the rationale for designing innovative pharmacological approaches to target the earliest events of tumorigenesis, with the long-term objective of eliminating metastatic prostate cancer and enhancing the well-being and health span of individuals suffering from this disease.

Proposal Title:	Comprehensive Investigation of AR mRNA Interactome in Advanced Prostate Cancer
Log Number:	PC220081
Current PI Name:	Yongyong Yang
Award Number:	HT9425-23-1-0491
Current Contracting Organization:	Northwestern University, Chicago, Illinois
Current Performing Organization:	Northwestern University, Chicago, Illinois
Web Approval Date:	11-03-2022

Scientific Objective: Prostate cancer is the most common non-cutaneous cancer and the second leading cause of cancer-related death among American men. The androgen receptor (AR) transcription factor is a master regulator of normal glandular homeostasis in the prostate, as well as growth and survival of prostate cancer cells. Therefore, AR-targeted therapies are effective for improving overall survival of patients with advanced prostate cancer that is incurable by surgery or radiation. However, the current primary treatment for advanced prostate cancer by hormone deprivation therapy eventually becomes castration-resistant prostate cancer with no effective treatment options, highlighting the urgent need to identify and develop better therapeutics to target advanced prostate cancer. The main cause leading to castration resistance is aberrant AR reactivation, including AR overexpression, mutation, changes in coregulator proteins, and steroid metabolism. Previous investigation identified AR gene amplification in tumor cells from castration-resistant patients, suggesting genomic changes of AR gene lead to increased AR mRNA transcript and AR protein expression. Gene expression can be regulated at different levels, such as genomic level, transcriptional level, post-transcriptional level, and post-translational level. Thus, genomic and transcriptomic changes insufficiently predict prostate cancer biology, and AR DNA, AR mRNA, and AR protein expression levels frequently do not correlate. Non-coding RNAs and RNA binding proteins could bind mRNA and regulate gene expression at the post-transcriptional level, thus participating in the cellular process and disease progression. Previous studies have reported the regulation of AR expression at the genomic level, transcriptional level, and post-translational level. However, the post-transcriptional regulation of AR mRNA by its interacted non-coding RNAs and RNA binding proteins, is largely unknown. In this proposed study, we will comprehensively profile AR mRNA interacted non-coding RNAs and RNA binding proteins for the first time and further identify key AR mRNA regulators among the interacted factors. Moreover, we will test the therapeutic efficacy of targeting these key AR mRNA interactors in the castration-resistance mice model.

The proposed study will address several FY22 PCRP Overarching Challenges. The investigation of AR mRNA interacted non-coding RNA and RNA binding proteins in castration-resistant prostate cancer cells will exceptionally expand the scientific knowledge about AR regulation at the post-transcriptional level for the first time, which will define the biology of prostate cancer progression to the lethal castration resistance prostate cancer. The bioinformatic analysis of the AR mRNA interactors' regulation of AR and their relevance to castration resistance will help to identify the biomarkers that could predict therapy resistance. What's more, the result from testing the therapeutic efficacy of targeting these key AR mRNA interactors will provide important preclinical data to support the initiation of clinical trials that target these AR mRNA interactors for the treatment of castration-resistant prostate cancer, leading to the development of new treatments that improve outcomes for men with the lethal castration-resistant prostate cancer.

Applicability of Research: Successful completion of the proposed studies will provide two immediate important clinical applications. This study will identify key AR mRNA interactors that participate in castration resistance. Some of these important AR mRNA interactors can be utilized as diagnostic and prognostic markers for prostate cancer patients who are receiving hormone deprivation therapy by analyzing large-scale transcriptomic and clinical data from prostate cancer patients. Second, the results of this study

will identify some AR mRNA interactors as promising new therapeutic targets for patients with castration-resistant prostate cancer. Ultimately, the preclinical data obtained from this proposal will provide a rationale to initiate new clinical trials of targeting AR mRNA interactors to inhibit prostate cancer cell growth and metastasis in patients with castration-resistant prostate cancer.

Contributions to Prostate Cancer Research: This proposal is highly innovative, as the interactome of the AR mRNA transcript is almost entirely unknown. Investigating the landscape of AR mRNA interactors for the first time will define new AR regulation mechanisms at the post-transcriptional level, which will significantly expand understanding of molecular mechanisms during prostate cancer progression. Furthermore, the proposed work will identify novel effective therapeutic targets for patients with advanced prostate cancer.

Proposal Title:	Turn the New Enemy to the Old Foe: Neuroendocrine-to-Luminal Redifferentiation
Log Number:	PC220087
Current PI Name:	Xin Lu
Award Number:	HT9425-23-1-0010
Current Contracting Organization:	Notre Dame, University of
Current Performing Organization:	Notre Dame, University of
Web Approval Date:	11-03-2022

This project addresses the FY22 PCRP Overarching Challenge, “Develop treatments that improve outcomes for men with lethal prostate cancer.”

The goal of our research is to develop treatments for the most aggressive and lethal form of prostate cancer, so-called neuroendocrine prostate cancer (NEPC). What is NEPC? Most of prostate cancer at diagnosis is characterized with a pathology feature called luminal adenocarcinoma, which expresses androgen-receptor (AR) and prostate-specific antigen (PSA) at high levels. Luminal prostate cancer can be treated with drugs that inhibit AR (for example, enzalutamide, apalutamide, and darolutamide, all approved by the FDA) because AR is required for luminal prostate cancer cells to survive and proliferate. However, about 2% of prostate cancers at diagnosis do not express AR or PSA; instead, they express molecular markers associated with neuroendocrine cells (cells that receive neuronal input and release various hormones), defining them as NEPC (i.e., de novo NEPC). What is more concerning for those patients with luminal prostate cancer and treated with AR-targeting drugs, 10-17% of their tumors shut down AR and PSA expression and start to express NEPC markers (i.e., treatment-induced NEPC). Because NEPC does not express AR, AR-targeting inhibitors show no activity on them. Currently, the only therapy option for NEPC is platinum-based chemotherapy, which is nonspecific, toxic, and easy to develop drug resistance. How should we develop effective strategies to treat NEPC?

Because NEPC can derive from luminal prostate cancer as an escaping mechanism from AR-targeted therapy, we argue that it is plausible to reverse this process and convert NEPC back to luminal prostate cancer by treating NEPC with certain drugs. This is an innovative and potentially high-gain idea that has not been explored previously. The objective of this project is to test this bold idea and find the drugs that revert NEPC back to luminal prostate cancer, at least the re-expression of AR so that the tumors are rendered sensitive to AR-targeting inhibitors again.

To achieve this objective, we propose to accomplish two specific aims. In Aim 1, we will perform high-throughput screening with a robotic system of a large-size drug library on a human NEPC cell line for drugs that can turn back on the AR expression and the AR signaling. In Aim 2, we will combine each of the top-ranked drugs that passed the screen in Aim 1 with the most widely used AR-targeting inhibitor enzalutamide to treat the human NEPC cell line so that we can determine whether NEPC cells can be killed by the combination treatment. Because each treatment alone is not expected to affect the survival of NEPC cells, if the combination treatment kills NEPC cells, that will prove our idea that NEPC cells can be sensitized to AR-targeting inhibitors.

Our project is highly relevant to the FY22 PCRP mission of eliminating death from prostate cancer and enhancing the well-being of men experiencing the impact of prostate cancer because treatment-induced NEPC has become a growing clinical threat due to the increasing use of AR- targeting inhibitors in the clinic to treat advanced prostate cancer. By taking a previously unexplored approach, our research may identify new drugs that turn NEPC back to the more tractable AR-dependent prostate cancer. Subsequent

translational studies may develop the combination treatment strategy discovered in this study into a new paradigm to treat both de novo and treatment-induced NEPC. We envision that the ultimate clinical application of the findings from our research will significantly prolong the survival and improve the well-being of most of the patients with NEPC.

Proposal Title:	Androgen Deprivation Therapy-Mediated Cardiovascular Disease and Vascular Aging in Men with Prostate Cancer: Racial Difference and Role of NAD+
Log Number:	PC220098
Current PI Name:	Avirup Guha
Award Number:	HT9425-23-1-0158
Current Contracting Organization:	Augusta University Research Institute, Inc.
Current Performing Organization:	Augusta University Research Institute, Inc.
Web Approval Date:	12-13-2022

Prostate cancer is the most common cancer in older men. However, heart diseases such as stroke and heart attack remain the most common cause of death 10 years following a cancer diagnosis. A lot of these heart issues happen in the first six months after starting hormonal treatment for prostate cancer. Black men tend to have a lot more of these issues compared to White men. A lot of these heart events are associated with non-medical factors that influence health outcomes (e.g., the conditions in which people are born, grow, work, live, and age, and the wider set of forces and systems shaping the conditions of daily life). These non-medical factors are also known as social determinants of health.

Age is one of the strongest predictors of heart events. Hormonal therapy can speed up aging and associated changes in the body. Aging is associated with a decline in a molecule in the body called nicotinamide adenine dinucleotide. This molecule is a building block for energy pathways in the human body. Through this study, we intend to study the role of the non-medical factors i.e., social determinants of health, in causing heart disease upon starting hormonal therapy in men with prostate cancer. In addition, we hope to find the biological reason for this increased risk of heart disease. This research will be able to tell us if hormonal therapy increases the aging of blood vessels in Black men compared to White men. We hope to also find out if the nicotinamide adenine dinucleotide molecule or social determinants of health play a role in the rapid aging process of hormonal therapy. In the following paragraph, we elaborate on the two aims of the study.

This research can answer a lot of questions regarding the health of men with prostate cancer. When men with prostate cancer get started on hormonal therapy, they undergo testing to determine the risk of heart disease. This study will tell us whether the non-medical factors have a role to play in heart disease noted after starting hormonal treatment. We will explore this question more by finding which of the five non-medical factors have a stronger role to play. The non-medical factors we are looking at include access to quality education, access to healthcare, and social relationships. We are also studying the patient's financial abilities and neighborhood. Through this study, we can pay closer attention to these non-medical factors while caring for men with prostate cancer to minimize the risk hormonal therapy may pose to their hearts.

We live in the era of personalized medicine. This means that each patient is special. The current methods to assess the risk of heart disease with hormonal therapy do not differentiate between patients. In other words, it calculates the risk similarly for all men by considering certain risk factors such as cholesterol levels. Although paying attention to risk factors such as cholesterol levels is important, we believe we can do better at the individual level. We know that hormonal therapy may speed up the aging process, but it may not do it the same way for all men. Given what we know about Black men and heart disease, they likely age faster than White men when started on hormonal therapy. This observation may be related to the difference between social determinants of health entirely and not related to race. Thus, we propose looking at the aging

process using special blood and cardiovascular tests. In addition, in this study, we will also examine the role of the energy molecule in the body called nicotinamide adenine dinucleotide. Given what we know about the ancestral differences in this molecule, it may be a reason why Black men age faster due to the addition of hormonal treatment for prostate cancer.

This study is focused on advancing health equity and reducing racial disparities among men with prostate cancer. This study is being conducted at Georgia Cancer Center, which is in the rural southeastern United States, where one can study underserved patients with limited access to clinical care and resources. Dr. Avirup Guha is a physician-scientist who is the main investigator in this study. He is a young scientist who, while conducting this research project under the guidance of his mentors, will develop skills to study questions such as this during his career. Dr. Guha has committed his career to care for men with prostate cancer in rural Georgia. He has gathered a group of very talented scientists who have experience in many aspects of the study proposed by Dr. Guha. Apart from providing resources for the study, his mentor group will facilitate his education regarding ways to conduct research that focuses on inequality and working with the community to encourage the recruitment of Black men into this study.

Proposal Title:	Targeting Schwann Cell-Mediated Prostate Cancer Progression and Metastasis
Log Number:	PC220104
Current PI Name:	Lizhong Wang
Award Number:	HT9425-23-1-0313
Current Contracting Organization:	Alabama, University of, at Birmingham
Current Performing Organization:	Alabama, University of, at Birmingham
Web Approval Date:	11-03-2022

Rationale: Perineural invasion, a neoplastic invasion of nerves, is common in 75% of prostatectomy specimens of prostate cancer and in 11% to 38% of prostate cancer biopsies. For patients treated by prostatectomy, perineural invasion is predictive of extraprostatic extension, seminal vesicle invasion, positive surgical margins, tumor metastasis, and prognosis. Thus, targeting perineural invasion is an attractive therapeutic option. Previous studies have attempted to cut nerves surgically or chemically to block growth, occurrence, and metastasis of prostate cancers. However, shortcomings related to the side effects of these therapies hinder their clinical application. In the peripheral nervous system, Schwann cells (SCs) surround neurons and protect them. In prostate cancers, SCs promote perineural invasion, tumor metastasis, and bone metastasis-related pain. Thus, for patients with prostate cancer, targeting of SCs appears to be an effective approach to block tumor progression and metastasis and to relieve cancer-related pain.

Objective: This study hypothesizes that targeting of SCs eliminates perineural invasion, inhibits tumor progression and metastasis, and relieves the cancer-related pain of prostate cancer.

Specific Aims: There are two specific aims: (1) targeting of SCs to inhibit tumor progression and metastasis and

(2) targeting of SCs to relieve bone metastasis-related nerve pain. This proposed work addresses one of the FY22 PCRP Overarching Challenges, “Develop treatments that improve outcomes for men with lethal prostate cancer,” which is relevant to the PCRP mission of eliminating death from prostate cancer and enhancing the well-being of Service Members, Veterans, and other men and their families who are experiencing the impact of the prostate cancer.

Ultimate Applicability of the Research: Although this research is proposed for preclinical animal models, in future clinical applications, this developed approach is expected to prevent lethal tumor metastasis and relieve cancer-related pain for prostate cancer patients with bone metastasis.

What Types of Patients Will It Help and How Will It Help Them?

This study will help (1) men with intermediate-risk and locally advanced prostate cancer who have perineural invasion diagnosed by prostate cancer biopsies and/or (2) men with high-risk prostate cancer who have bone metastasis. Targeting of SCs in this research will help patients (1) by preventing and reducing tumor progression and metastasis and/or (2) by relieving cancer-related pain from bone metastasis.

What Are the Potential Clinical Applications, Benefits, and Risks?

The potential clinical applications of this research are to improve existing therapeutics by (1) eliminating perineural invasion through targeting of SCs and preventing metastasis by (2) targeting SCs in bone to relieve cancer-related pain with few side effects, which will benefit patients with prostate cancer. Since this is a previously undeveloped therapeutic approach, potential risks will be addressed in this research.

What Is the Projected Time It May Take to Achieve a Patient-Related Outcome?

In the proposed study, if the developed therapeutic approach is effective with limited side effects in preclinical animal models, it will be immediately advanced to a clinical trial.

Proposal Title:	Noninvasive Diagnostic Imaging of Aberrant Sarcosine Biochemistry in Prostate Cancer
Log Number:	PC220114
Current PI Name:	Lloyd Lumata
Award Number:	HT9425-23-1-0062
Current Contracting Organization:	Texas, University of, at Dallas
Current Performing Organization:	Texas, University of, at Dallas
Web Approval Date:	11-03-2022

According to estimates from the American Cancer Society, there will be about 268,490 new cases of prostate cancer and 34,500 deaths from prostate cancer in the United States in 2022. This type of cancer is the second leading cause of cancer-related death of men in the United States, behind only lung cancer. Early and accurate diagnosis of prostate cancer is of paramount importance to guide for proper therapeutic intervention and reduce the mortality rate of this disease.

Responding to this diagnostic need, this study seeks to take advantage on the abnormal biochemistry in prostate cancer and turn it into its diagnostic Achilles' heel. Glycine is an amino acid that is endogenous or naturally present in our cells. It was reported previously that prostate tumors tend to produce abnormally copious amounts the biochemical sarcosine and the raw material needed for its biosynthesis is the amino acid glycine. To turn this metabolic anomaly in prostate cancer into a diagnostic advantage, this project will utilize a technology called hyperpolarization that has the ability to amplify the magnetic resonance imaging signals of biochemical such as glycine by >10,000-fold. The use of "hyperpolarized" glycine with supercharged MRI signals could potentially offer a new kind of diagnostic molecular imaging for prostate cancer by looking at the ensuing elevated sarcosine production as MRI biomarker for this malignancy. This project aims to improve the detection/diagnosis of this disease that may impact mission readiness and the health and well-being of military members, Veterans, their beneficiaries, and the general public. In addition, this research study addresses one of the FY22 PCRP Overarching Challenges, "define the biology of prostate cancer progression to lethal prostate cancer to reduce death."

The main innovation of this project is that, for the first time, the hyperactive sarcosine synthesis that is indicative of prostate cancer can potentially be "visualized" in vivo as it happens in the tissues using the combined high sensitivity and chemical specificity of hyperpolarized MRI technology. No other current technology can potentially provide this capability in a noninvasive and nonradioactive manner. Moreover, since glycine is a natural biochemical in the body and will be administered at physiological concentration as an injectable MRI agent, there is a high propensity that this potential MRI biomarker for prostate cancer can be translated for use in the clinic. The main impact of this research is that it could potentially provide a more clear-cut, non-invasive, and non-radioactive molecular imaging tool that will not only detect and locate prostate tumors, but also has a way to assess its malignancy with an MRI metabolic biomarker. This study could potentially provide a much-needed additional tool in the diagnostic arsenal for prostate cancer patients in the military and civilian sectors with high sensitivity and molecular precision.

Proposal Title:	CD44 Enhances Efficacy of the Chimeric Antigen Receptor T-Cell Therapy Against Prostate Cancer
Log Number:	PC220116
Current PI Name:	Qin Yu
Award Number:	HT9425-23-1-0021
Current Contracting Organization:	Icahn School of Medicine at Mount Sinai
Current Performing Organization:	Icahn School of Medicine at Mount Sinai
Web Approval Date:	11-03-2022

Even though there are high initial response rates of prostate cancer (PCa) patients to the androgen-deprivation therapy (ADT), castration-resistant prostate cancer (CRPC) often occurs with poor prognosis and limited treatment options. The chimeric antigen receptor (CAR)-T cell therapy offers curative potential for CRPC patients. In the CAR-T therapy, autologous T cells isolated from cancer patients are engineered with the chimeric receptors that recognize surface antigens of cancer cells. The engineered T cells are then infused back to the cancer patients to allow the CAR-T cells to track down, bind, and kill cancer cells. The CAR-T cell-based therapy has been successful in patients with B-cell leukemia/lymphoma and is being developed against solid tumors, including PCa, but still with limited success.

The mechanisms underlying the insufficient solid cancer responses to the CAR-T therapy include (1) inadequate selections of CAR-T targeted tumor antigens; (2) antigen escape of tumor cells; (3) immuno-suppressive tumor microenvironment (TME); (4) the inadequate ability of CAR-T cell trafficking, tumor infiltration, expansion, and persistence; and (5) CAR-T cell exhaustion. This proposal plans to offer an innovative solution for last two major problems. CD44 is a cell surface receptor for hyaluronan; is up-regulated in many cancer types; and promotes cancer progression including that of PCa. CD44 is also referred as a lymphocyte homing receptor and is a prominent marker that distinguishes memory and effector T cells from their naïve counterparts. CD44-related cancer studies have been exclusively focused on cancer cells, and little is done to investigate the contributions of CD44+ T/memory T cells to cancer progression and response to immunotherapy. Lymphocytes mainly express the standard or hematopoietic form of CD44 (CD44s/CD44H). Studies have shown that CD44 plays important roles in enhancing T cell receptor (TCR)-signaling and T cell migration, proliferation, and survival.

This proposal aims to develop treatments that improve outcomes for men with lethal PCa (a FY22 PCRP Overarching Challenge) by establishing that the CD44 armored CAR-T cells represent an innovative strategy to enhance efficacy of the CAR-T cell-based therapy against advanced PCa. The proposal is based on our novel findings: (1) anti-PD1 antibody (an immune checkpoint inhibitor, ICI) displayed reduced efficacy against PCa in CD44-knockout mice comparing to wild type (wt) mice; (2) loss of CD44 led to reduction of the numbers of intra-PCa CD3+/CD8+ T cells and the numbers of the anti-PD-1 antibody-induced increase of intra-PCa CD3+/CD8+ T cells; and (3) constitutive CD44H expression inhibits exhaustion and death of the CD19-targeting CAR-T cells. We hypothesize innovatively that CD44 expressed by T cells plays a critical role in enhancing the efficacy of the ICIs against PCa via promoting T cell infiltration into and expansion/survival in PCa and by inhibiting T cell exhaustion and that reduced or lost CD44 expression in T cells leads to the PCa resistance to the ICIs. We further hypothesize that constitutive CD44H expression in CAR-T cells, such as the prostate-specific membrane antigen (PSMA)-targeting CAR-T cells enhances the efficacy of the CAR-T cell-based therapy by promoting intra-PCa infiltration, expansion, and survival of the CAR-T cells.

Two Specific Aims are proposed: Aim 1 is to establish that CD44 expressed by T cells plays a critical role in enhancing the efficacy of the immune checkpoint inhibitors (ICIs) against PCa by promoting T cell

infiltration into and expansion/survival in PCa and by inhibiting T cell exhaustion. In this aim, CD44-null mice on C57BL/6 or FVB background will be intravenously implanted with pan-T/CD4+/CD8+ T cells that are CD44-null, wt, or constitutively express v5-tagged CD44H (CD44H-v5) and then orthotopically implanted with TrampC2 or Myc-CaP mouse PCa cells, which will be followed by treatments of control IgG or RMP1-14 anti- mouse PD1 antibody (Bio-X-Cell). Mouse survival rates will be determined using the LogRank analyses. Aim 2 is to establish that constitutive expression of CD44H in the prostate-specific membrane antigen (PSMA) targeting CAR-T cells enhances efficacy of the CAR-T cell therapy against PCa through promoting intra-PCa infiltration, expansion, and survival of the CAR-T cells. C4-2 and LACP4 (ATCC) human PCa cells that express PSMA will be orthotopically implanted into immunocompromised NSG mouse (Jackson Lab). The PCa-bearing mice will be implanted with control or the PSMA-targeting CAR-T cells (provided by the Creative- Biolabs) with or without constitutively expressing CD44H-v5. Mouse survival rates will be established and used to determine whether constitutive CD44H expression enhances efficacy of the CAR-T cell therapy.

Successful accomplishment of this proposal will identify CD44 expressed by T cells as a novel determinant of the efficacy of the ICI and the T cell-based immunotherapy and establish that CD44 plays a critical role in promoting CAR-T cell infiltration into and expansion/survival in PCa and in inhibiting CAR-T cell exhaustion, as well as that constitutive expression of CD44H in CAR-T cells enhances the efficacy of the CAR-T cell therapy against PCa (short-term impact). Positive results will lead to rapid clinic translation of the CD44 armored CAR-T cells to improve efficacy of the CAR-T therapy against advanced PCa and to improve clinical outcomes and survival rates of PCa patients (long-term impact). Thus, this proposal is of high biologic and clinical relevance and significance, as well as high therapeutic potential and impact.

Proposal Title:	Investigating Noncanonical Roles of Androgen Receptor in Driving Growth Inhibition by Supraphysiological Androgen in Prostate Cancer
Log Number:	PC220122
Current PI Name:	Rajendra Kumar
Award Number:	HT9425-23-1-0029
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	11-03-2022

Prostate cancer is the most common cancer in men. Androgens drive prostate cancer growth by binding to its receptor, known as the androgen receptor. Inhibiting androgen receptor signaling by androgen deprivation therapy (ADT) is the first line of therapy for prostate cancer. Initially, all patients respond, but later develop resistance to therapy. Once the castration resistance develops in patients, cancer advances further and is called metastatic castration-resistant prostate cancer. Bipolar androgen therapy (BAT) is a paradoxical approach that involves the administration of supraphysiological doses of testosterone (SupraT), which has been shown to dramatically decrease growth in a subset of prostate cancer cases. It involves the cycling of androgen levels between supraphysiological high and castration values. Patients in early trials of BAT showed promising clinical responses. Despite favorable outcomes in preclinical and clinical subjects, not all patients show clinical benefits and differ in the extent and duration of response. Also, the mechanism of action of BAT is not fully known and could be responsible for partial clinical success. Multiple studies are being conducted to understand how BAT works, and many researchers have reported partial success. This proposal aims to study a never-before-tested idea with a potential transformative (rather than incremental) impact on the field. In this exploration hypothesis development award, we will test the hypothesis that androgen receptor in the presence of SupraT leads to androgen receptor aggregates in the prostate cancer cells, very similar to that reported in Kennedy disease, a disease condition where an individual inherits mutated androgen receptors. These aggregates would result in a stressful cellular condition called ER stress that would lead to the death of prostate cancer cells. We further postulate that prostate cancer cells that are unable to handle the ER stress would be most susceptible to SupraT administration and BAT. We propose to explore this vulnerability by combining SupraT with clinical drugs that are known to cause ER stress. Our idea is that a combination of SupraT and ER stressor will overwhelm the ER stress pathway to cause prostate cancer cell death and inhibit growth, even in those subsets that do not respond favorably to BAT. Our aims are structured to test the hypothesis in prostate cancer cell lines and patient-derived organoid models that closely resemble original prostate tumors.

The proposed work will directly impact PCRP's FY22 Overarching Challenge, “develop treatments that improve outcomes for men with lethal prostate cancer.”

Proposal Title:	Intersection of Physical and Mental Health and Short- and Long-Term Outcomes Among Prostate Cancer Patients
Log Number:	PC220129
Current PI Name:	Sumedha Chhatre
Award Number:	HT9425-23-1-0027
Current Contracting Organization:	Pennsylvania, University of
Current Performing Organization:	Pennsylvania, University of
Web Approval Date:	11-04-2022

We are proposing a study that has strong relevance to one of the FY22 PCRP Overarching Challenges, "improve quality of life to enhance outcomes and overall health and wellness for those impacted by prostate cancer." The objective of our study is to develop a hypothesis regarding influence of depression on the health-related quality of life and life expectancy of elderly prostate cancer patients. Additionally, we will explore whether the influence varies based on race and ethnicity of a patient or geographic location of the patient (i.e., urban vs. rural patients). Our study will use linked data from Surveillance, Epidemiology and End Results (SEER) and Medicare Health Outcomes Survey (MHOS). Our study group will consist of Medicare elderly men who are aged 66 or older, were diagnosed with prostate cancer between 2000 and 2017, resided in SEER regions, and completed one MHOS survey before their prostate cancer diagnosis and one survey after their prostate cancer diagnosis. We will then analyze how depression affects quality of life and life expectancy in our cohort. In addition, we will also assess whether these observed effects are different based on factors such as race and ethnicity of the patient or the residence of the patient (urban vs. rural).

Relevance: In the United States, the exponential growth of elderly populations is posing a challenge to the health care system. For example, the United States is expected to see a doubling of the number and percentage of Americans over age 65. The elderly have unique health needs and generally experience a large number of health issues, one of them being prostate cancer. Among men in the United States, prostate cancer is the number one cancer. Thus, over the coming decades, we can expect a growing number of elderly men who have prostate cancer and are in need of health care. Several treatment options are available for prostate cancer. At the same time, beyond treatment, there exist multiple factors that can affect the quality of life and life expectancy of the elderly prostate cancer patients, for example, other concurrent health conditions and social detriments of health. The social determinants of health are the personal level and environmental level factors that influence a person's conditions of daily life and health and economic outcomes beyond biology or genetic inheritance. One important health condition that can affect the quality of life and life expectancy of elderly prostate cancer patients is depression. In general, depression in the elderly remains an overlooked issue. However, it is important to know what influence depression has on the quality of life and life expectancy of elderly prostate cancer patients. In addition, it is also important to know whether this influence varies based on the race and ethnicity of a patient or based on the geographic location of the patient (i.e., urban vs. rural patients).

Applicability: This knowledge will serve as a foundation on which future studies will be based. For example, future research studies can address how exactly depression affects quality of life and life expectancy, i.e., the mechanism; how and why this mechanism differs across patients from different racial and ethnic groups or those from urban areas vs. rural areas. In addition, our research can contribute to the development of an appropriate survivorship care plan for prostate cancer survivors to improve the outcomes of their care. Thus, our research can ultimately lead to patient-centered, tailored intervention strategies, all with an aim to improve the quality of life and life expectancy.

Proposal Title:	The Palmitoylating Enzyme DHHC11 Promotes Prostate Cancer Progression and Metastasis
Log Number:	PC220133
Current PI Name:	Wei Yang
Award Number:	HT9425-23-1-0028
Current Contracting Organization:	Cedars-Sinai Medical Center
Current Performing Organization:	Cedars-Sinai Medical Center
Web Approval Date:	11-04-2022

Once prostate cancer cells spread to distant organs and form metastases visible on an X-ray, the disease can no longer be cured, and most patients die within 2 to 3 years. Thus, it is critical to understand at the molecular level how prostate cancer cells spread and thrive in distant organs in order to prevent and treat lethal metastatic prostate cancer. Global analyses of mRNAs in prostate cancer tissue specimens showed that ~90% of metastatic prostate tumors (and ~30% of primary prostate tumors) express the ZDHHC11 gene at significantly higher levels than normal prostate tissues. Furthermore, patients with high levels of ZDHHC11 expression in their tumors are about five times more likely to have a metastatic recurrence than those with low levels of ZDHHC11 expression. ZDHHC11 encodes an enzyme called DHHC11, which attaches long-chain fatty acids (especially palmitic acid) to specific cysteines on proteins. This enzyme process is called protein palmitoylation, which dynamically changes the location and activity of many proteins. Studies have shown that abnormal protein palmitoylation contributes to many human diseases, including prostate cancer. Taken together, DHHC11 is potentially a novel key factor in prostate cancer metastasis and a promising drug target. But does enzyme-active DHHC11 increase prostate cancer spread and severity? If so, what mechanism does DHHC11 employ to increase the spread and severity of prostate cancer? These conceptually novel and clinically relevant questions will be addressed in the proposed research plan, utilizing our unique combination of various technical skills. First, we will use cell culture and mouse models to determine whether taking away DHHC11 from prostate cancer cells decreases the spread and growth of prostate cancer. We will also determine whether adding enzyme-active DHHC11, not enzyme-inactive DHHC11, to DHHC11-depleted cells allows prostate cancer to spread and grow again. Second, we will use powerful analytical methods to identify almost all of the proteins that bind to, are controlled by, or are palmitoylated by DHHC11. Some of these proteins are necessary for DHHC11 to increase prostate cancer spread and severity. Notably, the roles and mechanisms of palmitoylating enzymes have never been studied in prostate cancer spread before, so the proposed research will add a new dimension to our understanding of prostate cancer metastasis. Targeting DHHC11 should not cause any major side effects because it is rarely found in normal organs other than the testis. We envision that our research will lead to Phase 1/2 clinical trials to test specific, potent, and orally available inhibitors of DHHC11 enzyme activity if enzyme-active DHHC11 is needed for prostate cancer to spread and grow or inhibitors of DHHC11 interaction with specific binding partners if enzyme-inactive DHHC11 is needed. DHHC11 and its interacting or substrate proteins in tumor-derived extracellular vesicles can be turned into a non-invasive liquid biopsy biomarker to find the right patients who are most likely to benefit from anti-DHHC11 drugs. If successful, patients with non-metastatic castration-resistant prostate cancer (M0 CRPC) will live much longer without getting metastases and associated symptoms such as bone pain and breaks. Patients with only a few prostate cancer metastases (called oligometastases) will also benefit from these drugs, which inhibit cancer cells from spreading to more organs. If DHHC11 protects metastatic prostate cancer cells from dying, targeting DHHC11 can trigger cell death and thus shrink existing prostate cancer metastases. These early phase trials could lead to Phase 3 clinical trials, which would make it possible for DHHC11 inhibitors to be used regularly in the clinic. Thus, the proposed project is directly relevant to two FY22 PCRP Overarching Challenges: (1) define the biology of prostate cancer progression to lethal prostate cancer to reduce death and (2) improve quality of life to enhance outcomes and overall health and wellness for those impacted by prostate cancer.

Proposal Title:	Exploiting Retained Intron (RI)-Derived Neoepitopes for Innovative Prostate Cancer Immunotherapy
Log Number:	PC220137
Current PI Name:	Dean Tang
Award Number:	HT9425-23-1-0108
Current Contracting Organization:	Health Research Inc., Roswell Park Division
Current Performing Organization:	Health Research Inc., Roswell Park Division
Web Approval Date:	11-04-2022

Metastatic castration-resistant prostate cancer (mCRPC) claims the lives of >32,000 American each year, and there are few effective treatment options for mCRPC patients. The next-generation antiandrogens such as Enzalutamide (Enza) and abiraterone acetate (AA) only extend patients' lives by 3-4 months, even when used with chemotherapeutic agents or novel targeted therapeutics such as PARP inhibitors. Immunotherapies such as immune checkpoint inhibitors (ICI) are generally ineffective in PCa, mainly due to preexisting and therapy-induced immunosuppressive tumor microenvironment (TME) in most metastatic sites. Moreover, PCa is known to have low tumor mutational burden and low mutation rate (~0.5 per Mbp), and most driver mutations have low representations. These "indolent" genomic features and paucity of PCa-specific TAAs (tumor-associated antigens) and especially neoantigens (neoAgs) greatly contribute to the "cold" TME in mCRPC and to the "lukewarm" responses to ICIs.

However, PCa has prevalent and pervasive abnormalities in their transcriptomes, i.e., the entire repertoire of RNA transcripts from their genomic DNA. A recent publication may have identified a unique vulnerability in aggressive mCRPC. When systematically mapping the mRNA alternative splicing (the process whereby different isoforms of one mRNA are made) landscape in the spectrum of PCa evolution, the authors in this study observed that the severity of splicing dysregulation correlates with PCa progression and that mCRPC is particularly sensitive to chemical inhibitors that interfere with the splicing process (Nat Commu. 2020). More surprisingly, the authors discovered intron retention (IR) as a consistent hallmark of PCa stemness and aggressiveness with mCRPC possessing the highest numbers of Retained Introns (RI). Normally, introns are removed (spliced out) during generation of mature RNA products, but in a peculiar way, mCRPC produces a high number of mRNA transcripts with RIs.

Why does mCRPC have so abundant mRNA transcripts with RIs? More and more recent studies begin to link IR Program to development and stem cell functions and to cancer progression; of great interest, some studies suggest that many RIs in melanoma and colon cancer cells may be able to encode peptides that can bind MHC-I and potentially function as cancer neoantigens. Cancer neoantigens are cancer cell-specific "markers" that can engage the host immune system to kill cancer cells. Much inspired by these recent studies, in this project, we plan to systematically annotate the RIs in PCa and test the overarching hypothesis that some aggressive PCa-specific RIs are translated into peptides that can function as mCRPC-specific neoantigens.

This EHDA project tackles two PCRP Overarching Challenges, "define the biology of prostate cancer progression to lethal prostate cancer to reduce death" and "develop treatments that improve outcomes for men with lethal prostate cancer." Work here will provide the first evidence that some RIs in aggressive, Enza-refractory, and metastatic PCa can encode neoantigens to elicit specific T cell cytotoxicity. Our future goal is to translate this RI-neoAg discovery platform to PCa patients by developing personized neoantigen-specific vaccines and T cell therapies, which, together with ADT/Enza, should extend mCRPC patients' survival by co-targeting bulk differentiated (AR-dependent) PCa cells and the aggressive, stem-like, highly plastic, and therapy-resistant PCa cells.

Proposal Title:	Peptide-Drug Conjugate as Bone-Targeted Therapy for Bone-Metastatic Prostate Cancer
Log Number:	PC220150
Current PI Name:	Tsung Shih
Award Number:	HT9425-23-1-0203
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	11-04-2022

This project addresses the Area of Emphasis on “develop treatments that improve outcomes for men with lethal prostate cancer.”

Prostate cancer (PCa) bone metastasis is frequently associated with bone-forming lesions. Development of bone metastases significantly decreases survival time (5-year survival rate: ~30%). Currently, PCa bone metastases remain incurable. Considering the morbidity associated with late-stage disease, there is an urgent need to develop novel strategies.

One unique feature of PCa bone metastasis is the induction of aberrant bone overgrowth. Recent studies showed that tumor cells secreted BMP4 converts tumor-associated endothelial cells to become EC-to-OSB hybrid cells, which then become osteoblasts. EC-OSB hybrid cells in turn elicit paracrine effects on tumor cells to support PCa progression in bone. These studies revealed that EC-to-OSB hybrid cells are a unique cell type in PCa-induced bone lesion, suggesting it may serve as a target for treating PCa bone metastasis.

In this proposed research, we will develop and test a new type of therapeutic that could help treat PCa patients with bone metastasis. The proposed new therapeutic, peptide-drug conjugates (PDCs), is a chemical combination of two types of molecules – a “peptide” capable of selectively binding PCa-induced EC-OSB hybrid cells and a high potency chemotherapy drug that is toxic to cancer cells. Conjugation of bone targeting peptides to high potency medicines can provide tissue-specific delivery to concentrate the pharmacology at a preferred site. Therefore, PDCs can maximize therapeutic effects and reduce off-target toxicities to normal tissues. The successful development of PDCs as targeted therapeutics to specifically target and destroy PCa-induced bone lesions can improve the quality of life of patients by preventing the adverse side effects of existing chemotherapy.

At the end of this project, we will obtain proof in vitro that this approach works. We expect that another 4 years will be needed for further optimization and animal testing before putting into human clinical trials. If successful, PDC will make chemotherapy more efficacious and much safer and will have a tremendous impact on the improvement of survival rate and quality of life of patients with PCa bone metastasis.

Proposal Title:	Whole-Genome Structural Variation and CpG Methylation Analyses Through Long-Read Sequencing
Log Number:	PC220151
Current PI Name:	Changsheng Zhao
Award Number:	HT9425-23-1-0006
Current Contracting Organization:	Emory University
Current Performing Organization:	Emory University
Web Approval Date:	11-04-2022

Structural Variations (SVs), including DNA base deletion, insertion, inversion, and translocation, are hallmarks of prostate cancer (PCa). PCa is also critically regulated by epigenetic changes, such as DNA methylation. It has been challenging to obtain precise maps of structural variations and DNA methylations across the entire genome, as existing technologies depend on sequencing (e.g., Illumina) methods that generate short reads usually of only 50-300 base pairs long. Short reads have difficulty finding their position on the human reference genome, especially in chromosomal regions of major structural variations or repetitive elements. Further, current methods of detecting DNA methylation depend on bisulfite conversion and are unable to distinguish between DNA cytosine methylation (5mC) and hydroxymethylation (5hmC). For example, current standard whole-genome bisulfite sequencing measures the sum of 5mC and 5hmC as the level of DNA methylation. However, studies have shown that 5mC and 5hmC have opposite roles in regulating PCa gene expression.

The goal of the present study is to develop and benchmark an innovative technology called Nanopore Long-Read Sequencing (LRS) for PCa research. Nanopore LRS directly sequences native DNA and is thus able to distinguish between 5mC and 5hmC and provide their precise maps on the same DNA molecules. Nanopore provides long reads in an average of 50 kilobases but up to 4 megabases, if needed, and thus is often able to sequence across the entire region of SVs or repetitive elements, significantly improving accuracy in SV detection. In Aim 1, we will adapt Nanopore LRS technology in PCa cell lines and evaluate its performance in detecting DNA methylations relative to whole-genome bisulfite sequencing. Aim 2 will use LRS to analyze 10 pairs of matched benign and PCa samples and evaluate its ability to detect SV, 5mC, and 5hmC changes that are associated with PCa progression. We will also examine their association with TMPRSS2-ERG gene fusion, which occurs in approximately 50% of all PCa cases and has a lower prevalence in prostate tumors of African American men.

Ultimate Applicability of the Research: Once we optimize Nanopore LRS technology in PCa cells and establish all of the bioinformatics analysis pipelines, we will rapidly apply it to a large set of human PCa samples to obtain SV and methylation profiles with unprecedented accuracy. Such analyses will greatly advance our understanding of the biology of lethal PCa and of the genetic and epigenetic bases of health disparity. The project is thus of high relevance to the FY22 PCRP mission of defining the biology of lethal PCa to reduce death, advance health equity, and reduce disparities in PCa.

Proposal Title:	Regulation of PARP Activity by Ubiquitin E3 Ligase RNF6 in Prostate Cancer
Log Number:	PC220154
Current PI Name:	Yun Qiu
Award Number:	HT9425-23-1-0112
Current Contracting Organization:	Maryland, University of, Baltimore
Current Performing Organization:	Maryland, University of, Baltimore
Web Approval Date:	11-04-2022

Poly(ADP-ribose) polymerase (PARP) inhibitors act through synthetic lethality with mutations in DNA repair genes and are approved for treatment of cancers with BRCA1/2 mutation, including prostate cancer. The effectiveness of current targeted therapy for CRPC is limited by the lack of reliable markers to identify the patients who most likely benefit from PARP inhibitors (PARPi) and their combination therapy. Efficacy of PARPi as a monotherapy is limited to a small subset of prostate cancer patients. Identification of new PARP regulators relevant to PCa is critical for improving the efficacy of current PARPi and developing new effective combination therapy. This is a proof-of-concept study to investigate a novel functional interaction between PARP1 and RNF6 by characterization of RNF6- induced ubiquitination of PARP1 and test whether RNF6 modulates sensitivity to PARPi in prostate cancer cells. The effects of RNF6-induced ubiquitination on PARP1 polymerase activity, gene transcription regulation, chromatin association, and protein partner recruitment and sensitivity to PARPi will be examined in PCa cells. A new combination treatment of PARPi and RNF6 inhibitor will be tested in PCa cells. Successful completion of the proposed study will provide novel insights into mechanisms underlying PCa progression and therapeutic resistance and lay a foundation for the development of new effective combination treatment of CRPC.

Proposal Title:	Genome-Wide Analyses of Protein-DNA Interaction Using Long-Read Sequencing
Log Number:	PC220158
Current PI Name:	Changsheng Zhao
Award Number:	HT9425-23-1-0007
Current Contracting Organization:	Emory University
Current Performing Organization:	Emory University
Web Approval Date:	11-04-2022

Prostate cancer is marked by aberrant gene expression, which is controlled by proteins that bind to the DNA. It is therefore critical to map where on the genome a protein binds. The existing method, called ChIP-seq, requires PCR amplification of enriched DNA, followed by short-read, often 50-300 base pair, sequencing. PCR amplification causes sequence biases, while short reads have a problem mapping to repetitive regions, which accounts for 50% of the human genome. In Aim 1 of this study, we propose a new technology called DiMeLo- seq, which uses an enzyme to catalyze DNA adenine methylation (mA) at the binding sites of the target protein. The mA-modified DNA is then directly sequenced using Nanopore technology in long reads that are of average 50 kilobases. Long reads have no problem mapping to repetitive regions of the human genome. This method does not need PCR amplification. The accumulation of mA indicates protein-DNA binding, and long reads allow for the detection of co-binding on distant elements (e.g., promoters and enhancers), suggesting DNA looping. Further, it can detect endogenous DNA methylation, another major regulator of gene expression, along with exogenous mA on the same DNA molecule. Aim 1 will test the performance of DiMeLo-seq using androgen receptor (AR) as a typical transcription factor in prostate cancer cells. In Aim 2, we will further test the method for detecting histone modifications. We chose histone 3 lysine 9 (H3K9) methylation, which is known to bind at repetitive regions, with which conventional ChIP-seq has a problem achieving accurate mapping. We will compare DiMeLo-seq data with ChIP-seq data and evaluate how DiMeLo-seq outperforms ChIP-seq, especially in repetitive regions. We will further examine the ability of DiMeLo-seq to detect protein co-binding at the promoters and enhancers. We will integrate protein-DNA binding with DNA methylation data and determine whether AR binding at sites with extensive DNA methylation indicates gene repression. Last, we will examine whether H3K9 and DNA methylation are increased in Enzalutamide-resistant prostate cancer.

Ultimate Applicability of the Research: Our study will develop innovative technology for the prostate cancer research field. It will dramatically advance the studies of gene regulation, thereby increasing our understanding of the molecular mechanisms underlying prostate cancer progression and drug resistance. It might also identify potential targets that are involved in drug resistance that may be important therapeutic targets. Our study is thus paradigm-shifting. It addresses the PCRP overall challenge in defining the biology of lethal prostate cancer. By understanding the mechanism of Enzalutamide resistance, our study will help eliminate death from prostate cancer.

Proposal Title:	Defining Clinically Actionable Subtypes of Castration-Resistant Prostate Cancer Through Epigenetic Cell-Free DNA Analysis
Log Number:	PC220173
Current PI Name:	Jacob Berchuck
Award Number:	HT9425-23-1-0048
Current Contracting Organization:	Dana-Farber Cancer Institute
Current Performing Organization:	Dana-Farber Cancer Institute
Web Approval Date:	12-13-2022

Metastatic prostate cancer (mPC) – the term for when the cancer has spread beyond the prostate – is not curable and is the form of the disease that men die from. The backbone of treatment for mPC includes medications that block the actions of androgens (male sex hormones), such as testosterone. Unfortunately, all men with mPC eventually stop responding to anti-androgen therapy and become "castration-resistant." Fortunately, several new drugs significantly prolong survival for men with castration-resistant mPC. Further reducing morbidity (injury and suffering) and mortality (death) for men with mPC is contingent upon addressing two barriers. First, we lack the tools to predict how well an individual man will respond to specific drugs. The ability to do so would facilitate prioritizing the most effective treatments over those that may cause significant side effects with little to no benefit. Second, despite numerous effective therapies for men with mPC cancer, tumors eventually become resistant to all available treatments and men succumb to their illness. Developing tools to personalize therapeutic decision-making for individual men and understanding how tumors become resistant to current treatments so that we can develop new effective drugs are critical to prolonging survival for men with mPC.

Efforts to understand how mPC develops treatment resistance have largely focused on genetics, i.e., studying changes to the sequence of the DNA in important PC genes. It has become increasingly clear that changes in epigenetics also drive PC resistance. Epigenetics refers to the DNA modifications that cells use to turn genes on or off. Differences in epigenetics explain why, despite having the same DNA, a skin cell looks and functions differently from a muscle cell – or a PC cell is sensitive or resistant to one drug or another. Several challenges have prohibited major advances into our understanding of the role of epigenetics in contributing to response and resistance to treatments in mPC. First, traditional tools for epigenetic analysis require larger amounts of tissue than is often feasible to obtain from a mPC tumor biopsy. Second, there are no large datasets of paired tumor biopsies from the same patient collected before treatment and at the time of progression (i.e., when the treatment is no longer working).

The ability to detect cancer from a blood draw or urine sounds like science fiction. However, it is now possible to analyze DNA that is shed from tumor cells into the bloodstream. So-called "liquid biopsies" offer several advantages over conventional tumor tissue biopsies: (1) they can be performed as part of a routine blood collection; (2) they are less invasive and pose less risk to patients; and (3) they can be performed at multiple timepoints. Until recently, tools to perform epigenetic tumor analysis using liquid biopsies were lacking. To overcome this barrier, we have developed several innovative tools to study the epigenetics of mPC by analyzing tumor DNA circulating in the bloodstream.

Our objective is to utilize these exciting new tools for blood-based analysis of tumor epigenetics to develop liquid biopsy tests to optimize treatment decision-making for men with mPC and generate novel insights into epigenetic factors that drive treatment resistance. In Aim 1, we will develop a liquid biopsy test to detect epigenetic subtypes of mPC that, when identified, provide additional treatment options for patients. In Aim 2, we will develop a liquid biopsy test to identify men most and least likely to benefit from Abiraterone and Enzalutamide (the two most frequently used drugs in mPC) and determine how tumors change their

epigenetic landscape to become resistant to these drugs. Aim 3 has a similar goal to Aim 2, but focuses on Lutetium-PSMA, the most recent life-prolonging drug to be approved for men with mPC.

These aims will make substantial contributions towards defining the biology of and developing new, effective treatments for mPC. In the short term (2-3 years), this work will lead to blood-based tests to personalize care for men diagnosed with mPC, facilitating earlier use of effective treatments and minimizing exposure to toxic treatments that are unlikely to improve quality of life or prolong survival. It is critical to note that the blood samples required for this technology can be collected at any hospital – VA, community, or academic center – thus democratizing the availability of this powerful method to all patients. In the long-term (5-10 years), we believe that novel insights into how mPC adapts to develop treatment resistance by changing its epigenetics will identify tumor vulnerabilities and inform future efforts to develop new, effective PC drugs.

It is no longer science fiction that a blood draw can provide important information about cancer. We have assembled a team of dedicated clinicians, researchers, and computational biologists to use this revolutionary technology to meaningfully impact care for patients diagnosed with and treated for metastatic prostate cancer.

Proposal Title:	Study of the Role of EZH2 in the Regulation of Macrophage Polarization in Prostate Cancer Tumoral Microenvironment
Log Number:	PC220182
Current PI Name:	Beatriz German Falcon
Award Number:	HT9425-23-1-0304
Current Contracting Organization:	Cedars-Sinai Medical Center
Current Performing Organization:	Cedars-Sinai Medical Center
Web Approval Date:	10-02-2023

Scientific Objective and Rationale: Despite the advances in treatment options, metastatic prostate cancer (PCa) is the second leading cause of cancer-related deaths in men across the U.S. and Europe and remains incurable. Solid tumors, such as PCa are composed by neoplastic and non-neoplastic cells, including immune cells, and it has been widely reported that the tumoral microenvironment (TME) is an important regulator of primary tumor growth and metastatic spread to distal sites. Unfortunately, despite the introduction of immunotherapies like checkpoint inhibitors has changed cancer patient care, metastatic, and lethal PCa remains one of the poorest responders to these strategies. Therefore, activation of the immune system towards prostate tumor cells remains an attractive treatment strategy. Tumor associated macrophages (TAMs), the principal immune components of PCa TME, represent a major barrier for immunotherapy efficacy in PCa patients and there is a compelling need for understanding the mechanisms implicated in their polarization to improve the therapeutical arsenal available in clinic. Recently, the lab reported that the inhibition of the enhancer of zeste homolog 2 (EZH2), overexpressed in PCa patients, in combination with PD-1 treatment has significant therapeutic benefit. Specific to this application, they observed the reprogramming of TAMs from a tumor immunosuppressive phenotype to an anti-tumor phenotype following EZH2 inhibition. While the activity of EZH2 in TAMs has been reported, the importance of these processes and the exact mechanism/s whereby EZH2 reprograms TAMs has not been extensively studied in PCa. Further, given the potential of chimeric antigen receptor (CAR) mediated immunotherapy and the association of the PCa TME with TAMs, we propose to provide proof-of-concept for the use of CAR-iMacs to prevent PCa progression.

Applicability of Research: The proposed research project aims to help patients in two ways. First, will deliver a deeper molecular understanding for the epigenetic regulation of TAMs polarization in the development of an immunosuppressive TME which promotes lethal PCa stages and secondly generating and validating novel immunotherapy treatment strategies in preclinical models.

My Goals in Prostate Cancer Research: It is my goal to become an independent PCa researcher, to contribute to our understanding of lethal PCa development, and to generate novel and effective therapeutic strategies that can be translated successfully to the clinic. My proposal draws on previous work from our laboratory that highlights key role of the inhibition of epigenetic regulators to potentiate the efficacy of the check point inhibitor, anti PD-1 therapy, in PCa mouse models. Given my own experience with in vivo models of PCa, the study of different immune cells presented in the TME and the significant translational research expertise of my mentors, Drs. Leigh Ellis, Edwin Posadas, and Saul Priceman, I believe that I am well placed to pursue and achieve the goals of the proposed research. Further, the opportunity to pursue this project as a postdoctoral fellow at the Cedars Sinai Medical Center offers me an invaluable opportunity for professional development in the field of PCa research. I will also extend my knowledge of epigenetics and the generation of models of PCa that will add depth and breadth to the cancer genetics research that I conduct, as well as clinical relevance that will improve the chances of the clinical translation of my findings. I believe this training opportunity will allow me to make an impactful contribution to the field and demonstrate my potential as an independent researcher.

Contributions of This Study to Prostate Cancer Research: Significant progress has been made in characterizing the molecular tumor landscape that leads to lethal PCa; however, translation of these findings into clinical outcomes for patients has been limited. In the proposed research, we will study the mechanisms implicated in the TAMs polarization with are implicated in the development of resistant and lethal PCa. Our success will drive the PCa field forward, and significantly alter clinical management of patients with PCa. With highly relevant preclinical models and epigenetic targeting strategies used in combination with immunotherapy approaches proposed in this application, we anticipate the results of our work could lead to clinical trials in patients within 5 years.

Proposal Title:	Therapeutic Targeting of a Novel Tumor-Intrinsic Signaling Pathway for Lethal Prostate Cancer
Log Number:	PC220186
Current PI Name:	Hongwu Chen
Award Number:	HT9425-23-1-0302
Current Contracting Organization:	California, University of, Davis
Current Performing Organization:	California, University of, Davis
Web Approval Date:	07-11-2023

Most patients with prostate cancer disease are treated with androgen deprivation therapy or so-called androgen receptor signaling inhibitors (ARSIs) such as Xytiga (abiraterone), Xtandi (enzalutamide), or Casodex (bicalutamide). It is now known that, after prolonged treatments with the aforementioned drugs, tumor cells evolve or change from initially one cell type (called luminal) in the treatment-naïve tumors to other cell types such as cancer stem-like cells (CSCs) or neuroendocrine (NE) cells, something referred to as tumor plasticity. Studies also have strongly suggested that such tumor plasticity is the main reason why tumors become resistant to the different therapeutics. However, although recent studies revealed several important protein factors that mediate the plasticity and resistance, they yielded few opportunities for effective therapeutic targeting, as most of the factors or mechanisms that are therapeutically untargetable. Our recent study identified a membrane receptor ADR protein as a strong candidate driver for promoting tumor plasticity and ARSI resistance. As a receptor protein, it binds to small molecule with distinct chemical structures and is thus highly attractive for drug development. Indeed, drug candidates targeting this ADR are at clinical trials for Alzheimer's and other neurological diseases. We found that small molecule inhibitors of this ADR developed by us and others displayed high efficacy in inhibition of growth of several metastatic, castration-resistant prostate cancer (mCRPC) and ARSI-resistant cell and tumor models, including a patient-derived xenograft (PDX) tumor.

Our objectives are (1) to firmly determine its role in driving tumor plasticity and therapy resistance; (2) to demonstrate that its small molecule inhibitors, either alone or in combination with the current ARSIs, are highly efficacious and safe in blocking tumor growth and metastasis and thus effectively delaying the emergence of lethal form; and (3) to address for clinical relevance and help identify suitable cohorts of patients for future treatment, we will develop markers and assays.

One short-term applicability of this study (once this study is completed) is nomination of an adrenergic receptor (ADR) inhibitor/drug candidates such as the ORM compound for clinical trials for treatment of androgen receptor signaling inhibitor (ARSI)-resistant prostate cancer patients and patients with high risk of developing resistance. Our results from prostate cancer cells showed that the ORM drug candidate, which is currently at phase II trial for Alzheimer's disease, is highly effective in killing ARSI-resistant cells in cell culture. We are confident that the ORM drug candidate will be highly effective in treating ARSI-resistant tumors in our preclinical models including ones derived from patients' tumors (PDXs). With the ORM drug candidate at a phase II trial with a favorable safety profile, it can be quickly moved by us or others to phase 1 /2 trials for prostate cancer patients when our study establishes the safety and efficacy of the drug candidates.

Another short-term applicability of this study is that the markers (i.e., the ADR and its controlled genes and proteins in the prostate cancer tumors) and assays developed by this study will be highly valuable for selection of patients for the clinical trial with drug candidates targeting the ADR.

One long-term applicability (hopefully in the next 5-8 years) is to develop highly effective and safe drugs that selectively target the ADR hyper-signaling in lethal prostate cancer. The candidate compound we

developed has much improved potency than the ones from pharma in killing advanced prostate cells and tumors in a limited number of preclinical models. This candidate drug compound is identified using the prostate cancer cells where the ADR functions in a unique way that is different from its normal function and is thus more selective in targeting the ADR in prostate cancer tumors, which will likely increase its therapeutic window for its use as a cancer drug.

Patients who will likely benefit from the outcomes of this study will include ones who have already developed resistance to or are at high risk of developing resistance to the current ARSI drugs such as enzalutamide/Xtandi, abiraterone/Zytiga, and their related drugs. We expect that the treatment will effectively delay the resistance and thus disease relapse in the high-risk patients and block the growth and metastasis of the resistant tumors.

Based on the short-term and long-term applicability and the objectives described above, our study will make significant contributions to solutions to the PCRP challenge, “Develop treatments that improve outcomes for men with lethal prostate cancer.” As our study will also provide new scientific/conceptual insights for better understanding of the disease progression to the lethal forms, our study will also directly address the challenge, “Define the biology of lethal prostate cancer to reduce death.”

Proposal Title:	Defining Androgen Receptor Function and Disease Dependencies in BRCA1 /2-Deficient Prostate Cancer
Log Number:	PC220188
Current PI Name:	Nicole Traphagen
Award Number:	HT9425-23-1-0910
Current Contracting Organization:	Dana-Farber Cancer Institute
Current Performing Organization:	Dana-Farber Cancer Institute
Web Approval Date:	10-02-2023

Objective and Rationale: Mutations in the genes BRCA1 and BRCA2 are common in prostate cancer and are associated with poor response to treatment and progression to the incurable form of disease. These mutations can be inherited ("germline" mutations) or acquired by prostate cancer cells as the disease progresses ("somatic" mutations). Most prostate tumors rely on androgens and the androgen receptor (AR) for tumor growth, so blocking AR is the foundation of prostate cancer treatments; however, prostate tumors with mutations in BRCA1 and BRCA2 are often resistant to these treatments. Activated AR is a transcription factor that binds to DNA to regulate which genes are expressed within the cell. The sets of genes that AR regulates is dependent on many factors, including how the DNA is organized ("chromatin structure"). Both BRCA1 and BRCA2 have potential roles in regulating this DNA organization; therefore, our first objective is to determine how loss of BRCA1 and BRCA2 alters chromatin structure, and how this relates to changes in AR activity. PARP inhibitors can be used to treat tumors with BRCA1 and BRCA2 mutations, but most patients eventually develop resistance to this treatment as well. Although PARP inhibitors have been used mainly as a late-line treatment after prostate cancer progresses on anti-AR treatments, combination treatment with PARP inhibitors and anti-AR therapies is now being investigated as a first-line treatment for metastatic prostate cancer. PARP plays a role in the regulation of gene expression, and the DNA damage induced by PARP inhibition changes chromatin structure. It is not known how PARP inhibitor treatment alters AR function, and what treatments will be effective for prostate cancer patients who progress on this new combination therapy. Therefore, our second objective is to determine how PARP inhibitors alter AR function. Our third objective is to determine which genes are required for the growth of prostate cancer cells that have acquired resistance to PARP inhibitor and anti-AR combination therapy, and to validate these genes as potential therapeutic targets.

Research Applicability: This proposal addresses the PCRP overarching challenges to define the biology of prostate cancer progression to lethal prostate cancer to reduce death and develop treatments that improve outcomes for men with lethal prostate cancer. This work will help define how BRCA1 and BRCA2 mutations and PARP inhibitors alter AR function in prostate cancer cells. This is important to understand since most prostate cancer treatments are based on targeting AR. It may also provide direction for the development of new treatments for BRCA1 and BRCA2 mutant prostate cancer. This study will also identify the therapeutic vulnerabilities of prostate cancer cells which have acquired resistance to PARP inhibitor and anti-AR combination treatment. Since nearly all patients treated with this combination treatment will eventually experience disease progression, this study will provide important insight into which treatments will be effective for these patients.

Principal Investigator Career Goals: My career goal is to join the faculty at a cancer research center, where I plan to run a research program focused on androgen receptor function and resistance to anti-AR treatments in prostate cancer. The resources provided by this award will allow me to develop skills in generating and analyzing chromatin-based data and genome-wide screens, which will be crucial to my development as an independent prostate cancer researcher. My mentor, Dr. Myles Brown, is widely recognized as a leader in the field of AR biology and chromatin structure in prostate cancer, and he has trained >30 previous

postdoctoral fellows who have gone on to successful careers as independent researchers. As part of the Brown lab, I will have the opportunity to connect with other prostate cancer researchers as part of the Dana-Farber/Harvard Cancer Center Prostate Cancer Program and take part in the many research seminars and career development workshops offered by Dana-Farber.

Proposal Title:	Targeting Lipid Kinase PIKfyve in Neuroendocrine Prostate Cancer
Log Number:	PC220193
Current PI Name:	Yuanyuan Qiao
Award Number:	HT9425-23-1-0084
Current Contracting Organization:	Michigan, University of
Current Performing Organization:	Michigan, University of
Web Approval Date:	10-02-2023

Rationale, Objective, and Aims of the application: Treatment decisions and prognoses vary across cases of prostate cancer depending on many factors. For instance, prostate cancer confined to the prostate is less aggressive and can be treated with surgery or radiation. More advanced prostate cancer often requires a protein called the androgen receptor (AR) to fuel cancer cell growth and survival, and patients in this setting are prescribed androgen deprivation therapy (ADT) to inhibit AR activity or reduce levels of circulating androgens. However, many patients progress on ADT to a state known as castration-resistant prostate cancer (CRPC). Since AR remains a major driver of CRPC, additional AR inhibitors (e.g., enzalutamide and abiraterone) were developed to decrease remaining AR activity in CRPC. Although these drugs improve CRPC survival, they are not curative, and CRPC cells can develop further mechanisms of resistance. One such type of prostate cancer that can develop from CRPC AR inhibitor treatment is called neuroendocrine prostate cancer (NEPC). NEPC often has no or little AR expression, and the only standard therapy option for NEPC is chemotherapy (e.g., cisplatin). Although many NEPC patients initially respond to chemotherapy, resistance invariably occurs after a short time, and the disease remains incurable. Thus, there is an urgent need to develop new therapies to decrease mortality from NEPC.

Through preliminary studies, we have identified a promising new therapeutic target for NEPC called PIKfyve. Using cell lines and patient-derived xenografts (PDXs; tumors removed from patients that are then grown in mice), we found that NEPC is preferentially sensitive to PIKfyve inhibition with the clinical compound ESK981. PIKfyve is known to play essential roles in processes called autophagy and lysosome adaptation that are vital for cell growth and survival in oxygen-depleted (a state known as hypoxia) and nutrient-poor environments, both being settings found in NEPC. We, therefore, hypothesize that PIKfyve is indispensable for NEPC development and progression through maintenance of adaptive lysosome and nutrient recycling pathways under hypoxic and nutrient-deprived conditions, which occur from loss of androgen signaling.

Specific Aims: To test this, we propose three specific aims.

Aim 1. Determine the role of PIKfyve in NEPC progression. Our preliminary data suggest that NEPC develops an enhanced dependency on PIKfyve during progression from AR-dependent CRPC. We will use genetic and pharmacologic targeting methods in cell lines and animal models to ascertain whether PIKfyve is indeed required for NEPC development and proliferation.

Aim 2. Examine the mechanisms by which PIKfyve regulates cell survival in NEPC. The high proliferative rates of NEPC coupled with hypoxia and nutrient depletion requires a coordinated set of responses for tumor survival. We will delineate the pathways through which PIKfyve mediates survival in NEPC by inhibiting PIKfyve and examining activation of key players in nutrient survival pathways (e.g., mTORC1 and TFEB) as well as effects on oxygen consumption and stress on the cellular protein synthesis machinery.

Aim 3. Determine whether PIKfyve inhibition combined with standard of care therapies for NEPC and CRPC enhance outcomes in preclinical models. We will perform preclinical animal studies with multiple NEPC models treated with cisplatin, ESK981, or the combination to determine whether PIKfyve inhibition can enhance NEPC response to chemotherapy. Our preliminary data also found that ESK981 and AR

inhibitors can synergize in AR-dependent CRPC; thus, we will also employ animal models to determine whether combined ESK981 and standard of care enzalutamide decreases tumor growth of AR-dependent CRPC.

Clinical Applicability of the Research: This research addresses two of the FY22 PCRP Overarching Challenges:

1. Define the biology of prostate cancer progression to lethal prostate cancer to reduce death. NEPC is a particularly lethal form of CRPC, with only chemotherapy as an option to temporarily control the cancer. Our research will define the biological roles of PIKfyve in progression of AR-dependent CRPC to NEPC and in maintenance of NEPC cell survival. In addition to PIKfyve, these experiments may uncover other therapeutic targets to be developed for future precision oncology approaches to decrease NEPC lethality.

2. Develop treatments that improve outcomes for men with lethal prostate cancer. PIKfyve is a prime candidate to be therapeutically targeted in NEPC. Importantly, we have a clinical PIKfyve inhibitor, ESK981, that is ready for phase II trials, and our clinical colleagues (led by Dr. Elisabeth Heath) are ready to help quickly initiate future clinical trials with ESK981 based on our pre-clinical findings. Our data suggest that ESK981 will prevent tumor growth in NEPC patients, and our pre-clinical studies will provide data to support its use alone or in combination with standard chemotherapy. Additionally, AR inhibitors synergize with ESK981 in AR-positive CRPC cell lines, so it is likely that this will translate to enhanced therapeutic outcomes in future clinical trials of men with AR- positive CRPC. Together, our studies will credential PIKfyve inhibitors as promising new drugs to add to the limited therapies available for men with NEPC and ultimately reduce mortality from this devastating disease.

Proposal Title:	Targeting NUAK2 in Neuroendocrine Prostate Cancer
Log Number:	PC220197
Current PI Name:	Everardo Macias
Award Number:	HT9425-23-1-0125
Current Contracting Organization:	Duke University
Current Performing Organization:	Duke University
Web Approval Date:	05-01-2023

Neuroendocrine prostate cancer is an aggressive and lethal subtype of prostate cancer. While neuroendocrine can develop from the onset as the primary lesion, it is rare. More commonly, neuroendocrine prostate cancer develops as a resistance mechanism in heavily treated, late-stage, metastatic, castrate-resistant prostate cancer referred to as treatment emergent neuroendocrine prostate cancer. This is due to heavy and earlier use of Androgen Receptor-targeted therapies such as enzalutamide. It is estimated that 20-25% of late-stage prostate cancer patients will develop neuroendocrine prostate cancer. Neuroendocrine prostate cancer patients often present with high metastatic burden, and the prognosis is poor, with a 5-year survival rate of less than 20 percent. There are currently limited treatment options for neuroendocrine prostate cancer aside from platinum-based chemotherapies. Thus, new actionable molecular targets to combat neuroendocrine prostate cancer are urgently needed. Our published and new preliminary data propose that NUAK Family Kinase 2 (NUAK2) is a highly actionable protein that can be exploited as a target for neuroendocrine prostate cancer therapy.

We found that NAUK2 expression progressively increases as prostate cancer becomes more and more advanced. In patient-derived preclinical models, the expression of NUAK2 was most elevated in neuroendocrine prostate cancer subtypes. Our preliminary experiments show that genetic or pharmacological targeting of NUAK2 in neuroendocrine prostate cancer cells slowed their growth rate. The objective of our proposal is to test the potential of NUAK2 as a therapeutic target for neuroendocrine prostate cancer. NUAK2 is from a class of enzymes called kinases, and the scientific community has been very good at making medicines that block the activities of these type of proteins. Indeed, we have reported use of an investigational NUAK2 inhibitor to slow the growth of prostate cancer in mice with no observable toxicities. We now aim to test this compound in the more advanced and lethal prostate cancer subtype. We have also identified compounds that are already FDA-approved and in clinical trials that bind NUAK2 very potently and slow neuroendocrine prostate cancer cell growth in preliminary studies. We propose to exploit these clinical drugs for their beneficial NUAK2 off target activity. If our studies are successful and our hypothesis is proven, progression to clinical applications should occur quickly. These studies may greatly benefit patients diagnosed with lethal neuroendocrine prostate cancer.

Proposal Title:	Mechanisms Controlling the Progression of Prostate Adenocarcinoma to Neuroendocrine Cancer
Log Number:	PC220202
Current PI Name:	Jane Johnson
Award Number:	HT9425-23-1-0285
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	07-12-2023

This project directly addresses the FY22 PCRP Overarching Challenge to “Define the biology of prostate cancer progression to lethal prostate cancer to reduce death.” It will contribute to our understanding of how neuroendocrine prostate cancer forms and will test multiple molecular pathways as vulnerabilities for future therapies targeting this form of prostate cancer. Neuroendocrine prostate cancer (NEPC) is an aggressive form of prostate cancer that may arise de novo (less than 1% of prostate cancer), or it may arise in patients previously treated with hormonal therapies for prostate adenocarcinoma as a mechanism of resistance. The latter is an emerging clinically relevant problem since so called therapy- emergent neuroendocrine prostate cancer (tNEPC) is a lethal disease with a median overall survival of less than 1 year from the time of detection. Given the aggressive clinical course and poor outcomes of tNEPC patients, it is essential to better understand the biological basis for the progression from prostate adenocarcinoma to lethal tNEPC. Identifying the molecular drivers of the progression from an adenocarcinoma to a neuroendocrine cancer will provide new strategies for development of therapeutic agents to block the progression and/or growth of the cancer.

Development of new therapeutic approaches to tNEPC is severely limited by a lack of molecular insight into factors required for the transition of non-neuroendocrine tumors to the neuroendocrine identity. This project provides development of a new mouse model to be used to gain biological insights and identify vulnerabilities in tNEPC. In doing so, new opportunities for therapeutic intervention will be identified, which is critical for changing the mortality curve for therapy- resistant prostate cancer. We will leverage our knowledge of key molecular drivers of neuroendocrine lineages in normal development and disease and our expertise with genetically engineered mouse models to gain access to this understudied area.

The first aim of this project will be to identify the types of cells that transition to tNEPC using newly developed genetically engineered mouse models to study this process in the intact animal. We will use these same mouse models to test whether a factor that is known for its function in controlling the generation of neuroendocrine cell types in multiple organs during normal development is required for the transition of prostate cancer to NEPC. Finally, we will explore the role of a signaling pathway that connects molecular information from outside the cell to changes in the nucleus that may be required for the aggressive characteristics of tumors with neuroendocrine features. The importance of the latter aim is that the signaling pathways comprise the largest multigene family of known drug targets and, thus, provide potential therapeutic implications for future exploitation.

The anticipated near-term impact of this program includes providing much-needed new models to study the progression of prostate adenocarcinoma to a neuroendocrine tumor in an intact system to probe key molecular vulnerabilities. These advances will provide a foundation for future research projects to develop therapeutic strategies directly targeting identified vulnerabilities that will enable progress towards eliminating death from prostate cancer and enhancing the well-being of all the men and their families who are experiencing the impact of the disease.

Proposal Title:	Targeting Oncogenomic Function of N-Myc to Inhibit Neuroendocrine Prostate Cancer Visceral Metastasis
Log Number:	PC220217
Current PI Name:	Qianben Wang
Award Number:	HT9425-23-1-0072
Current Contracting Organization:	Duke University
Current Performing Organization:	Duke University
Web Approval Date:	01-27-2023

Cancer cells and normal cells from the same patient have long been recognized as genetically different. Medical research has studied these differences to identify genetic vulnerabilities that are unique to cancer cells. Unfortunately, the search for new medicines to attack these genetic vulnerabilities has proven to be slow and difficult. Most existing cancer treatments are chemotherapies, and the process of finding small chemical molecules to effectively exploit a particular genetic vulnerability is technically challenging and frequently undermined by safety concerns. As a result, very few therapeutic options exist to treat the most advanced and lethal forms of prostate cancer, and additional treatment options are badly needed for patients whose cancer has spread to a critical organ such as the liver or lungs.

The goal of this proposal is to develop a new treatment to attack a genetic vulnerability that is particularly found in an extremely aggressive form of prostate cancer known as neuroendocrine prostate cancer (NEPC), which frequently spreads to the liver or lungs. The ability of NEPC cancer cells to spread to the liver and lungs and survive in those locations appears to rely upon a gene known as MYCN, which encodes the N-Myc protein that belongs to a key group of molecules called transcription factors (TF). TFs can dramatically influence cellular behavior by binding to DNA itself and controlling the use of genetic information, but how N-Myc binds to DNA and controls the process of metastasis is largely unknown. This proposal describes a plan to discover in detail how N-Myc regulates the use of genetic information and enables the metastasis of NEPC cells to the liver or lungs using a combination of cutting-edge approaches, including CRISPR/Cas13d RNA-targeting technologies and high throughput genome sequencing.

Traditional efforts to develop chemotherapies to inactivate TFs that promote cancer have repeatedly failed, leading to the reputation that TFs may be “undruggable.” We propose to develop a non-traditional form of CRISPR/Cas13d-based “gene therapy” to block the ability of NEPC cells to produce N-Myc. The CRISPR /Cas13 RNA therapy will be packaged into the center of tiny delivery capsules called nanoparticles that can travel through the bloodstream and deliver the therapy to prostate cancer cells that have spread to the liver or lungs. However, it will be absolutely critical to deliver the therapy directly to cancer cells in those organs, avoiding accidental delivery to nearby healthy cells or other unaffected organs. Technological breakthroughs in the nanoparticle field have now made it possible to accomplish these goals more effectively than ever before. The nanoparticles containing the CRISPR/Cas13d therapy must be surrounded with an outer coating or capsule during intravenous or oral administration to the patient. Researchers have recently discovered two modified recipes for this outer coating that can steer the nanoparticles towards either the liver or lungs, and away from other organs where therapy is not needed. At the same time, this capsule can be coated with a specially designed material that attracts them to lethal prostate cancer cells, helping them physically attach and deliver maximum therapeutic benefit to the patient, with minimal side effects. Our preliminary studies indicate that this strategy appears to be both effective and safe. Interestingly, these preliminary studies have also found that this gene therapy can sensitize NEPC cells to platinum-based chemotherapy, enhancing the effectiveness of an existing treatment. In this proposal, we propose to conduct more extensive studies to rigorously test both efficacy and safety of our gene therapy and combined gene therapy/chemotherapy in multiple laboratory models of metastatic prostate cancer.

While the short-term goal is to provide a safe and effective new therapy to benefit patients with one particularly lethal form of metastatic prostate cancer, the larger strategy of using nanoparticles to deliver CRISPR/Cas13d RNA therapy is highly adaptable to other forms of prostate cancer. Thus, our findings will support the future development of new prostate cancer therapies as researchers continue to discover new genetic vulnerabilities of the disease. The proposed study will directly address the overarching challenges of the FY22 PCRP to “develop treatments that improve outcomes for men with lethal prostate cancer” and to “define the biology of prostate cancer progression to lethal prostate cancer to reduce death.”

Proposal Title:	Targeting Serine and 1-Carbon Metabolism for the Treatment of RB-Deficient Aggressive-Variant Prostate Cancer
Log Number:	PC220221
Current PI Name:	Daniel Frigo
Award Number:	HT9425-23-1-0424
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	10-02-2023

Rationale: Prostate cancers marked by the loss of the tumor suppressor RB (encoded by the RB1 gene) represent a highly aggressive, genetically defined cancer subtype that is largely incurable. Hence, there is a pressing need to identify novel therapeutic approaches for the treatment of RB-deficient prostate cancers. While prior work has focused on RB's role in cancer via its regulation of the cell cycle, emerging data from our group and others indicate that RB is also a major regulator of cancer cell metabolism. Using unbiased approaches to assess metabolism in preclinical models and patient cohorts, we identified the serine, glycine, and one-carbon pathway (SGOCP) to be upregulated following the loss of RB in prostate tumors. Biochemical and functional data from our laboratory demonstrate that the SGOCP is required for aggressive prostate cancer cell growth and survival. Further, RB loss sensitizes prostate cancers to the pharmacological inhibition of an enzyme called phosphoglycerate dehydrogenase (PHGDH), the first rate-limiting step of serine biosynthesis. These findings indicate that the SGOCP may represent a mechanistically alternative therapeutic target. To drive the development of new SGOCP-directed therapies, several issues still need to be addressed: (1) it is not clear why RB-deficient prostate tumors require increased SGOCP metabolism; (2) we do not know the best way to target the SGOCP (ex. there might be better ways than PHGDH inhibition); and (3) it is unknown whether targeting the SGOCP will sensitize aggressive prostate cancers to existing standard of care treatments. These issues (defining an optimal targeting strategy and understanding the fundamental biology) need to be addressed to evaluate any mechanistically new therapy to determine whether it can be safely and effectively administered to the correct patient population.

Objectives: The primary objective of this proposal is to characterize how increased serine and one-carbon metabolism caused by the loss of RB promotes prostate cancer progression. Further, we will rigorously test whether different parts of the SGOCP are bona fide therapeutic targets in preclinical models of RB-deficient prostate cancer. In addition, we will characterize whether this approach enhances the efficacy of existing drugs and assess whether there are potential side effects (positive or negative).

Aims: Aim 1 will evaluate the unrealized metabolic functions of RB loss in controlling the SGOCP in rigorous, preclinical models of advanced prostate cancer. Here, we will use new and established cell and mouse models that mimic different stages of the disease (RB-positive and RB-negative) and that will allow us to test whether genetic inhibition of PHGDH blocks disease progression. Aim 2 will test whether RB1 depletion drives epigenetic changes and lineage plasticity, drivers of therapy resistance, via the SGOCP. To do this, we will use integrated metabolomic, proteomic, and epigenomic approaches to link RB loss-mediated SGOCP activity to epigenetic modifications and changes in cellular identity. Finally, Aim 3 will evaluate the safety and efficacy of targeting the SGOCP at different points in the pathway. We will also test if a specialized diet can improve the efficacy of SGOCP inhibitors in validated models of aggressive prostate cancer.

Contributions to the Field and Patients: The development of any mechanistically novel therapeutic approach faces more barriers compared to the development of drugs that regulate old, established targets such as the androgen receptor (AR). In this regard, established targets are viewed as safer options for drug development

because there is validated biology with known side effects. As such, most new therapies focus on well-known drug targets. However, current targeted therapies, such as those targeting AR, are not curative in advanced prostate cancer and new AR-directed agents typically only improve patient prognosis by a matter of months over existing therapies. To truly make significant leaps in the treatment of advanced prostate cancer, we think that entirely new approaches/therapeutic targets need to be established. This study will provide the comprehensive validation needed to move SGOCP-targeted therapies towards the clinic and establish a roadmap for evaluating their effectiveness in advanced prostate cancer. Of note, several inhibitors of the SGOCP are already FDA-approved for the treatment of other cancer types and will be tested in this study. Positive results from this study stand to have near-term clinical benefit because it could lead to the repurposing of existing drugs for a genetically defined cohort of men with lethal prostate cancer that we propose would benefit greatly from this treatment modality.

Applicability of the Research: This study directly addresses the Overarching Challenges to develop treatments that improve outcomes for men with lethal prostate cancer and define the biology of lethal prostate cancer to reduce death. We will test a new therapeutic target for the treatment of an identifiable subtype of aggressive prostate cancers that are often refractory to all AR-targeted therapies. Our preclinical models will also test whether this new treatment approach can safely enhance the effectiveness of current standard of care treatments.

Proposal Title:	Characterization of a novel class of non-competitive androgen receptor antagonists
Log Number:	PC220238
Current PI Name:	Zhou Wang
Award Number:	HT9425-23-1-0295
Current Contracting Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Current Performing Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Web Approval Date:	10-02-2023

Castration-resistant prostate cancer (CRPC) is currently incurable and makes prostate cancer the second most common cause of cancer death in U.S. males. Second-generation androgen receptor (AR) targeting agents such as enzalutamide and abiraterone can prolong CRPC patient life for several months on average. CRPC develops resistance to these AR targeting agents, and AR reactivation is the major mechanism leading to the resistance. Thus, novel strategies to suppress reactivated AR in CRPC are urgently needed.

One strategy to suppress reactivated AR is to reduce AR level in the nuclei of CRPC cells, since a prerequisite for AR to function is its presence in the nucleus where it activates the expression of its target genes. We have developed a high-throughput screen to discover small molecules that can inhibit AR levels in the nuclei in CRPC cells. A screen of a library of ~220,000 small molecules identified two compounds that can reduce AR levels in the nuclei of CRPC cells. One of them can also inhibit ARv7, an AR splice variant that is insensitive to enzalutamide or other FDA-approved AR targeting agents. This compound and its analogs are well tolerated and can be used via oral gavage in animals in preclinical studies. Preliminary studies also showed that this AR antagonist and its analogs are effective in the inhibition of CRPC, including enzalutamide-resistant CRPC.

A major goal of our research is to develop more potent analogs of this novel class AR antagonists with improved physicochemical properties and bioavailability that can be used as a single agent or in combination with enzalutamide for the treatment of CRPC. However, it is not yet clear how this class of novel AR antagonists binds to AR, which limits the discovery of more potent analogues. The proposed structural and functional analysis of this novel class of AR antagonists will greatly facilitate the rational development of more potent analogues that may lead to a new therapy for CRPC.

This multidisciplinary project will (1) determine how this class of novel non-competitive AR antagonists bind to AR using structural and other analytical techniques, (2) design, synthesize and analyze more effective drug-like analogues, and (3) determine the efficacy of promising new analogs, and their synergy with enzalutamide, to inhibit CRPC tumor growth.

The success of this project will provide a strong foundation and guidance for future clinical studies of this class of novel AR antagonists in patients with CRPC, as a single agent or in combination with enzalutamide. Thus, the proposed project will address the following FY22 PCRP Overarching Challenge, “Develop treatments that improve outcomes for men with lethal prostate cancer.”

Proposal Title:	Interrogating the Intersection of PSMA and PI3K Pathway Signaling as a Novel Treatment Approach in Treatment-Resistant Prostate Cancer
Log Number:	PC220240
Current PI Name:	Marina Sharifi
Award Number:	HT9425-23-1-0164
Current Contracting Organization:	Wisconsin, University of, Madison
Current Performing Organization:	Wisconsin, University of, Madison
Web Approval Date:	07-10-2023

Scientific Objective and Rationale: Despite advances in treatment, metastatic prostate cancer is a lethal disease that remains the second leading cause of cancer-related death in men. Chemotherapy and hormone therapy are the most effective treatments for men with metastatic prostate cancer and can cause tumors to shrink, improve symptoms from the cancer, and help men live longer with the cancer. Unfortunately, these treatments are not effective for all men with metastatic prostate cancer, and even when they do work, every patient's cancer will eventually develop resistance to all therapies and become lethal. In recent years, researchers have identified multiple new targeted therapies that can be effective in metastatic prostate cancers after hormonal therapy stops working. These therapies target specific molecular changes in a patient' s cancer cells, leading to fewer side effects compared to chemotherapy. One type of therapy takes advantage of DNA changes present in more than 50% of metastatic prostate cancers, which can cause abnormal activation of a protein known as phosphatidylinositol 3- kinase (PI3K). Another type of therapy takes advantage of a protein called prostate-specific membrane antigen (PSMA), which is found in high levels on the surface of prostate cancer cells. While these both represent additional non-chemotherapy options for metastatic prostate cancer, each therapy is effective in less than half of patients who have the target, and even when they work, a cancer will inevitably develop treatment resistance, usually over the course of months. Data from prostate cancer cell lines in the lab suggests that these two proteins work together in ways that could promote treatment resistance, so combining treatments may be more effective for some patients than either one alone. However, the tests that we currently use to identify which individual patient would benefit from one of these therapies alone, both together or through a completely different approach, are not very effective. Because of this, patients may be exposed to side effects from a drug that will not control their cancer.

We have taken advantage of the fact that cancer DNA and cells can be isolated from a simple blood draw in patients with metastatic cancer, sometimes called a "liquid biopsy," to develop a unique set of tests to measure the activity of these proteins and related signaling pathways in cancer cells in the blood of metastatic prostate cancer patients, and through the incredible generosity of our patients, we have found differences in protein activity that could explain why some patients respond to each drug and some do not and could potentially predict which treatment approach will be effective for an individual patient. The goals of this project are (1) to use our liquid biopsy blood tests to determine which patients will benefit from each type of therapy on its own, and to understand why some patients benefit and some do not and (2) to use prostate cancer cells grown in the lab as well as liquid biopsy tests in prostate cancer patients to understand whether combining these PI3K and PSMA targeted therapies will increase the effectiveness of these therapies and whether we can use the liquid biopsy blood tests to determine which patients will benefit from the combination of both therapies.

Applicability: If successful, this project will help men with metastatic prostate cancer, who are at the highest risk of developing therapeutic resistance and dying from prostate cancer, through the development of a new combination therapy approach to overcome PI3K- and PSMA-targeted therapy resistance, with integrated

liquid biopsy testing to select patients most likely to benefit, increasing effectiveness and reducing unnecessary exposure to side effects from ineffective therapies. In addition to directly helping these patients, this research will contribute to the field of prostate cancer research by increasing our understanding of the mechanisms underlying the development of treatment resistance for these therapies, which will allow us to identify additional ways to target and overcome treatment resistance. We anticipate that the research from this project will translate into new clinical trials for men with metastatic prostate cancer in 3-4 years.

Career Goals: My career goal is to develop treatment strategies to overcome treatment resistance and improve survival for men with metastatic prostate cancer. The plan we have developed will provide me with clinical and translational research experience and training under the mentorship of Dr. Lang, an accomplished genitourinary medical oncologist and physician scientist with extensive expertise in prostate cancer liquid biopsies; Dr. Zhao, a genitourinary radiation oncologist and physician-scientist with expertise in prostate cancer biomarker development; and Dr. Carver, an experienced urologist and surgeon-scientist focused on PI3K-targeted therapy resistance in prostate cancer. The skills that I gain through completion of this project will provide me with an excellent foundation for a career as an independent physician scientist treating prostate cancer patients in the clinic and developing new treatment approaches to improve outcomes for patients with metastatic prostate cancer.

Proposal Title:	Ecologically Informed Treatment of the Polyaneuploid Cancer Cell (PACC) State to Halt the Emergence of Castrate-Resistant Prostate Cancer
Log Number:	PC220242
Current PI Name:	George Butler
Award Number:	HT9425-23-1-0157
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	05-03-2023

Scientific Objective and Rationale: Cancer is lethal because tumors evolve resistance to all forms of systemic therapy. That is, after a series of attempts, cancer eventually “solves” the resistance puzzle. For prostate cancer patients, this is apparent by resistance to hormone therapy, marked by the development of castrate-resistant tumor growth. Yet, the evolutionary path that cancer follows to solve the resistance puzzle remains broadly undefined. Likewise, the ecological features that define the path to resistance are poorly understood.

Specifically, it is unclear whether uncertainty and volatility in oxygen availability accelerates the search for a resistant solution through the accession of a transient cell state, the Polyaneuploid Cancer Cell (PACC) state. The PACC state is accessed by cancer cells in response to stress (including in the tumor microenvironment or due to therapy) that is impervious to conventional therapeutics, e.g., hormonal therapy and chemotherapy. Once the stress subsides, the cancer cells can then exit from the PACC state and repopulate the tumor(s) within a treated patient, which is clinically observed as cancer recurrence following treatment failure. As a result, we hypothesize that, unless cells that enter the PACC state are eliminated, cancer will recur in treated patients and continue to lead to the deaths of men with prostate cancer.

The overarching goal of this proposal is to determine the effect of oxygen availability on the evolutionary path to castrate-resistant growth via accession of the PACC state and to test the effectiveness of a novel PACC-targeted therapy. In Aim 1, we will quantify the effect of evolutionary impact of changes in oxygen availability on the emergence of castrate-resistant growth via accession of the PACC state. In short, we will test whether continual fluctuations in oxygen availability accelerate the search for a resistant solution. In Aim 2, we will evaluate the use of a novel therapeutic approach in which we force cells to prematurely exit the PACC state, making them vulnerable once again to hormonal therapy. By achieving these aims, we will address two of the PCRP overarching challenges to define the biology of prostate cancer progression to lethal prostate cancer to reduce death and to develop treatments that improve outcomes for men with lethal prostate cancer. Furthermore, the findings from this project will be applicable to drugs that are already FDA-approved for other purposes and therefore have the potential for rapid translation and impact for high-risk patients.

Principal Investigator Career Goals in Prostate Cancer Research: I plan to eventually lead a multidisciplinary research lab to characterize the different evolutionary paths that drive the emergence of lethal and incurable metastatic prostate cancer and to identify vulnerabilities that can be exploited with targeted therapies. My Ph. D. in Mathematical Biology focused on understanding the individual traits that make a cell likely to successfully metastasize. My postdoctoral work builds upon my quantitative background while also honing my practical cell and molecular biology lab skills to understand how individual cellular traits and behaviors evolve to drive the emergence of lethal cancer. Combining my unique mathematical background in single cell modeling with my postdoctoral research in prostate cancer therapy resistance provides me with a powerful skillset to build a successful multidisciplinary research program centered around understanding the

evolutionary dynamics of prostate cancer resistance. In addition to rigorous training, I have developed a bespoke individual Researcher Development Plan that includes specific training in translation research (e.g., attending weekly Tumor Board meetings at Johns Hopkins), research leadership and laboratory management (e.g., involved in “go/no-go” decisions regarding the continuation of low-productivity projects within the wider lab), and establishing productive collaborations with expert scientists at John Hopkins and at other national and international research institutions to accelerate research discovery. This, together with the excellent guidance from my world-renowned mentors, Drs. Sarah R. Amend and Robert Gatenby, will leave me poised to lead my own research group and solve the outstanding questions in lethal prostate cancer.

Proposal Title:	Determining the Contribution of Lineage Intermediate Tumor Cells During Progression to t-SCNC
Log Number:	PC220255
Current PI Name:	David Mulholland
Award Number:	HT9425-23-1-0186
Current Contracting Organization:	Icahn School of Medicine at Mount Sinai
Current Performing Organization:	Icahn School of Medicine at Mount Sinai
Web Approval Date:	07-07-2023

Targeting of the androgen receptor (AR) provides significant benefits for individuals with localized and metastatic prostate cancer. However, an increasing clinical observation is the prevalence of prostate cancers that have adapted to the continued suppression of AR expression and function. Clinical examples of such adapted therapies include treatment related small cell neuroendocrine prostate cancer (t-SCNC) and prostate cancers with mesenchymal signatures. Since t-SCNC is poorly responsive to most standard-of-care therapies and has poor clinical outcomes, there is a need to model and understand how ADT (androgen deprivation therapy) induces the evolution of prostate adenocarcinomas to lethal disease subtypes that are no longer responsive to AR targeting agents. An emerging mechanism for the occurrence of neuroendocrine and mesenchymal tumors is through lineage switching or plasticity—a process whereby one cell type can take on features of another lineage or cell type. During this progression, we propose that tumor cells must transition through intermediate cellular phenotypes including the coordinate expression of luminal-epithelial and neuroendocrine or mesenchymal lineage markers. Intermediate subpopulations may be identified by unique transcriptional, epigenetic, and pathological signature(s). Thus, we propose that intermediate subpopulations may present a rate-limiting step that is required for cells destined to become neuroendocrine or mesenchymal. We have developed new patient-derived tumor models and lineage reporters in efforts to target and reprogram luminal-neuroendocrine, intermediate cell populations through hormone replacement therapy, genetic approaches, and novel small molecule inhibitor targeting repressive epigenetic events. Our studies will address the complex clinical challenge of treatment resistance and lethal disease.

Proposal Title:	Targeting the Unique Biology of Ductal Prostate Cancers Using Poly ADP-Ribose Polymerase (PARP) Inhibitor and Androgen Signaling Inhibitor Combination Therapy
Log Number:	PC220261
Current PI Name:	Weranja Ranasinghe
Award Number:	HT9425-23-1-0225
Current Contracting Organization:	Monash University
Current Performing Organization:	Monash University
Web Approval Date:	05-18-2023

Ductal prostate cancer (DAC) is an aggressive type of cancer occurring in up to 12% of all prostate tumors. When present in a prostate tumor, DAC causes a poor response to traditional treatments such as surgery, radiation, and hormonal treatment. Even in its early stages, DACs have properties similar to very advanced prostate cancers causing early spread (metastasis) into organs such as the lung and are almost universally fatal. Therefore, understanding the cellular pathways driving these ductal cancers is crucial in treating this disease. Dysfunction of the DNA damage repair pathway in particular is seen in approximately half of the patients with ductal prostate cancer. A type of medication called PARP enzyme inhibitors has been used successfully in patients with other cancers (including prostate cancer) who have dysfunction of the DNA damage repair pathway. Our preliminary work has also shown that, when combined with newer-generation hormone therapies, these PARP enzyme inhibitors effectively kill DAC cells even when there is no dysfunction of the DNA damage repair pathways. Therefore, we hypothesize that targeted treatments with PARP enzyme inhibitors with newer-generation hormone therapies can improve the response of DACs.

Contributions of This Study to PCRP Overarching Challenges: No current therapies effectively treat these lethal DACs. This study will provide an understanding of its biology, identify any genes driving these cancers, and assess whether targeted PARP enzyme inhibitor therapy with hormone therapy can be used to treat these lethal cancers. If proven to be effective, these already FDA-approved treatments can help improve the effectiveness of surgery when treating patients with DAC and improve survival. A clinical trial for using these medications for DAC before surgery has already been planned and is awaiting this data so that it can be readily expedited at a large tertiary U.S. academic center that regularly treats patients with DAC.

Ultimate Applicability of the Research:

What Types of Patients Will It Help? Our research will benefit patients with DAC who are highly likely to spread and die from their disease.

How Will It Help Them? Our study will identify whether the targeted therapies of PARP enzyme inhibitors combined with newer generation hormone therapies are successful in treating patients with DAC.

Potential Clinical Applications, Benefits, and Risks. PARP enzyme inhibitors combined with newer-generation hormone therapies have already been successfully used in clinical trials. Therefore, if our results demonstrate that these therapies can effectively treat DACs, this will help start an already designed clinical trial where patients with DAC can receive these therapies before surgery to improve the outcomes of surgery and survival of the patients.

Projected Time to Achieve a Patient-Related Outcome. In 3 years, we will know whether using PARP inhibitors with hormone therapy can target DACs. Our collaborative large tertiary academic center has a history of rapidly translating scientific advances to clinical trials in men with DAC, so our results could lead to patient benefit soon after completion of our studies, should they prove successful.

Principal Investigator (PI) Career goals: The PI is a surgeon-scientist (urologist) who has expertise and has published high-impact papers in the field of DAC. He currently holds several academic, clinical, local, and national leadership roles and actively engages in prostate cancer consumer groups. His short-term academic goal is to build on this project to establish a fully functional scientific lab and a 10-year plan for achieving an international leadership role in urology with a professorial appointment at an academic center.

Research Plan: The primary mentor holds professorial appointments at two of the country's top eight universities and has more than 30 years of scientific research experience. The PI’s co-mentor is a urologist with a distinguished career in academic urology who was the president of a national urology society. These mentors have mentored ~100 students, including Ph.D.s, Master’s degree, and clinical fellows. Formal mentorship and professional development plans have been implemented to help achieve the PI's goals. These focus on (1) impactful, novel translational research with timelines and (2) running a large research program using leadership, people management, supervision of students, financial management, networking, commercialization opportunities, and awareness of critical legal and ethical issues. Through weekly meetings and mentorship plans, the mentors will offer the PI vast scientific, clinical, and leadership experience to ensure his milestones and goals are met. Upon completing this award, the PI will have a framework to develop into an independent investigator to achieve his goals.

Proposal Title:	Slow-Cycling Prostate Cancer Cells in Therapy Resistance, Disease Relapse, and Metastasis
Log Number:	PC220273
Current PI Name:	Dean Tang
Award Number:	HT9425-23-1-0163
Current Contracting Organization:	Health Research Inc., Roswell Park Division
Current Performing Organization:	Health Research Inc., Roswell Park Division
Web Approval Date:	05-18-2023

Likely Contributions of This Study to a 2022 Overarching Challenge: This project directly tackles the Overarching Challenge, “Define the biology of prostate cancer progression to lethal prostate cancer to reduce death,” and has the potential to identify novel a prostate cancer (PCa) cell “Achilles heel” for new clinical trials.

What Types of Patients Will It Help and How? This study is pertinent to treatment-naïve advanced and metastatic PCa patients and especially mCRPC patients. mCRPC still claims the lives of >32,000 Americans each year, representing a huge health care burden.

Why Is mCRPC So Lethal? Patients with advanced/metastatic PCa are treated with androgen receptor (AR)-targeting therapies such as androgen-deprivation therapy (ADT) and enzalutamide (Enza), as well as chemotherapeutic drugs such as docetaxel. However, therapeutic responses are generally short-lived, and tumors enter an “adaptation/dormancy” period with clinically defined minimal residual disease (MRD), and most treated PCa patients will eventually develop therapy resistance with recurrent CRPC in the prostate and, more frequently, in metastatic sites. This pattern of treatment response/MRD/tumor recurrence in PCa patients is remarkably similar to breast cancer patients treated with anti-estrogens/chemotherapeutic drugs. In the case of breast cancer, there is now strong evidence for a population of dormant slow-cycling cells (SCCs) to survive therapeutics, populate the MRD, and mediate disease recurrence and metastasis. However, in the case of PCa patients, it is generally unclear which PCa cells survive ADT and chemotherapeutic drugs and enter the adaptation/dormancy phase and which PCa cells come out of the MRD and mediate tumor relapse and metastatic dissemination. Here we propose that “Dormant (slow-cycling) PCa cells with prostate cancer stem cell (PCSC) properties preferentially survive treatments (ADT and chemodrugs) and mediate therapy resistance, tumor recurrence and metastatic dissemination,” which is also our overarching hypothesis for the current DOD IDA project.

This hypothesis was based on: (1) our >20 years of focused studies on various PCa cell populations that have implicated functionally defined stem-like PCa cells (i.e., PCSCs) in mediating therapy resistance, CRPC, and metastasis; (2) our observations that the PCSC pool is heterogeneous, with only a small subset of very dormant PCSCs being able to generate and propagate CRPC; and (3) our recent studies in a genetic mouse model demonstrating very dormant luminal epithelial progenitor cells being inherently castration-resistant. By overcoming technical hurdles, we have now developed a novel genetic mouse model called trigenic Hi-Myc that allows identification, purification, and prospective studies of LIVE PCa slow-cycling cells (PSCCs) from both intact and castrated mouse prostate. We propose to test our overarching hypothesis in the novel trigenic Hi-Myc tumor model (Aim 1) as well as in human PCa xenograft and PDX models (Aim 2).

What Are the Potential Clinical Applications, Benefits, and Risks? There are several potential clinical applications. For example, the “dormancy gene signature” derived from this study may be used in patients’ biopsies to help assess whether the patient on treatment has entered the “adaptation/dormancy” stage. The gene expression profiles identified herein to be specific to PSCCs may be utilized to gauge the probability of

when/whether the responsive tumors may eventually stop responding and mediate recurrence. In the long term, the “Achilles heel” of PSCCs identified in this study will likely be clinically actionable targets.

What Is the Projected Time to Achieve a Patient-Related Outcome? We estimate it to be within 5-7 years. This estimation is based on the timeline of several of our lab studies that have been translated to novel clinical trials (e.g., NCT03751436).

What Are the Interim Outcomes? By integrating the studies in the two Aims, we expect to obtain the most updated and comprehensive molecular portrait of the PSCCs and to greatly advance our understanding of these cells in PCa therapy resistance, relapse, and metastasis. Overall, the biological studies proposed herein will provide the most definitive evidence for PSCC involvement in mediating PCa dormancy and tumor relapse as well as metastasis.

Proposal Title:	Role of Oxidative Mitochondrial Metabolism in Resistance to Androgen Receptor Inhibition
Log Number:	PC220295
Current PI Name:	Andrew Goldstein
Award Number:	HT9425-23-1-0379
Current Contracting Organization:	California, University of, Los Angeles
Current Performing Organization:	California, University of, Los Angeles
Web Approval Date:	10-02-2023

Prostate cancer (PC) is the second leading cause of cancer death in American men. Various therapies for PC are available, such as androgen deprivation therapy, chemotherapy, radionucleotides, and immunotherapy. However, despite these therapies, PC often progresses and becomes lethal especially once it metastasizes. We hypothesize that a drug delivery system that efficiently and selectively targets metastatic PC will greatly improve PC management. In fact, [177Lu]Lu-PSMA-617, a novel drug that targets prostate-specific membrane antigen (PSMA), showed prolonged progression-free and overall survival in the VISION trial, suggesting that selectively delivering drugs to PC can be a powerful way to improve the outcome of PC therapy.

We recently discovered a peptide named CTK that appears to efficiently target metastatic PC. We identified CTK through an extensive in vivo phage display screen in mice bearing metastatic PC tumors. Our preliminary data show that CTK homes efficiently to PC, especially to metastatic PC in various sites, such as the bone (e.g., tibia, ribs, jaw), brain, and lymph nodes. In contrast, CTK has not shown obvious homing to pancreatic and breast cancer in mice, suggesting that it targets a molecule selectively expressed in PC. Here, we will characterize the CTK peptide in detail to study its potential as a drug delivery scaffold for metastatic PC therapy. We will further confirm the PC-specific homing of CTK in humanized mice that carry human PC in the presence of human immunity (huPC mice) to study CTK biodistribution under a translational setting. We will also search for the receptor(s) of CTK that determines the in vivo biodistribution of the peptide. To study the significance of CTK in PC therapy, we will perform a proof-of-concept treatment study using CTK-coated docetaxel (Doc) liposomes in the huPC mice. We will apply various techniques we have developed through the studies of the iRGD peptide, another tumor-homing peptide that we had identified through phage display and is now in clinical trials for pancreatic cancer therapy.

Specific Aims and Study Design:

Specific Aim 1. To study the biodistribution of the CTK peptide in humanized prostate cancer mice. We will develop huPC mice using a human PC cell line and patient-derived PC tissue. Humanized mice bearing human leukocyte antigen (HLA) matched with their human immune cells and PC cells will be used. We will first characterize the huPC mice by various means including tissue staining, flow cytometry, and RNA-seq. Fluorescein-labeled CTK and control peptides such as scrambled CTK will be injected into the tail vein of the huPC mice, and the biodistribution will be studied by macroscopic and microscopic fluorescence imaging.

Specific Aim 2. To identify the receptor(s) of the CTK peptide. Affinity chromatography coupled with mass spectrometry will be used to identify the receptor(s) of CTK expressed on the PC cells or in the PC microenvironment. We will then study the expression profile of the receptor(s) in the huPC tumors and a panel of patient-derived PC and normal tissue. To confirm the binding of CTK to the receptors, we will perform in vitro binding assays using purified receptor(s) and cells in which the receptor(s) was eliminated or forcefully expressed. The affinity between CTK and the receptor(s) will also be measured.

Specific Aim 3. To perform a proof-of-concept treatment study to assess the therapeutic efficacy of CTK-coated docetaxel liposomes in humanized prostate cancer mice. We will treat huPC mice using Doc-loaded liposomes coated with either CTK or a control scrambled CTK. The liposomes will be prepared using our original protocol. Therapeutic efficacy based on the primary and metastatic tumor burden and the potential side effects will be studied. Its effect on the immune microenvironment of the tumors will also be studied for potential future development of synergistic immunotherapies.

We envision that establishing a novel PC-targeting system will have a major near-term impact on PC research by providing a platform to develop enhanced PC therapies and diagnostics. Identifying the receptor (s) of CTK may help us uncover a novel mechanism of organotropic metastasis. The huPC mice will provide an innovative platform to study tumor immunity in PC, an area that requires further scientific attention. Long-term impact would include next-generation PC-specific therapies and immunotherapies that result from the use of the CTK peptide and from mechanistic studies of organotropic metastasis and immunomodulation in PC. We believe that the proposed project will provide a solution to the FY22 PCRP Overarching Challenges by facilitating “Development of treatments that improve outcomes for men with lethal prostate cancer.”

Proposal Title:	Tumor Immunotherapy by Gene-Circuit Engineered Response (TIGER) for Neuroendocrine Prostate Cancer
Log Number:	PC220301
Current PI Name:	Ming-Ru Wu
Award Number:	HT9425-23-1-0152
Current Contracting Organization:	Dana-Farber Cancer Institute
Current Performing Organization:	Dana-Farber Cancer Institute
Web Approval Date:	05-02-2023

Scientific Objective: Our goal is to develop a transformative new strategy - Tumor Immunotherapy by Gene-circuit Engineered Response (TIGER) for treating neuroendocrine prostate cancer (NEPC). NEPC is the most aggressive subtype of prostate cancer and usually occurs during hormonal therapy. TIGER forces tumors to recruit immune cells to kill primary tumors and metastasis. To achieve this goal, we will design artificial gene circuits activated explicitly in NEPC cells. These gene circuits will command NEPC cells to secrete immune modulators that attract immune cells to target the tumors for destruction. This effect will also induce long-term immune memory against tumor relapses. Here, we will develop, optimize, and validate the effectiveness of TIGER within in vitro and in vivo mouse models of NEPC.

Rationale: The immune system has been harnessed to treat a variety of blood cancers, including acute leukemia and multiple myeloma, via cell-based therapies. These strategies require isolation, engineering, and expansion of immune cells from each patient, which is expensive and labor-intensive. Furthermore, these approaches have not yet been applied successfully against NEPC, which poses additional therapeutic challenges due to the heterogeneity found in the tumors. Thus, there is an urgent need for novel, safe, and effective therapies.

We aim to develop novel therapies that act from within tumors to recruit and activate immune cells into tumors – a Trojan horse approach. Specifically, we will design genetic circuits that can be delivered locally or systemically, sense when they are inside cancer cells, and respond by producing combinations of complementary immune modulators from within tumors. These immune modulators will condition the tumor microenvironment to favor immune response, recruit immune cells into the tumors, thus harnessing the immune system to target NEPC and establishing long-lasting protection against metastasis and recurrent cancer. TIGER can be modulated and shut off if needed, thus providing controllable safety switches. Furthermore, TIGER does not require custom cellular engineering for every patient, thus enabling greater patient access and reduced burden on healthcare infrastructure. TIGER can also be used with other cancer therapies to achieve enhanced efficacy.

Aims: In Aim 1, we will engineer synthetic gene circuits to specifically express immunomodulators within tumors to recruit immune cells to kill tumors. We will validate the effectiveness of these gene circuits in vitro in NEPC patient-derived organoid models. We will also optimize TIGER to target heterogeneous tumors. In Aim 2, we will identify the optimal therapeutic output combination that confers the strongest efficacy. We will also determine the minimal percentage of cancer cells that need to be targeted by TIGER to achieve therapeutic efficacy in mouse models of NEPC. In Aim 3, we will elucidate the immune response triggered by TIGER. We will also test the ability of TIGER to eliminate primary and metastatic NEPCs in fully immunocompetent mice. Furthermore, we will optimize the capacity of TIGER to trigger immune memory to prevent tumor relapses. This work will establish key parameters needed for successful immunotherapy against NEPC and enable the optimization of designs for future preclinical and clinical trials.

Overarching Challenges: We aim to address the following FY22 PCRP overarching challenge - develop treatments that improve outcomes for men with lethal prostate cancer.

What Types of Patients will be Helped and How? This work will benefit NEPC and castration-resistant prostate cancer patients, especially ones with metastatic disease or cancer relapse, with a new and potentially powerful therapy.

What are Potential Clinical Applications, Benefits, and Risks? Our strategy has the potential to become a new clinical therapy for NEPC. The potential benefits of this technology include providing highly effective treatment for NEPC and protection against future relapse. The potential risks of this technology include the challenge of targeting heterogeneous solid tumors, although we have outlined a comprehensive plan to minimize this risk with a set of alternative strategies.

What is the Projected Time for Patient-Related Outcomes/Interim Outcomes? We anticipate that, by the end of the 3 years of this grant, we will have optimized our therapeutic circuits and validated their therapeutic efficacy in multiple mouse NEPC models. If successful, we anticipate that preclinical and clinical development can commence immediately after that, thus accelerating the time scale to impact patient health. If successful, this research will provide a novel therapeutic strategy for NEPC, with the potential to replace existing treatments that have toxicities and target metastatic and recurrent NEPC, which are major causes of mortality.

Proposal Title:	The Role of EZH2-ADAR1 Axis in Advanced Prostate Cancer
Log Number:	PC220305
Current PI Name:	Yang Yi
Award Number:	HT9425-23-1-0661
Current Contracting Organization:	Northwestern University, Chicago, Illinois
Current Performing Organization:	Northwestern University, Chicago, Illinois
Web Approval Date:	10-02-2023

Prostate cancer is one of the most common cancer types and ranks as the second leading cause of cancer-related death among American men. Although therapeutic strategies such as androgen deprivation therapy (ADT) has been successfully used to treat prostate cancer, the majority of patients still eventually relapse and result in dismal outcomes. To this end, it is of paramount importance to identify novel viable targets to help patients diagnosed with lethal prostate cancer go back to normal.

RNA editing is a widely conserved modification which could change the specific nucleotide of RNA transcript from one to another. The most prevalent type of RNA editing in humans is adenosine-to-inosine (A-to-I), which is catalyzed primarily by an enzyme named adenosine deaminases acting on RNA-1 (ADAR1). In addition to the RNA editing function, which is mainly restricted in the cell nucleus, ADAR1 protein could also translocate to the cytoplasm under stress and protect its interacting mRNAs from degradation. Cumulative evidence suggests that aberrant ADAR1 expression and A-to-I RNA editing profiles are implicated in prostate cancer, but the underlying mechanism remains unknown. Therefore, understanding the link between ADAR1 and prostate cancer and uncovering the mechanism by which ADAR1 is modulated in response to oncogenic signals during prostate cancer progression will contribute to define the biology of prostate cancer progression to lethal prostate cancer to reduce death.

Enhancer of zeste homolog 2 (EZH2) is a well-known enzymatic protein that has histone methyltransferase activity. Elevated expression of EZH2 has been reported in many solid tumors including prostate cancer. The unique roles of EZH2 in promoting cancer progression and metastasis make it a potential therapeutic target for cancer treatment. However, scientists found that targeting the enzymatic activity of EZH2 alone is ineffective in treating EZH2-dependent solid tumors. Instead, emerging findings including ours support the notion that EZH2 could promote cancer development through multiple ways that do not rely on its enzymatic activity. Hence, deeply investigating the multifaceted tumorigenic functions of EZH2 will shed new light on understanding the etiology of cancer.

Here, we report for the first time that, EZH2 plays novel enzymatic-independent roles in regulation of A-to-I RNA editing and RNA stability via targeting ADAR1. Our preliminary data suggested that EZH2 could directly interact with ADAR1 to modulate its preference to choose editing substrates. Consequently, the global A-to-I editing pattern in prostate cancer cells is largely re-shaped due to the upregulation of EZH2 to facilitate cancer initiation and progression. More importantly, we further suggested that EZH2 could keep the ADAR1 proteins stay inside the nucleus by promoting the translation of Transportin-1 (TRN1), a protein that is responsible for the transportation of ADAR1 from cytoplasm to nucleus. Therefore, depletion of EZH2 in prostate cancer cells will lead to the accumulation of cytoplasmic ADAR1 in prostate cancer cells, which subsequently prevents a number of ADAR1-bound mRNAs from degradation. Among the group of EZH2-mediated mRNAs, we identified Ataxia-Telangiectasia Mutated (ATM), which encodes a protein that helps cancer cells develop therapeutic resistance. Accordingly, combinational targeting of EZH2 and ATM using specific inhibitors could achieve a synergistic effect in killing prostate cancer cells.

In this application, we will build upon our novel preliminary findings to understand the role of EZH2 in modulation of ADAR1 during the progression of prostate cancer. Our proposed study will not only enrich our knowledge on the molecular mechanism by which EZH2 drives prostate cancer, but also shed new light

on the development of novel therapeutics to benefit prostate patients. To be specific, although the EZH2-targeting strategy has been utilized in a number of ongoing clinical trials for prostate cancer treatment, our preliminary data indicated a crucial side effect of this strategy: induces the export of ADAR1 proteins from nucleus into cytoplasm and adversely promotes the expression of prostate oncogenes such as ATM through extending their mRNA half-lives. Therefore, targeting both EZH2 and ATM may serve as a better therapeutic method than the currently used EZH2-single targeting strategy to improve outcomes for men with lethal prostate cancer. We expect that this project would take 2-3 years to achieve the aims, which will finally provide impetus to initiate a future phase I clinical trial testing combination therapy.

Proposal Title:	Studying Intratumor Heterogeneity in the Evolution of Metastatic Hormone-Naive Prostate Cancer to Castration Resistance After Intensified Hormonal Therapy
Log Number:	PC220307
Current PI Name:	Joaquín Mateo
Award Number:	HT9425-23-1-0209
Current Contracting Organization:	Vall d'Hebron Institute of Oncology
Current Performing Organization:	Vall d'Hebron Institute of Oncology
Web Approval Date:	05-23-2023

Metastatic prostate cancer (mPC) is the advanced form of prostate cancer, when the disease has spread to other organs. mPC growth is primarily dependent on androgens. Intense targeting of the androgen signaling pathway, combining anti-hormone injections and drugs targeting the androgen receptor protein in the tumor cell, is the new standard of care for men with newly diagnosed mPC. However, a lethal form of the disease ("castration-resistant") eventually emerges. We hypothesize that understanding the drivers of this resistance would be critical to further improve the outcome of men with newly diagnosed metastatic prostate cancer.

To do so, we plan to study biopsies acquired from mPC patients at initial diagnosis, once they have started therapy and after their disease has become resistant. We will use novel molecular techniques to assess the heterogeneity within the tumor, with regards to the emergence of resistance mechanisms by providing a map of how changes occur in different tumor areas. These techniques will also give us information on how the tumor can evade the immune system by co-mapping the expression of immune checkpoints and the presence of immune cells in the tumor and exploring differences between organs where prostate cancer spreads.

Some of these samples will come from men participating in a clinical trial that combines hormonal therapies with PARP inhibitors, a new family of drugs being investigated in prostate cancer. We have also generated tumor mice models using biopsies from these patients, allowing us to test different treatments in the same tumor; we will use these models to study in the laboratory how the tumor reacts when we give these treatments either together or sequentially, to better understand biological drivers of drug resistance.

Contributions to the PCRP Overarching Challenges: This project will contribute to improving our understanding of the biology behind progression to lethal metastatic castration-resistant prostate cancer. Moreover, we aim to support the development of new treatment combinations that can prevent, or at least significantly delay drug resistance, to improve outcomes of men with lethal prostate cancer.

What types of patients will it help and how will it help them? About half of men with metastatic prostate cancer present with distant metastasis at first diagnosis. These are the ones more likely to receive these therapies but unfortunately the tumor will relapse over time. They would be the first target population where a better understanding of resistance can be leveraged towards designing new therapies. Furthermore, this knowledge will also be applicable to treatments given to men in later stages of lethal prostate cancer.

What is the projected time it may take to achieve a patient-related outcome? We will be testing the impact of drug combinations that are already being used in clinical practice and/or in late-stage clinical trials, so we anticipate a rapid translation of our findings into clinical trials that can improve outcomes of men with prostate cancer at the completion of this 4-year project.

The Investigator: Joaquin Mateo is a prostate cancer physician-scientist who studies how mPC patients have distinct tumor molecular profiles and how these differences can guide personalized treatment selection. He has now launched his own laboratory, where he will carry out this project. He will be supported by co-mentors, Professor Johann de Bono, a world-renowned prostate cancer investigator, and Professor Josep Tabernero. They will ensure the applicant has all the necessary resources to conduct this project and provide scientific guidance and career-development support. Understanding intra-tumor heterogeneity and drug resistance is key to delivering precision medicine; this award would enable the applicant to expand his molecular testing and computational analysis skills and to consolidate his emerging independent group.

Proposal Title:	Feasibility of Establishing Community Living Labs to Improve Access to Prostate Health Resources, Education, Amenities, and Community Health (REACH) for Black Men
Log Number:	PC220312
Current PI Name:	Folakemi Odedina
Award Number:	HT9425-23-1-0110
Current Contracting Organization:	Mayo Clinic and Foundation, Jacksonville
Current Performing Organization:	Mayo Clinic and Foundation, Jacksonville
Web Approval Date:	10-02-2023

Objective and Rationale: According to the American Cancer Society, Black men are more likely to die from prostate cancer compared to any other racial or ethnic group. For example over 41,000 Black men will be told they have prostate cancer in 2022. Without any preparation, these men will abruptly begin a prostate cancer care and survivorship journey they never knew they would take before their diagnosis. The transition from prostate cancer diagnosis phase to survivorship can be mentally and physically trying, especially for those who lack emotional and financial support. It is thus important to have prostate health services that will support Black men wherever they are, in their communities. In this study, our goal is to make prostate health Resources, Education, Amenities, and Community Health (REACH) services available to Black men in their community.

Mayo Clinic Comprehensive Cancer Center (led by Dr. Folakemi Odedina, an experienced global cancer disparities scientist) has partnered with the American Legion Post 197 (led by Mr. Arnold Merriweather, a 12-year prostate cancer survivor and globally recognized prostate cancer advocate) to develop a community-based health system that we are calling Community Living Lab, or “CoLLab” for short. A CoLLab Learning Health System is an innovative community setting that provides health-related education and navigation services and includes research to be able to improve the services provided immediately and continuously. As with most of our projects, our research group in Florida focuses on answering the questions raised by our community. In this study, the primary questions that we want to answer are:

Are we able to establish Community Living Labs in highly accessible community sites, such as the American Legion Post?

Can the CoLLab sites improve access to prostate health resources, education, amenities and community health for Black Men?

What is the impact of the services provided at CoLLab sites on Black men’s prostate cancer-related and clinical trials-related knowledge, attitude, health beliefs, perceived control, intentions and cues to action?

Methods: Our long-term goal is to eliminate the prostate cancer morbidity and mortality disparities experienced by Black men through community-based and culturally appropriate interventions. For this IDEA award, we will:

1. Develop all the materials needed for the CoLLab Health System, design the program to meet the needs of Black men and conduct a preliminary assessment to ensure that the program is tailored to the community.

2. Set up the program at three American Legion Posts in Duval County (FL) and compare the health outcomes of participants at these Posts to an American Legion Post in St Johns County (FL).

3. Evaluate the impact of the program among 200 Black men and the impact on the community. We are proposing a 30-month exploratory study.

Applicability of the Research: Once we establish that the CoLLab Health System works well in supporting Black men's prostate health, we will continue to study the broad effectiveness in multiple American Legion Posts nationally. The American Legion is the nation's largest wartime Veterans service organization aimed at advocating patriotism across the United States through diverse programs that includes enhancing the well-being of communities. The American Legion has more than 12,000 posts in communities throughout the United States and over 200 in Florida. It is well positioned to support prostate health services and navigation for Black men.

Study Contributions: Compared to White men, Black men experience great disparities relative to prostate cancer incidence and deaths. They also experience disparities relative to prostate cancer survival. Closing the disparity gap requires an innovative way to address the problem of prostate cancer among Black men. More importantly, we need to have the solutions to these problems in the community as the problems exist in the community. We have chosen to leverage the outstanding partnership between the Mayo Clinic Comprehensive Cancer Center and American Legion Post 197 to find the best way to support Black men in Black communities. Our innovative application is relevant to the DoD Prostate Cancer research Program focus areas of "Improving quality of life" and "Advancing health equity." By providing immediate access to prostate health services in Black communities, the program will impact the prostate cancer care continuum, from prevention to survivorship in the following ways: increasing prostate cancer knowledge and awareness; influencing/reinforcing perceptions, beliefs, and attitudes; prompting action; and refuting myths and misconceptions. It will also assist Black men to make informed decisions about participating in clinical trials. In the long term, it will facilitate sustained change and help users to overcome barriers/systemic problems associated with prostate cancer. Overall, this study will contribute to the elimination of prostate cancer disparities experienced by Black men.

Proposal Title:	Regulation of Cellular Plasticity by Androgen Receptor Splicing Variant in Prostate Cancer
Log Number:	PC220339
Current PI Name:	Yun Qiu
Award Number:	HT9425-23-1-0142
Current Contracting Organization:	Maryland, University of, Baltimore
Current Performing Organization:	Maryland, University of, Baltimore
Web Approval Date:	05-15-2023

One in nine men will be diagnosed with prostate cancer in his lifetime. Understanding the mechanisms underlying regulation of the androgen receptor (AR) activity is critical for the development of effective treatment for incurable castration-resistant prostate cancer (CRPC) and, optimally, reducing or delaying lethality in Veterans who suffer from prostate cancer. We and others previously discovered several AR splicing variants lacking the ligand-binding domain in hormone-refractory prostate cancer cells. One major splicing variant, AR3 (also known as AR-v7), is upregulated during prostate cancer progression, and its expression level predicts the risk of tumor recurrence and enzalutamide resistance. This project aims to elucidate the role of androgen receptor splicing variant (AR3/AR-v7) in the therapeutic resistance of prostate cancer. The precise mechanisms by which AR3 regulates transcription program in prostate cancer cells and its role in transition to more aggressive neuroendocrine prostate cancer (NEPC) remain to be fully understood.

Our recent study showed that the AR3 splicing variant, but not the prototype full-length AR (AR-FL), positively regulates transcription factor E2F1 expression in prostate cancer cells resistant to both Enzalutamide and Docetaxel. E2F1 in turn regulates AR3 expression, and these two proteins form a positive regulatory feed-forward loop. These findings suggest that AR3 may play a distinct, yet essential, role in disease progression and drug response through regulating a unique set of genes that are not regulated by the prototype AR. We have identified a number of proteins preferentially binding to AR3 through a proteomic analysis.

We will delineate the mechanisms by which AR3 exerts its transcriptional activity and specificity in prostate cancer cells and test the effects of miR-877 on blocking AR3 activity on drug sensitivity (Aim 1). In addition, we have established a new transgenic mouse model, fAR3pTG, by targeted expression of AR3 in prostatic epithelium to recapitulate pathological changes occurring in human prostate cancer. RNA-seq analysis on fAR3pTg prostate reveals that multiple genes involved in neuroendocrine differentiation (NED) and metastasis processes are altered. We will establish a compound mouse model and examine the role of AR3 in NED and prostate cancer metastasis in Pten-deficient background (Aim 2). Successful completion of the proposed research will enable us to gain new insights into molecular mechanisms underlying prostate cancer metastasis and therapeutic resistance, as well as to develop new effective therapeutics targeting AR variants to optimally reduce or delay lethality for patients who suffer from prostate cancer.

Proposal Title:	Novel Role and Therapeutic Targeting of LMO4 in Lethal Prostate Cancer
Log Number:	PC220341
Current PI Name:	Boyang Wu
Award Number:	HT9425-23-1-0118
Current Contracting Organization:	Washington State University, Pullman
Current Performing Organization:	Washington State University, Pullman
Web Approval Date:	05-02-2023

Approximately 35,000 men are projected to die from prostate cancer (PC) in the United States in 2022, making it the second commonest cause of cancer death in men of all ages and the commonest cause of death from cancer in men over age 75. Hormone therapy, which prevents androgens such as testosterone from promoting PC's biological effects, is the current dominant therapy. Though PC patients benefit from this type of therapy at first, PC inevitably progresses to castration resistance frequently associated with cancer spread to distant body sites (metastasis) and is then treated with more potent hormone therapies such as enzalutamide (Xtandi). However, PC can rapidly develop drug resistance to the newer hormone therapies and develop neuroendocrine features, a phenomenon that has been reported in up to 25% of therapy-resistant relapsed PC. Currently, the survival of patients who suffer from neuroendocrine disease is only ~7 months. These dismal facts underscore an urgent clinical need to develop new and effective targeted therapies against metastatic castration-resistant and neuroendocrine types of PC, which currently remain incurable.

Recent studies, including ours, have discovered that PC cells can evolve or switch from a therapy-responsive luminal cell type to a therapy-resistant neuroendocrine cell type, a process referred to as tumor plasticity, under the selective pressure of newer hormone therapies like enzalutamide. Unraveling and targeting the molecular drivers of tumor plasticity will help us develop new molecularly targeted therapies for treating lethal PC by restoring their luminal cell identity and sensitivity to hormone therapies. We recently identified a transcription cofactor protein, LMO4, as a strong candidate driving factor promoting tumor plasticity and hormone therapy resistance. We found that LMO4 is overproduced in human prostate tumors associated with castration resistance, neuroendocrine features, higher incidence of metastasis and shorter overall survival times. Our preliminary studies also showed that silencing LMO4 effectively suppressed castration-resistant and neuroendocrine prostate tumor growth in experimental models. Based on these exciting findings, we propose to test the hypothesis that LMO4 plays an active role driving metastatic castration-resistant and neuroendocrine PC and that targeting LMO4 is a feasible strategy to treat these lethal forms of PC.

We designed three specific aims to test our hypothesis. Aim 1 will characterize the role of LMO4 in driving metastatic castration-resistant and neuroendocrine PC. In Aim 2, we will elucidate the mechanisms by which LMO4, likely through partnering with other transcriptional regulatory proteins to produce coamplified effects and activate expression of many target neuroendocrine and pro-proliferative genes, induces lethal PC, as well as further establish the clinical relevance of the mechanistic findings in a large cohort of patient samples. In Aim 3, we will evaluate the safety and effectiveness of our newly identified first-in-field LMO4 inhibitors for treating lethal PC in a range of prostate tumor mouse models. Notably, one of the tumor mouse models we propose to use for efficacy testing is a patient-derived xenograft model where tumor tissue from patients is engrafted into mice, thus retaining the key features of human tumors to more faithfully reflect or predict clinical responses to LMO4 inhibitors.

Our research addresses the FY22 PCRP Overarching Challenges of "define the biology of prostate cancer progression to lethal prostate cancer to reduce death" and "develop treatments that improve outcomes for men with lethal prostate cancer." We can contribute to resolving one of the most important questions

remaining to be answered in the PC research field – how castration-resistant and neuroendocrine PCs develop

– by specifically investigating the previously unrecognized role of LMO4 in driving tumor plasticity and PC progression toward terminal stages and elucidating the corresponding molecular mechanism. This will significantly advance our current understanding of lethal disease progression. We will also rigidly characterize and evaluate the therapeutic potential of our LMO4 inhibitors in cancer cell cultures and a variety of tumor mouse models, for the development of a novel and effective therapeutic strategy for treating lethal PC.

The proposed study emphasizes late-stage PC patients who no longer respond to even the most potent hormone therapies and develop castration-resistant and neuroendocrine traits that currently lack effective interventions. The proposed study, if successfully executed with the expected results, will provide compelling preclinical evidence for further developing our LMO4 inhibitors and moving forward to human clinical trials in collaboration with clinicians to apply a LMO4-targeted therapy against lethal PC for the first time in the near future, with the ultimate goal of eradicating PC death and enhancing the well-being of men suffering from this disease.

Proposal Title:	The BRIDGE Project: Broadening the Reach of Imaging, Diagnostics, and Genetic Evaluation
Log Number:	PC220342
Current PI Name:	Alexander Cole
Award Number:	HT9425-23-1-0531
Current Contracting Organization:	Brigham and Women's Hospital, Inc.
Current Performing Organization:	Brigham and Women's Hospital, Inc.
Web Approval Date:	10-02-2023

Recent years have seen an expansion in imaging and molecular diagnostics for prostate cancer. These tools allow more precise diagnosis and treatment of prostate cancer, limit toxicities and overtreatment, and may open the door for new interventions that are guided by patients' unique genetic background. Unfortunately, racial and ethnic minority groups often lag behind white men in the receipt of new technologies. We don't yet know whether this is also the case for advanced imaging and diagnostic testing in prostate cancer. What's more, as these tools become more widely used, strategies to ensure adequate dissemination of these services are needed.

There are reasons to believe advanced testing (incorporating advanced imaging and genetic testing) and targeted interventions (incorporating image guided therapeutics and molecularly targeted therapies) are likely to be underused but also of particular importance for Black men at risk from prostate cancer. For example, Black men are more likely to meet criteria for genetic testing. Imaging can reduce overdiagnosis and limit toxicities of treatment—issues implicated in our finding that Black men are 22% less likely to be treated for potentially lethal cancer than white men.

We will begin by using national insurance claims data to assess patterns of these services. First, are Black men truly less likely to receive these advanced services? What other patient characteristics play a role? Second, we will use national data to assess whether difference in use of these tests are due to sociodemographic factors, to the physician or hospital where these men are treated, or to regional practice patterns. Once potential contributory factors have been identified we will move on to an assessment of the diagnostic and treatment pathway for Black men at a group of local clinics in our region. We will involve stakeholders including patients, providers, clinic employees and patient advocates to understand potential barriers to performing these tests. Ultimately, we believe this work will provide a list of potential "targets" that can be addressed health policy and novel implementation projects.

Prior work by our team strongly supports the thesis worse access to care (and specifically worse access to high quality care) is likely to be a major determinant of unequal prostate cancer outcomes in Black men. When Black men are treated in equal access systems and have access to uniform, high-quality care (e.g., in the context of an equal access health system or in a clinical trial) we find they the experience outcomes which are similar to those of white men. This means that efforts to ensure both quality and equity of cancer care have the potentially to dramatically improve outcomes for Black men at risk from prostate cancer.

I am a urologist and health services researcher at Brigham and Women's Hospital/Dana Farber Cancer center. My clinical focus is on the treatment of prostate cancer with an emphasis on image-based approaches, including MRI-based diagnostics, image-based surveillance, and image-guided therapeutics. As a researcher, I have developed a body of work that strongly supports access to care as a critical determinant of racial disparities in prostate cancer outcomes among Black men. Having shown the critical importance of access to high quality care as a strategy to overcome the quality gap in prostate cancer care, the logical next step is to move directly into the clinic and use a mixture of quantitative and qualitative data gathering to define the

landscape of care around advanced testing and targeted interventions. This project will do this. It will provide a roadmap for innovating and implementing change to ensure equitable use of advanced testing and targeted interventions for prostate cancer.

My mentorship team and research development plan are designed to support me in moving beyond my existing body of health services research in “descriptive” disparities research towards “interventional” health services research. Not only does the BRIDGE project aim to connect underserved community members with high quality, modern prostate cancer diagnostics, it will yield knowledge skills and training that will serve as a bridge for the next step in my own career which is to move from descriptive health services research to implementation.

Proposal Title:	Impact of Maryland's All-Payer Model on Racial and Socioeconomic Disparities in Prostate Cancer Management
Log Number:	PC220346
Current PI Name:	Meiling Ying
Award Number:	HT9425-23-1-0141
Current Contracting Organization:	Michigan, University of
Current Performing Organization:	Michigan, University of
Web Approval Date:	07-07-2023

Scientific Objective and Rationale: Prostate cancer is the most common nonskin cancer and the second leading cause of cancer-related death in U.S. men. There are important disparities in appropriate treatment, postoperative outcomes, and access to care for prostate cancer. Specifically, Black men and men with low socioeconomic status (SES) have greater barriers to receiving appropriate intervention, experience higher rates of readmissions and complications, travel longer distances to seek care, and have a higher likelihood of facing delayed care, compared to other patients.

Maryland's All-Payer Model of 2014, a payment reform, was introduced to improve outcomes and optimize costs of hospital services by making hospitals accountable for the entire hospital revenues, readmissions, and complications. If Maryland hospitals meet payment and quality targets, they can keep their revenues and get additional bonuses from Medicare; if they fall short, they have to repay the difference between the payment target and their total revenues to Medicare and receive financial penalties (up to 50%) in the following year. These changes may have positive and negative effects on existing disparities in prostate cancer care. First, to constrain total revenues, Maryland hospitals may consider cheaper therapies (i.e., conservative treatment) to expensive interventions (i.e., curative treatments, including surgical or radiation therapies). This might be harmful for men with high-risk, localized prostate cancer, where curative treatments are appropriate, particularly for Black and low-SES men who already face substantial challenges to receiving curative treatment. Second, the Model may spur hospitals to select appropriate surgical candidates and improve their care practices to meet the payment and quality targets (i.e., readmissions and complications) for the sake of savings. This may enhance surgical outcomes for prostate cancer patients who undergo prostatectomy, particularly among Black men and SES disadvantaged patients who already face worse outcomes, reducing the existing disparities. Third, Maryland hospitals may just shift the high-cost and high-need patients from their facilities to achieve cost containment and quality targets. Therefore, Black men and men from low-SES strata may need to travel longer distances for prostate cancer care and be more likely to face delayed treatment.

Ultimate Applicability of the Research: Alternative payment models like Maryland's All-Payer Model, in which participating entities take on considerable financial risk to deliver high-quality care at lower cost, have been mainstream strategies of improving the value and efficiency of health care delivery since the passage of the Patient Protection and Affordable Care Act of 2010. This proposal will provide scientific evidence that will inform the further design of alternative payment reforms to include inequality/disparity-focused metrics, reducing racial and SES disparities for prostate cancer. This will benefit all men with prostate cancer in general and Black and low-SES men in particular. Such beneficial changes will ultimately advance prostate cancer care, which may generate positive spillover effects on other groups, such Service Members, Veterans, and military beneficiaries.

Principal Investigator's Career Goals in Prostate Cancer Research: My short-term career goal is to become an independent prostate cancer researcher who is dedicated to facilitating health equity and reducing disparities in prostate cancer. My long-term career goal is to emerge as a policy leader in the fields of health

policy, health disparities, and cancer care. To realize these goals, I am seeking support in the form of an Early-Investigator Research Award. This award will afford me a great opportunity to devote 100% of my effort to purse formal coursework, mentorship, and project-based learning-activities preparing me for a long career of independent scientific investigation. The researcher development plan builds on my foundation in health services research. These activities will further my education and skills in cancer registry-Medicare linked data use, necessary clinical knowledge/practices, and advanced econometrics as they apply to prostate cancer care. My research plan will provide an opportunity to execute and enhance skills detailed in the researcher development plan. My mentors play a key role in successfully implementing the development and research plans. My mentorship team includes a physician- scientist, Dr. Vahakn B. Shahinian, who is an expert in use of administrative and clinical data for prostate cancer research and alternative payment reforms; a clinician health services researcher, Dr. Brent K. Hollenbeck, who has tremendous clinical and research experience in prostate cancer care and health disparities in prostate cancer management; and a health economist, Dr. Richard A. Hirth, who is proficient in quantitative methods, health policy evaluations, empirical study design. The combination of analytic expertise and clinical nuisance will help ensure that I will use robust approaches to address both clinically meaningful and policy-relevant issues, as well as gain necessary training to achieve my goals.

Proposal Title:	Stromal Modulation by the Secretory Factors of Neuroendocrine-like Prostate Cancer
Log Number:	PC220349
Current PI Name:	Juhi Mishra
Award Number:	HT9425-23-1-0920
Current Contracting Organization:	Nebraska, University of, Medical Center
Current Performing Organization:	Nebraska, University of, Medical Center
Web Approval Date:	10-02-2023

Applicability of the Research: Tumors in castration resistant prostate cancer patients are heterogenous, which contain different types of cancer cells (such as neuroendocrine, adenocarcinoma), immune cells, nerve cells and others. These cells communicate with each other, which is required for the metastatic progression of the disease. Since, metastatic dissemination of castration resistant prostate cancer is the root cause of death due to prostate cancer, we would like to understand the process of metastatic dissemination in this proposal by studying the communication between cancer cells and other non-cancer cells. Our preliminary studies have indicated that neuroendocrine-type prostate cancer can secrete several biomolecules that promote neuronal growth in the tumor and also help macrophages (a specific immune cell) to be activated following stimulation by the newly recruited neuronal cells. The activated macrophages then send signals to the cancer cells and transform them to become metastatic prostate cancer cells. In this proposal, we will study the mechanism by which neuroendocrine cancer cells promote neuronal growth in prostate tumor tissue. We will further study using animal models of prostate cancer, how neuron-macrophage axis in tumor tissue helps to generate metastatic and therapy-resistant prostate cancer, which leads to patient demise. A thorough understanding of this process will help to identify appropriate targets for drug development, which can prevent the metastatic dissemination of castration-resistant prostate cancer and thus can protect the lives of the patients from this lethal disease.

Principal Investigator’s (PI) Career Goal in Prostate Cancer Research: As a young researcher, it intrigues me to undertake this challenge and study the biology of advanced Prostate Cancer (PCa) and its microenvironment that could be instrumental in designing better therapeutic approaches which could lead to improved survival and quality of life of PCa patients. In the proposed study I wish to seek the role of advanced PCa whether in altering the functions of stromal cells, such as nerve and macrophages in tumor microenvironment, which can then enhance the therapy resistance ability and metastatic dissemination of tumor cells. During the award period and beyond, mentors will provide guidance and suggestions to become oriented to the duties and responsibilities. Initial emphasis will be to improve scientific knowledge, experimental techniques, and design, as well as carry out hypothesis-driven research, followed by developing skills required for managing a project on a day-to-day basis, and I will be guided to analyze, interpret, and present data efficiently and articulately. Altogether, the PI intends to take advantage of this opportunity to establish her as an independent investigator in prostate cancer research. The outcomes of the study will make a significant contribution to the fight against prostate cancer in the future by exploring the biology of advanced PCa to reduce death. My multidisciplinary research training after successful completion of this proposal will establish me as an independent investigator and pursue PCa research. After completion of this award, I plan to expand my knowledge and utilize my research outcomes to devise therapeutic targets for advanced PCa and solve more deep-rooted mysteries behind aggressive PCa and therapy resistance.

Proposal Title:	A Viral Mimicry Approach to Target Prostate Cancer
Log Number:	PC220354
Current PI Name:	Charles Spruck
Award Number:	HT9425-23-1-0135
Current Contracting Organization:	Sanford Burnham Prebys Medical Discovery Institute, La Jolla
Current Performing Organization:	Sanford Burnham Prebys Medical Discovery Institute, La Jolla
Web Approval Date:	05-03-2023

Although strides have been made in the clinical management of prostate cancer, most patients with hormone-refractory recurrent or overt distant metastases are incurable. Treatment options for these patients are primarily restricted to toxic chemotherapy and pain management. Even with aggressive treatment, the 5-year survival rate for patients with metastatic prostate cancer is only ~30%. Therefore, there is an unmet clinical need to develop innovative, more effective, and less toxic treatment options for these patients. In this proposal, we explore an entirely new way to target metastatic prostate cancer by awakening ancient viruses in the DNA of prostate cancer cells. This approach, which has been termed by us and others as “viral mimicry,” has the potential for widespread clinical applications in metastatic prostate cancer treatment as a standalone therapy and enhancer of certain antitumor therapies, including PARP inhibitor and immune checkpoint blockade (ICB) therapies. Ancient viruses compose ~40% of the human genome and are normally transcriptionally silenced in our cells. We discovered that a histone methyltransferase called SUV39H1 is essential for the silencing of ancient viruses in prostate cancer cells. SUV39H1 inhibition, such as through treatment with the newly developed drug F5446, reactivated these ancient viruses in prostate cancer cells, which stimulated viral defense pathways that normally function to protect cells from infection by exogenous pathogens (e.g., viruses). F5446 also promoted DNA replication stress, stimulated interferon (IFN) signaling, and potently killed prostate cancer cells in vitro. Importantly, SUV39H1 was found not to be required for ancient virus silencing in normal cells, indicating a therapeutic window for metastatic prostate cancer treatment. In this study, we will evaluate three potential therapeutic applications for F5446 in metastatic prostate cancer treatment: (1) as a standalone therapy through induction of DNA replication stress and natural killer (NK) cell cytotoxicity; (2) synergy with ICB therapy through stimulation of IFN signaling and cytotoxic T cell tumor infiltration; and (3) as an enhancer of PARP inhibitor antitumor activity through induction of DNA replication stress in prostate cancer cells. Validation of efficacy of F5446 in any of these therapeutic applications could revolutionize the way that metastatic prostate cancer patients are treated, which would undoubtedly have a significant impact on reducing disease mortality. Moreover, we are actively developing more potent and selective SUV39H1 inhibitors, suggesting that the results of this study could be rapidly translated into the clinic for prostate cancer patient care in a 2-3-year time frame.

Proposal Title:	Development of Targeted Chemotherapy for the Treatment of Metastatic Prostate Cancer
Log Number:	PC220355
Current PI Name:	Adrian Chrastina
Award Number:	HT9425-23-1-0148
Current Contracting Organization:	Proteogenomics Research Institute for Systems Medicine
Current Performing Organization:	Proteogenomics Research Institute for Systems Medicine
Web Approval Date:	05-18-2023

Prostate cancer remains one of the deadliest cancers. Advanced and metastatic prostate cancer is initially controlled with androgen deprivation therapy. Unfortunately, most cases eventually progress into metastatic castration-resistant prostate cancer and become refractory to this treatment. Current chemotherapies for metastatic castration-resistant prostate cancer provide no cure or are aimed at palliations of symptoms. Suboptimal efficacy of currently used therapeutics is in part related to their limited tumor-specificity and insufficient levels of delivery. Given intravenously, most of the therapeutics are diluted over the entire body and only small amounts reach diseased tissue with even less able to penetrate solid tumors where they can be most effective. This leads not only to poor treatment outcome, but also severe side effects. Therefore, there is an urgent critical need for development of more effective prostate cancer therapies with improved therapeutic effectiveness and safety profile to patients.

Our previous research has identified an active cellular transport pathway that can pump antibodies armed with therapeutic cargo directly into solid tumors and metastatic lesions. We have identified a specific disease biomarker that is concentrated in the tumor vasculature and unique to this cellular pump and have subsequently developed the humanized recombinant antibody specific to this biomarker that is capable of penetrating deeply and specifically inside prostate tumor tissue as the result of pumping. Here, we propose to use this highly specific and unique antibody for precision delivery of select, very potent chemotherapeutics directly into prostate tumors and metastatic lesions. Depending on the particular therapeutic cargo and linker chemistry evaluated in the study, we expect to identify those therapeutic reagents that are most effective in treatment of metastatic prostate cancer. Extending our previous work, we will evaluate the potential of this strategy for targeted chemotherapy of prostate cancer in several preclinical models of metastatic prostate cancer, including tumors that are castration-resistant or unresponsive to conventional chemotherapy treatment.

If successful, this novel targeting strategy could significantly enhance therapeutic efficacy and reduce undesirable side effects in patients suffering from prostate cancer. The preclinical research proposed here will establish a path for the development and potential clinical translation of this type of novel biotherapeutics for treatment of metastatic prostate cancer.

Proposal Title:	Targeting the Immunosuppressive Microenvironment of Prostate Cancer to Enhance Therapeutic Efficacy of the Immune Checkpoint Inhibitors
Log Number:	PC220363
Current PI Name:	Qin Yu
Award Number:	HT9425-23-1-0169
Current Contracting Organization:	Icahn School of Medicine at Mount Sinai
Current Performing Organization:	Icahn School of Medicine at Mount Sinai
Web Approval Date:	05-12-2023

Even though there are high initial response rates of prostate cancer (PCa) patients to the androgen-deprivation therapy (ADT) and anti-androgens, the castration-resistant prostate cancer (CRPC) invariably occurs with poor prognosis and limited treatment options, which urges for more efficacious and longer-lasting novel therapies and combinations to achieve better clinical outcomes and improve survival rates for PCa patients. Cancer evades host immune system attack by inhibiting T cell activation through the interactions of the immune checkpoint proteins (ICPs), such as the programmed cell death protein 1 (PD-1) expressed by T cells and their ligands, such as PD ligand 1/2 (PD-L1/2) expressed by tumor and other cells.

Immune checkpoint inhibitors (ICIs) have been developed against a variety of cancers. Unlike other cancer types with high levels of genetic mutations and infiltrating lymphocytes, PCa is relatively immunologically “cold” and poorly responsive to immunotherapy. So far, Phase III clinical trials of the ICIs failed to show improved overall survival in PCa patients, suggesting that identifying and targeting the determinants govern the PCa response, and resistance to the ICIs are the keys for future success in the ICI-based immunotherapy in PCa.

Tie2 is a receptor tyrosine kinase (RTK) for the Angiopoietin family members, Angiopoietin-1 (Angpt1), Angpt-2, and Angpt-3/4, and is expressed by endothelial cells, cancer associated fibroblasts (CAFs), and tumor cells as well as a subset of tumor-infiltrating myeloid cells, the TIE2-expressing monocytes (TEMs). Angiopoietins (Angpts) are known to promote tumor angiogenesis and tumor progression. In a castration-resistant prostate cancer (CRPC) data set, we found that Angpt1 is amplified in 51% of CRPC patients, but only in 3% of primary and androgen-deprivation therapy (ADT)-naïve PCa patients, and that Angpt1 protein is up-regulated in advanced PCa and CRPC samples. We showed that increased Angpt1 enriches immunosuppressive TEMs and reduces CD8+T cells in PCa, whereas Angpt1 knockdown displays the opposite effects. In addition, Angpt1 induces expression of SDF-1 and IL-10 in PCa and an Angpt1 inhibitor, soluble Tie2 (sTie2), sensitizes the CRPC response to an anti-PD-1 antibody, suggesting an important novel role of Angpt1 in negatively regulating the immune response of CRPCs and the potential of Angpt1 inhibitors in sensitizing the CRPC response to the ICIs.

Based on our novel results, we hypothesize that Angpt1 is a major contributor of the immunosuppressive PCa microenvironment and plays a key role in inhibiting the PCa response to the ICIs by activating Tie2 RTK to (1) promote accumulation/activation of the immunosuppressive TEMs; (2) induce SDF-1 secretion by CAFs in PCa microenvironment, which in turn promotes further recruitment of the TEMs and other myeloid-derived suppressor cells (MDSCs); and (3) induce IL-10 expression by TEMs, which in turn inhibits CD8+ T cell proliferation and activation. We further hypothesize that elevated Angpt1 leads to the reduced PCa response to the ICIs, whereas Angpt1 inhibition reverses its immunosuppressive effects and sensitizes the PCa response to the ICIs, and that combined inhibition of Angpt1 and the PD-1/PD-L1 together with or without anti-androgens such as enzalutamide constitutes a novel and more efficacious therapeutic strategy against the CRPC.

Three Specific Aims are proposed. Specific Aim1 is to establish that Angpt1 enriches and activates the immunosuppressive Tie2+ monocytes (TEMs) and inhibits accumulation and activation of CD8+ T cells in PCa and that Angpt1 inhibition reverses its effects and enhances the PD-1/PD-L1-inhibitor mediated anti-PCa immunity. Aim 2 is to determine the mechanisms underlying the effects of Angpt1 on the immune cells and on the PCa response to the ICIs. More specifically, we will (1) determine the effects of Angpt1 on the Tie2+ monocytes and the mechanisms underlying these effects; (2) establish that the Angpt1-induced up-regulation of SDF-1 plays an important role in mediating the immunosuppressive effect of Angpt1 in vivo; and (3) determine whether the Angpt1-induced up-regulation of IL-10 mediates the effects of Angpt1 on the immune cells and on therapeutic efficacy of the ICIs. Aim 3 is to establish that combined inhibition of Angpt1 and PD-1/PD-L1, with or without anti-androgens such as enzalutamide, constitutes a novel and more effective strategy against CRPCs. In this aim, both mouse CRPC models in their syngeneic mice and our newly established innovative castration-resistant and prostate cancer patient-derived xenografts (CR-PCa-PDXs) in humanized mice will be used.

This proposal aims to develop treatments to improve outcomes for men with lethal PCa (an overarching challenge). The proposal is well supported by novel preliminary results and designed to test the well-reasoned, innovative, and highly translatable hypotheses. Successfully accomplishing this proposal will establish that Angpt1 is a novel key contributor of the immunosuppressive CRPC microenvironment; establish the mechanisms underlying the inhibitory effect of Angpt1 on the CRPC response to the ICIs; provide novel therapeutic targets for sensitizing the CRPC response to the ICIs and novel/more efficacious combined treatments by using Angpt1 inhibitors and the ICIs together with or without anti-androgens; and provide guidance to predict the PCa patient’s response to the ICIs and the combination therapy using our innovative CR-PCa-PDX models (short-term impact in 3 years). The positive results will lead to rapid (immediately after accomplishing this proposal) clinic translation of the combinational treatments to significantly improve efficacy of the immunotherapy and clinical outcomes and survival rates for men with lethal PCa (long-term impact). Thus, this proposal is of high clinical relevance and significance and high therapeutic potential and impact.

Proposal Title:	Targeting Prostate Cancer Metabolic Vulnerabilities in the Bone Microenvironment to Circumvent Lethal Metastatic Disease
Log Number:	PC220365
Current PI Name:	Hai Wang
Award Number:	HT9425-23-1-0417
Current Contracting Organization:	Health Research Inc., Roswell Park Division
Current Performing Organization:	Health Research Inc., Roswell Park Division
Web Approval Date:	10-02-2023

Challenge Addressed: This application addresses the Prostate Cancer Research Program's (PCRP) overarching challenge to "Define the biology of prostate cancer progression to lethal prostate cancer to reduce death."

Rationale: Prostate cancer is the second leading cause of cancer death in American men. Despite medical advances over the past decade, prostate cancer (PCa) continues to cause over 30,000 deaths per year in the United States. The vast majority of PCa-related deaths are caused by metastases, not by primary tumors. Compared with the relative low occurrence of visceral metastases (i.e., cancer cells that have spread to soft organs like the brain, liver, lung) in PCa, bone metastases (BoM) predominantly occur in up to 90% of patients at autopsy and represent the most lethal form of PCa. Based on a cohort study of 23,087 patients in Denmark, the 5-year survival rate is 56% in PCa patients without BoM, but it is as low as 3% in patients with BoM. Therefore, BoM represent an attractive focus for research aimed at improving survival rates in PCa patients, for whom current clinical treatments do not work well. In particular, research is needed to better understand the biology of PCa during BoM progression, which will be critical for identifying novel druggable targets in cells that can form the basis for new therapies. Presently, knowledge about the progression processes involved in the growth and persistence of BoM is extremely limited, probably because of the lack of appropriate models to study PCa BoM. Here, we propose to use our newly established BoM models to study the biology of PCa during the progression of BoM, with the hope that this knowledge can ultimately be used to address the unmet needs of patients that do not respond well to current treatments.

In this BoM-focused application, we propose to address this need for more information by exploiting our recent discovery about how PCa cells adapt to the environmental stresses in the bone milieu in a non-conventional way and gain the capacity to convert nutrition to energy and basic cellular building blocks, namely, metabolic adaptation. We hypothesize that this process is double-regulated by the hyper-activation of organ-specific, oncogenic NFAT (nuclear factor of activated T cells) signaling in combination with the suppression of metabolic repressor Sirtuin 3 (SIRT3). Interestingly, our early work disclosed an unexpected role for SIRT3 in the regulation of NFAT levels. Additionally, our studies, for the first time, have identified a collaborative and mutually interfering regulatory cascade as a critical mechanism that supports the metabolic adaptation and metastatic progression of PCa cells, thereby allowing PCa cells to survive amidst a limited nutrition supply and environmental stresses in the bone milieu. The involvement of the indicated genes was supported by public genomics data and pilot biological studies. These findings provide a compelling rationale to investigate and target the indicated metabolic axis for BoM treatment.

This study represents pioneering work on the metabolic biology in PCa BoM, which has remained an extremely unappreciated focus area in cancer research. Importantly, new understanding of the unorthodox metabolic behavior in bone metastatic PCa could ultimately lead to the discovery of novel therapeutic strategies to improve survival rates and the quality of life in patients with lethal/metastatic PCa. We expect to complete this mechanistic study within 3 years. The proposed effort, albeit at a basic cancer biology level, has promising potential to lead to rapid translation with a clinical impact. Most compounds tested in this

project have currently entered clinical trials—in fact, some have already been approved by the U.S. Food and Drug Administration to treat other diseases. Thus, success in our studies would have near-term clinical relevance.

Proposal Title:	Deciphering the Complete Oncogenic Proteome and Its Role for Prostate Cancer Progression
Log Number:	PC220370
Current PI Name:	Crystal Conn
Award Number:	HT9425-23-1-0082
Current Contracting Organization:	Pennsylvania, University of
Current Performing Organization:	Pennsylvania, University of
Web Approval Date:	07-31-2023

After curative surgery and therapy, the majority of prostate cancer patients will remain cancer-free, and yet prostate cancer remains the second leading cause of cancer-related deaths in men in the United States. This is partly due to the severity of the metastatic disease and the acquired resistance to drug therapies as the cancer progresses. Currently, our diagnostic options fail to classify non-aggressive from aggressive tumors starting from the primary tumors removed at surgery. This can lead to potentially missed treatment options after early detection, with poor therapeutic response for advanced disease. Despite these limitations, there arise cellular adaptations that oncogenic cells rely on for their survival and tumor progression that may offer new avenues for diagnosis and future therapies. We discovered an adaptive pathway that is required for tumor survival, enhanced by current clinical therapies for promoting resistance, and linked to metastasis in patient samples. The biological activation of this stress adaptation, the factors at play, and the downstream alterations on the cancer epigenome augmented remain unknown.

The focus of this proposal is to understand the biological mechanisms of cell adaptations that occur early in primary tumors to promote aggressive prostate development and therapy resistance. Our aim is to understand and map these stress response pathways, which are known to reconstitute gene expression through regulating mRNA translation. We will utilize several state-of-the-art technologies including deep sequencing and mass spectrometry coupled to bioinformatics to study this adaptation and the factors involved in the prostate cancer stress responses required for tumor progression. Collectively, we seek to map the adaptive post-transcriptional aspects of gene expression, identify full protein sequences missing from our current annotated genomes, and determine the factors and oncogenic signaling that command this adaptive response utilized by aggressive prostate cancer. Our results will gain access to a tremendous window of opportunity for understanding prostate cancer progression and this targetable vulnerability of aggressive tumors. By discovering the mechanism of regulation for these processes, we have the potential to identify novel functional biomarkers for diagnostics in primary tumors that can improve our current therapeutic strategies, while revealing innovative targets.

Proposal Title:	Defining the Molecular Basis for the Immunogenicity of ADT-Resistant Locally Advanced Prostate Tumors
Log Number:	PC220394
Current PI Name:	Haydn Kissick
Award Number:	HT9425-23-1-0318
Current Contracting Organization:	Emory University
Current Performing Organization:	Emory University
Web Approval Date:	07-16-2023

Rationale, Objective, and Aims of the Application: About 15-20% of all patients diagnosed with localized prostate cancer are considered high-risk. This means that there is a higher probability that after surgery or radiation, their cancer will come back. These numbers are based on historical averages of patients with specific clinical diagnostic criteria, such as higher Gleason scores in their biopsy sample. Patients generally recur due to hidden micrometastases that are present at the time of initial therapy—these are not detected by even the most sensitive of imaging, and because they are outside the prostate, neither radiation nor surgery treats them. Neoadjuvant therapy, in which drugs are given prior to surgery or radiation, certainly has effects on prostate-localized disease, but is believed to also act on those hidden micrometastases. In our clinical trials, we have evaluated moving hormonal therapies earlier in the disease course to the neoadjuvant setting, and they are tremendously effective in about 40% of patients with otherwise high-risk disease. Of course, the question then becomes “why didn’t 60% of patients respond well?”

We have examined the biopsy and posttreatment specimens from patients in our study. First, we found that patients who respond poorly always have at least some immune infiltration around the cancer that is left in the prostate. This is remarkable because untreated prostate cancer with immune infiltration is extremely rare. Second, we found that, in patients whose cancers do not respond completely to neoadjuvant hormone therapy, there is a genetic signature that predicts that they would have also responded to HER2-targeting therapies, a class of drugs that is safe and effective in patients with certain types of breast and ovarian cancer. The first objective of this application is to study tissue samples from patients who have immune infiltration in their cancer after treatment, and ask what genomic or genetic features of the tumor correlate with that. The second objective of this application is to use freshly-acquired prostate tumor specimens from patients receiving radical prostatectomies to test whether those tumors can be killed by adding immunotherapy (specifically PDL1 blockade), HER2 inhibition, or both, to hormone therapy.

Thus, our application has two aims. The first aim is to characterize the immunology of posttreatment tumors and how they compare to untreated tumors from the same patient, with a goal of understanding the relationship between genetics and immune response. The second aim is to test validate our findings using drug studies of patient tissue samples in mice. We are using a mouse xenograft protocol that also uses cord blood derived stem cells enabling us to study an immune response in the mouse that is matched to the patient.

Ultimate Applicability of the Research: Likely contributions of the study to the FY22 PCRP overarching challenges. This study addresses two of the PCRP overarching challenges. First, locally advanced prostate cancer will progress to lethal cancer in about 60% of patients, even with aggressive neoadjuvant therapies. The patients in our study have been diagnosed with NCCN high-risk disease. Thus, this project responds to the challenge to develop treatments that improve outcomes for men with lethal prostate cancer. Second, we are making an effort to define the biology of prostate cancer progression to lethal prostate cancer to reduce

death. Our application responds to this challenge by developing a greater understanding of how these lethal cancers evade intense androgen deprivation and what can promote an immune response, making them more sensitive to immunotherapy.

Types of Patients This Project Will Help and How It Will Help Them: This project will help patients with newly diagnosed disease that currently has a very unfavorable prognosis – high-risk, with a significant change of recurrence if treated by surgery or radiation. If the combination of hormonal therapy, HER2-directed therapy, and immunotherapy kills all tumor cells in our patient samples, we would use this data to support the development of a new clinical trial at the National Cancer Institute. If that trial is successful, patients with newly diagnosed high risk may not face the threat of progression to lethal disease as frequently as before.

Potential Clinical Applications, Benefits, and Risks: Combined treatment with intense hormonal therapy, HER2 inhibition, and immunotherapy has not been tried in prostate cancer before, but all of these treatments are safe and approved by the FDA on their own. If the work described in this application is successful, we will initiate Phase 1 studies to assess safety prior to a randomized Phase 2 study.

Projected Time It May Take to Achieve a Patient-Related Outcome: The timeline of this application is 3 years. If successful, a new clinical trial (with outcomes) may be realized within 5 years.

Proposal Title:	Clinical Identification and Targeting of Non-Neuroendocrine Lineage Plasticity in Castration-Resistant Prostate Cancer
Log Number:	PC220395
Current PI Name:	Ekta Khurana
Award Number:	HT9425-23-1-0074
Current Contracting Organization:	Joan and Sanford I Weill Medical College of Cornell University
Current Performing Organization:	Joan and Sanford I Weill Medical College of Cornell University
Web Approval Date:	05-02-2023

Untreated prostate cancer is characterized by dependence on a set of proteins called androgen receptors. Hormonal therapy that abrogates testicular testosterone production has been the centerpiece for treatment of advanced prostate cancer for over 75 years. However, most patients progress to a cancer stage that is resistant to hormonal therapy, and many of these patients do not depend on androgen receptors anymore. We have recently identified a group of such tumors that constitute a novel subtype of prostate cancer. Our calculations show that 30% patients with advanced metastatic prostate cancer that is resistant to hormonal therapy belong to this subtype, called stem-cell-like. We have also identified a set of proteins that drive the growth of these tumors. These proteins, FOSL1, TEAD, YAP, and TAZ, become overly active and alter the state of tumor cells to drive their growth.

In this proposal, we will test the impact of clinical grade small molecule inhibitors of an important protein in these stem-cell like tumors, TEAD, in in vitro and in vivo studies. We will also develop computational methods to identify prostate cancer patients whose tumors belong to this subtype in a non-invasive manner using their plasma samples. By sequencing the DNA of tumors from this subtype, we will identify the genetic drivers of the growth of such tumors.

If successful, the results of this proposal will pave the way for clinical trials of TEAD inhibitors to treat tumors of this subtype. The computational method developed for clinical identification of patients with tumors of this subtype will be validated in larger patient cohorts and, if it shows high sensitivity and specificity, can be translated to the clinic in a relatively short term. If we identify new genetic drivers from this proposal, the next steps would be to perform their functional validation and they may reveal novel proteins for targeted therapy of such tumors, while also helping their clinical identification.

This proposal will help men with advanced prostate cancer by revealing the biology of lethal prostate cancer and it will help develop treatments that improve outcomes for men with lethal prostate cancer.

Proposal Title:	Targeting KIF20A, a Kinesin with a Novel, Noncanonical Role in Castration-Resistant Prostate Cancer
Log Number:	PC220396
Current PI Name:	Kerry Burnstein
Award Number:	HT9425-23-1-0369
Current Contracting Organization:	Miami, University of, Coral Gables
Current Performing Organization:	Miami, University of, Coral Gables
Web Approval Date:	10-02-2023

Rationale: Castration-resistant prostate cancer (CRPC) is currently incurable and presents a formidable obstacle to treatment. With time, CRPC tumors almost always develop the ability to evade the second-generation androgen receptor-targeted drugs, such as enzalutamide, resulting in reinitiation of tumor growth. Androgen receptors are proteins responsible for regulating the effects of androgens (e.g., the hormone testosterone) and for promoting uncontrolled tumor growth in CRPC. To overcome this significant obstacle, new treatment strategies are required to specifically combat CRPC and metastatic CRPC (when tumors have escaped the prostate and initiated growth at remote sites, most commonly the skeleton).

By analyzing publicly available data obtained from patients with prostate cancer (PC) and by testing PC samples in our lab, we identified a protein termed KIF20A that is present at high levels in CRPC and even more so in metastatic CRPC (compared to primary [early stage] PC and normal prostate tissue). In our studies conducted in mice bearing human prostate tumors, we demonstrated that increasing KIF20A (by genetic manipulations) causes primary PC cells to undergo the devastating transition to CRPC tumors. We have also demonstrated that KIF20A can promote lethal PC by causing tumors to secrete yet-to-be-defined biological substances that stimulate tumors to grow under “castration-resistant” conditions (similar to the conditions experienced by men undergoing androgen-deprivation therapy). Most importantly, our cell-based data showed that blocking KIF20A (either by genetic experiments or by treatment with pre-clinical drugs) stopped the growth of CRPC cells but spared normal cells.

Objective and Aims: We hypothesize that KIF20A represents a therapeutic vulnerability in lethal PC. Given the current availability (and planned development) of preclinical drugs that inhibit KIF20A, we propose that targeting KIF20A offers a promising and novel approach for treating CRPC and metastatic CRPC.

Study Design: Focusing the expertise of our multi-disciplinary team of scientists, our strategy confronts KIF20A using three powerful approaches. First, we will determine how KIF20A promotes the emergence of CRPC. We will use an array of advanced techniques (including mass spectrometry) to identify the active ingredients in the undefined mixture secreted by PC cells with elevated KIF20A. Equally promising, cataloging these ingredients may identify a circulating biomarker for diagnosing early stages of CRPC. Second, we will examine a preclinical drug known to selectively inhibit KIF20A and its function in a spectrum of aggressive CRPC models. Specifically, we will evaluate the anti-tumor effects of a KIF20A inhibitor both in human CRPC cell lines growing in mouse prostates and in human tumors transplanted to mice (aka, patient-derived xenografts), collectively representing an array of PC features found in men. Using big data to guide our understanding (and frame future clinical research), we will analyze patient-specific variations in KIF20A levels in a large collection (the Prostate Cancer Biorepository Network) of tumor samples from PC patients – focusing on CRPC and metastatic CRPC – to ultimately identify those patients most likely to benefit from drugs that inhibit KIF20A. Third, collaborating with a medicinal chemist and a computational drug modeler, we will generate improved KIF20A inhibitors.

PCRP Overarching Challenges: In targeting KIF20A for drug treatment, we advance a therapeutic strategy for those with incurable CRPC, directly addressing the PCRP challenge, “Develop treatments that improve outcomes for men with lethal prostate cancer.” In defining the specific role of KIF20A in newly emergent CRPC, we advance a more nuanced understanding of CRPC – necessary for optimal clinical use of KIF20A inhibitors and to reveal potential new biomarkers – that explicitly addresses the PCRP challenge, “Define the biology of lethal prostate cancer to reduce death.”

Patient Applicability: This proposal focuses on treatment of lethal and incurable PC. By directly targeting KIF20A (for which non-toxic inhibitors are currently available for proof-of-concept studies), successful discoveries will translate to clinical trials and near-future new therapies.

Clinical Applications, Benefits, and Risks: While our studies are entirely preclinical, we focus our drug investigations on using well-characterized pharmacological agents that are well-tolerated in mice and on developing improved inhibitors. This approach is designed for eventual translation to clinical trials.

Projected Time to Patient-Related Outcomes: Compounds that inhibit KIF20A are currently undergoing clinical trials for cancers other than prostate. Accordingly, this work may be rapidly translated to patient-ready treatments for lethal PC.

Proposal Title:	Targeting Dysregulated Histone-Modifying Enzymes Driving Prostate Cancer Cell Survival After Androgen Deprivation Therapy
Log Number:	PC220401
Current PI Name:	David Jarrard
Award Number:	HT9425-23-1-0127
Current Contracting Organization:	Wisconsin, University of, Madison
Current Performing Organization:	Wisconsin, University of, Madison
Web Approval Date:	05-02-2023

An underappreciated area of prostate cancer management is the persistent cancer cells that remain after treatment with androgen-deprivation therapy (i.e., hormone removal) for advanced prostate cancer. Eradication of these persistent prostate cancer cells is critical to improve treatment outcomes. Our research has demonstrated that these persistent cells after androgen removal upregulate specific genes that help with survival and growth. One major regulator of cell behavior involves a series of enzymes called histone modifiers. We have discovered that unique histone patterns result in androgen-resistant cells that are controlled by these enzymes. In this proposal, we will examine the hypothesis that these enzymes represent a potential “Achilles heel” that plays a key role in the resistance response of prostate cancer cells to the combination of hormone removal and histone modifier inhibitors. Specifically, we will test this novel approach in cell culture models and clinically relevant human prostate cancers. Our studies could lead to a new treatment paradigm for prostate cancer to eradicate persistent cells after hormone removal that has the potential to dramatically improve treatment outcomes. This study addresses the development of cancer resistance in prostate cancer patients, and furthermore should be readily translated into the clinic since many histone modifiers inhibitors are already and androgen-deprivation therapy is currently in use.

Proposal Title:	Synergistic Combination Treatment of Prostate Cancer Using CD46-Targeted Antibody-Drug Conjugate and Radioimmunotherapy
Log Number:	PC220404
Current PI Name:	Anil Parsram Bidkar
Award Number:	HT9425-23-1-0139
Current Contracting Organization:	California, University of, San Francisco
Current Performing Organization:	California, University of, San Francisco
Web Approval Date:	05-01-2023

Despite important research advances, late-stage, metastatic, castration-resistant prostate cancer is a disease with poor outcomes. For this reason, there is a need to develop improved therapeutic strategies to treat prostate cancers effectively. Additionally, many current cancer treatments have side effects that create difficulties for patients and decrease quality of life. Therefore, tumor-specific therapy preventing the side effects on normal body organs is vital. A therapeutic dose of the drugs can be delivered to destroy tumor cells by targeting specific proteins expressed on the tumor tissue. The goal of this grant application is to develop a treatment strategy for patients with prostate cancer. A high dose of a single drug is needed to get a good response for therapy, but this results in a toxic reaction to the body organs. We plan to combine the two-prostate cancer-targeted molecules to achieve a synergistic response for tumor killing at a lower dose. This combination therapy will result in a synergistic anti-tumor response with lower side effects. Therefore, this study contributes to the overarching challenge to “develop treatments that improve outcomes for men with lethal prostate cancer.”

Recently, the concept of “theranostics” has revolutionized prostate cancer care, by combining high-sensitivity scanning together with targeted therapy. The current theranostic agents for prostate cancer are based on the presence of prostate cancer membrane antigen (PSMA) on tumor. However, many patients do not show the presence of PSMA. As we target the novel biomarker CD46, this therapeutic strategy could also help these patients with low or no PSMA on the tumor tissue. We are combining the CD46 targeted actinium-225 labeled antibody with an already known therapeutic molecule in clinical trials (CD46 ADC). We have conducted preliminary studies on combining these therapies with very promising results. Specifically, when used in combination, these two molecules show synergistic response at the lower doses as compared to their individual response. However, this needs to be confirmed with additional experiments using different tumor models, for example, tumors collected from patients and tumor spreading (metastasizing) to other body organs. Additionally, we have planned to carry out the toxicity study for individual drug molecules, as well as for combination therapy. As of now, we will be working with mouse models, but the results of these studies will form a solid ground for designing the therapy for men with prostate cancer.

Another important fact of this study is the use of patient tumor tissues and metastatic tumor models in mice. The primary tumor at one location is different from the metastasized tumor in other body organs. We have adopted the strategy to make these metastatic tumor models in mice to study the effect of our combination therapy. This way, we will have knowledge about the action of combination therapy in advanced diseases, where it will be used in patients. The anti-cancer effect of therapeutic molecules will be studied in these advanced tumor models to find out the ways to improve anti-cancer response for men with advanced or metastatic tumors.

We will study the molecular mechanism of the synergistic anti-cancer response. The mechanistic information will be utilized to change the doses of individual drugs to achieve maximum therapy response with minimum toxicity or side effects. Current experiments are at the preclinical stage; thus it will take 2

years to collect all of the data and analyze the results. However, the CD46 ADC is currently under a clinical trial at UCSF; thus, data from this study will help us to expedite our experiments and possibly work on human trials.

The Principal Investigator, Dr. Anil Bidkar, is already working on some projects in Dr. Flavell's lab at the Radiology Department, University of California, San Francisco. With the current proposal, Dr. Bidkar will have a project where he will learn about almost everything needed to become an independent researcher. Dr. Bidkar has completed all the pieces of training required for laboratory mice handling and radiation safety. Dr. Bidkar participated in the departmental retreat and attended an international workshop on the radiobiology of molecular radiotherapy. The established mentors at UCSF, Dr. Flavell, Dr. Liu, and Dr. Vanbrocklin will provide guidance and help in designing experiments, assays, and interpreting the data. This grant would provide great training opportunities for Dr. Bidkar, enabling him to transition into an independent prostate cancer researcher.

Proposal Title:	Defining the Endocrinology of Neuroendocrine Prostate Cancer to Identify Microenvironment Drivers of Treatment Resistance
Log Number:	PC220408
Current PI Name:	Tarana Arman
Award Number:	HT9425-23-1-0096
Current Contracting Organization:	Fred Hutchinson Cancer Center
Current Performing Organization:	Fred Hutchinson Cancer Center
Web Approval Date:	01-27-2023

Scientific Objective and Rationale: While the vast majority of prostate cancers (PCs) are adenocarcinomas with secretory epithelial features and an active androgen receptor (AR) program, PCs with a spectrum of other histological characteristics also occur. Among these are PCs with characteristics of neuroendocrine (NE) cells. These prostate neuroendocrine carcinomas (NEPCs), representing

While pure NEPC is evident in some tumor biopsies, including a subset with small cell histology that is indistinguishable from small cell carcinomas arising in other organs such as the lung, other tumors show mixtures of ARPC and NEPC cells indicating a degree of intratumor heterogeneity. Neuroendocrine small cell carcinomas are primarily characterized by morphological features, lack of AR expression, and a higher expression of several canonical markers reflecting NE cell differentiation, e.g., the transcriptional factors ASCL1, NEUROD1, INSM1, as well as function, e.g., the secreted proteins synaptophysin (SYP), chromogranin A (CgA) and neuron specific enolase (NSE). To date, the role of these and other NE secreted factors in terms of endocrine and paracrine effects toward tumor cells and local microenvironments has not been established. This research project is specifically designed to bridge this knowledge gap. The research objectives are to: (1) identify the spectrum of NEPC-derived secreted factors with the potential for adverse paracrine/endocrine activity, (2) determine the influence of NEPC cells on the growth and treatment resistance of ARPC cells, and (3) determine the role of individual secreted factors in the ARPC growth and survival program with an intent to identify druggable targets to develop novel therapies.

Applicability of Research: This research is applicable to patients diagnosed with mPC and specifically those PCs with small cell and NE features – either exclusively, or intermixed with conventional ARPC. Currently, there are no therapies that can effectively treat metastatic NEPC beyond platinum-based chemotherapy. Further, the output of this project may be applicable to patients with ARPC undergoing AR-directed therapy. It is evident that AR antagonism can promote the emergence of NEPC, and co-existing NEPC cells have the potential to drive AR pathway resistance. Finally, there are a subset of patients that exhibit “paraneoplastic” syndromes whereby the secretory component of tumor cells produces adverse systemic effects. The successful outcome of this project could produce strategies that repress such events. The research plan involves studies in highly relevant preclinical models that reflect the biology of contemporary ARPC/NEPC. A subset of candidate therapeutic targets have clinical grade inhibitors already developed. The advancement into clinical testing could occur within 3-5 years.

Principal Investigator’s Goals in Prostate Cancer Research: My career goal is to conduct research in the field of basic and translational oncology. I would ideally like to lead a small research group – comprised of mature scientists and young trainees – that is focused on understanding key interactions that occur in tumor microenvironments and between heterogeneous cancer subtypes. PC is an ideal malignancy to study in this regard. I would also strive to develop interactions with clinical researchers through a team-based approach that leverages my skills with experts in pathology and clinical therapeutics. Through this project, I aim to acquire key core skills – such as biostatistics and basic coding/programming – that will enhance my immediate and long-term productivity. I also plan to gain proficiency in several research methods, including

mass spectrometry/proteomics and the use of vertebrate animals and tissue slice cultures for studies of drugs and tumor microenvironment interactions. My career development plan also includes specific plans to enhance written and verbal communication skills, critically analyze literature, develop relationships with key thought-leaders in the field, and develop effective approaches for assembling research proposals. The output of my research project should provide new insights into the pathobiology of PC and produce important data with the potential to impact patient treatment and lead to new ideas that can serve to nucleate my independent research career.

Proposal Title:	Developing New Therapeutic Strategies to Target Trop2 in Aggressive Prostate Cancer
Log Number:	PC220415
Current PI Name:	Alifiani Hartono
Award Number:	HT9425-23-1-0237
Current Contracting Organization:	California, University of, Los Angeles
Current Performing Organization:	California, University of, Los Angeles
Web Approval Date:	05-18-2023

Prostate cancer is the leading cause of cancer among men worldwide. Current treatment for prostate cancer diagnosis is through surgical removal of the tumor and radiation therapy, with androgen-deprivation therapy (ADT) given if the cancer returns after prostatectomy and radiotherapy. Over the course of ADT, some tumors can mutate and evolve to become castration-resistance prostate cancer (CRPC), a metastatic and lethal form of prostate cancer. The current therapies for CRPC include treatment with second-generation anti-androgen drugs, but they only improve median overall survival by a few months and new therapeutical methods are needed to address metastatic CRPC. Furthermore, a subset of CRPC gains neuroendocrine phenotype and expresses neuroendocrine markers after being treated with second-generation anti-androgen drugs. These neuroendocrine prostate cancer (NEPC) cells are not sensitive to any anti-androgen drugs because their growth is independent of androgen signaling, and they are considered the most difficult to treat and a lethal form of prostate cancer. Therefore, there is an immediate need to establish new therapeutical methods against mechanisms that promote advanced prostate cancer.

We previously found a high level of Trop2 expression in advanced prostate cancer patients' tumors. Trop2 is a cell membrane protein that is also highly expressed in advanced breast, colorectal, and lung cancer. When activated, Trop2 is cleaved into two parts: the extracellular domain (TECD) and the intracellular domain (TICD). We found that active Trop2 leads to faster cell growth and increases the ability of these Trop2 high-expressing cancer cells to invade and metastasize in locations such as bones and lungs. Depletion of Trop2 expression leads to inhibition of cell growth and a decrease in the numbers of metastases in our preclinical metastatic prostate cancer patient-derived xenograft (PDX) models. Therefore, we hypothesize that targeting Trop2 and inhibiting Trop2 signaling represent a novel therapeutic strategy for targeting advanced prostate cancer.

We utilized a high-throughput compound screen for Trop2 inhibitors at the Stanford High-Throughput Bioscience Center. Over 2,500 compounds were screened, and 30 potential Trop2 inhibitors were found that have specific toxicity towards prostate cancer cell lines. Our goals for this study are (1) to develop small molecule inhibitors that target Trop2 activation and function and (2) to test these compounds' therapeutic potential through inhibiting Trop2-driven tumor growth and metastasis. The results from this study will directly impact the prostate cancer community by providing the health community with new therapeutic methods in targeting a protein, Trop2, which is necessary for advanced prostate cancer to grow. Developing a Trop2-specific inhibitor will also be a useful tool for the research community in investigating the mechanisms by which Trop2 exerts its oncogenic role in cancer.

My future goal is to establish an academic translational research lab as an independent investigator. As a molecular biologist, I am excited to investigate the oncogenic properties of Trop2 in advanced prostate cancer development. The proposed project focuses on developing novel inhibitors to Trop2, and the translational aspect of the project is in line with my growing interest in exploiting disease-specific biomarkers as therapeutic targets. My mentor, Dr. Tanya Stoyanova, has over 12 years' experience in prostate cancer signaling and has characterized some of Trop2's oncogenic properties in prostate cancer. Her

guidance and expertise in prostate cancer preclinical models will be invaluable for this project. I will also collaborate with Dr. James Brooks, a surgical urologist with over 20 years' experience in mentoring young scientists. His expertise in prostate cancer patient treatment will be useful as I learn more about translating findings in the laboratory setting and into a clinical trial. I will participate in weekly data presentation meetings with the Stoyanova lab as well as a joint biweekly meeting with Dr. Brooks and his lab members. At this joint biweekly meeting, we will discuss current advancements in prostate cancer through journal clubs, data presentation, and show-and-tell topics. Additional grant writing seminars and professional development seminars/courses will be taken via the Office of Postdoctoral Affairs at Stanford University to prepare my transition to a faculty position.

Proposal Title:	Personal SPECT: A Wearable Sensor Array for Continuous Real-Time Dosimetry for Theranostics
Log Number:	PC220421
Current PI Name:	Mekhail Anwar
Award Number:	HT9425-23-1-0159
Current Contracting Organization:	California, University of, San Francisco
Current Performing Organization:	California, University of, San Francisco
Web Approval Date:	05-12-2023

The goal of this project is to create a wearable, at home, SPECT capable of continuous dosimetry monitoring to tumors and organs at risk (OARs) after targeted radionuclide therapy (TRT) for prostate cancer. This will solve a major unmet need to optimize the TRT dose for each patient based on their unique and widely varying biodistribution and tumor uptake and retention profiles – moving away from the current "one size fits all" dosing approach to a personalized method maximizing the dose to the tumor, while not causing toxicity.

Despite a swell of new drug approvals over the past 10 years for the treatment of metastatic castration-resistant prostate cancer (mCRPC), these therapies are not truly curative. Thus, developing new strategies to ablate mCRPC is an urgent unmet need. Radiotherapy is an effective and promising treatment for prostate cancer

– in part due to its simplicity; delivery of high doses to the tumor will ablate it, but the challenge resides in delivering that dose safely without exceeding the dose to surrounding critical organs. Unfortunately, standard (external beam) radiotherapy cannot be targeted to many areas in the body at the same time, precluding treatment for mCRPC. However, radioligand therapy, in which a radioactive drug is administered systemically and carries radiation to all tumors within the body simultaneously, is a treatment strategy that has been used for decades to cure deadly tumors like thyroid cancer. Recently, promising clinical trial results have emerged for the use of TRT targeting prostate-specific membrane antigen (PSMA) on mCRPC cells. That said, many patients experience no benefit or recur quickly. This is due primarily to the inadequate dose delivered to the tumor, driven by using a "one-size-fits-all" approach that is based on data models that do not consider the biological variability between tumors that we know to exist among mCRPC patients. Thus, the administration of the radioligand therapy is in no way personalized to the patient. On this basis, we hypothesize that monitoring dose distribution continuously will enable optimization and personalization of TRT – maximizing the therapeutic ratio for each patient. This will be even more important as alpha radionuclides (300X more powerful than 177Lu) and synergistic TRT-drug combinations are introduced into clinical trials, such as with DNA damage response agents or immunotherapy drugs.

Currently, it is not possible to monitor in real time how much radioligand is depositing within tumors, and the field is therefore blind to drug-tumor interactions and simply must wait weeks to months for evidence to tumor responses. Developing new technologies to study the interaction between tumors and radioligand therapy continuous and in real time is a major unmet need that we aim to address during this project.

Real-time, ultrasensitive dosimetry remains an important, yet elusive, goal due to the inherent physical properties of radioactive particles; long half-lives (~7 days) require continuous monitoring to obtain accurate dosimetry, and the current state of the art (SPECT imaging) is not available at all centers and cannot feasibly be done on a repeated basis. We solve this problem by introducing a wearable, at home, SPECT dosimetry platform enabled by millimeter-scale nano-electronic dosimetry chips that will enable us to measure radiation in both tumors and normal tissues remotely. In this proposal, we build on our validated prototype

capable of detecting gamma emissions from 177Lu, by making it more sensitive, embedding it in a wearable array, and using an algorithm that takes data from a PSMA-PET to allow reconstruction of all dosimetry from a few strategically placed wearable sensors. We validate this prototype on a mouse model of prostate cancer, which is readily scalable to a patient, as the chips are made using computer-chip technology (and easily mass fabricated), and, while patient measurements distances are 10X more than a mouse (i.e., 10 cm from tumor to skin), the dose to patients is ~200X that of a mouse, making our results in a mouse directly applicable to patients.

The success of this proposal will result in a prototype at-home, wearable dosimeter array and proof of concept in a mouse model using a targeted radiolabeled small molecule (PSMA-617) targeting prostate cancer cells. We anticipate an additional 1 year will be needed for manufacture of a patient-sized prototype and preparation for first-in human-testing. At that time, we will begin the first-in-human trials, targeting metastatic castrate-resistant prostate cancer undergoing 177Lu-PSMA-617 treatment. In future efforts, this paradigm can be employed for other theranostics.

Proposal Title:	The Role of Polyamine Metabolism in Advanced Prostate Cancer
Log Number:	PC220429
Current PI Name:	Laura Sena
Award Number:	HT9425-23-1-0107
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	05-02-2023

Research Objectives and Potential to Help Patients: The goal of our research is to develop innovative and effective therapies to prolong the lives of patients with advanced prostate cancer. Currently available therapies are only effective for a limited duration of time due to the cancer developing resistance. Moreover, current therapies for prostate cancer have failed to recruit the assistance of the body's immune system to help fight the cancer. We believe that the metabolism of prostate cancer, i.e., the way that nutrients are processed and utilized, becomes programmed to promote resistance to therapy and silencing of the immune system. Specifically, our preliminary data suggest that the central driver of prostate cancer, the androgen receptor, stimulates the production of polyamines. These polyamines function to promote cancer cell resilience under the stress of therapy and are potently immunosuppressive. Using prostate cancer models, we observed greater death of prostate cancer cells when polyamine synthesis is inhibited by a drug called difluoromethylornithine (DFMO) in combination with other therapies. On this basis of these data, we are opening a clinical trial targeting the androgen receptor in combination with DFMO for patients with advanced prostate cancer. DFMO is an approved medicine for a parasite infection called trypanosomiasis and has very few side effects. The overall goal of this proposal is to define how polyamine metabolism regulates prostate cancer cell fate and evasion of the immune system following hormonal therapies. We will study patient biopsy samples obtained from our clinical trial to validate mechanistic work using prostate cancer models. These studies will enable rational development of novel therapeutic strategies that overcome resistance and immunosuppression for patients with lethal prostate cancer.

Career Development and Training of the Candidate: Laura Sena is the candidate principal investigator for this award. She is currently a medical oncologist at Johns Hopkins University who cares exclusively for patients with advanced prostate cancer. She has a Ph.D. in molecular biology and cellular metabolism. She completed internal medicine and oncology subspecialty training at Johns Hopkins Hospital and became a faculty member in October 2021. Her career goal is to develop better therapies for patients with prostate cancer to improve their duration and quality of life by targeting cellular metabolism. This proposal includes a career development training plan with dedicated mentorship, a scientific advisory committee, didactic and hands-on research, and interaction with the wider prostate cancer community to provide Dr. Sena with skills and experience to become a leader in this field. Her mentors for this award are Samuel Denmeade and Robert Casero. Dr. Denmeade is the director of GU medical oncology at Johns Hopkins, is a world-renowned translational prostate cancer researcher, and will serve as Dr. Sena's clinical/translational mentor with specialty in prostate cancer. Dr. Casero is a molecular biologist with extensive expertise in polyamine metabolism in cancer and other disease settings and will serve as Dr. Sena's laboratory mentor with specialty in polyamine metabolism. Overall, this project will integrate two distinct areas of expertise by these mentors, provide numerous training opportunities for Dr. Sena, and drive a new line of investigation that has significant potential to help patients.

Proposal Title:	Development of Next-Generation STEAP1 Chimeric Antigen Receptor T-Cell Therapies to Combat Antigen Escape and T-Cell Dysfunction
Log Number:	PC220440
Current PI Name:	Vipul Bhatia
Award Number:	HT9425-23-1-0089
Current Contracting Organization:	Fred Hutchinson Cancer Center
Current Performing Organization:	Fred Hutchinson Cancer Center
Web Approval Date:	01-27-2023

Prostate cancer is the second most common cause of cancer-related death in men in the United States. It relies on sex hormones called androgens for survival and growth, and drugs blocking these hormones are the first line of treatment for advanced prostate cancer. However, prostate cancers eventually become resistant to these drugs. When the cancer spreads to the bones, distant lymph nodes, and other organs, it is known as metastatic disease and is incurable.

A patient's immune T cells can be genetically engineered to carry "chimeric antigen receptors" (CAR) that recognize cancer-specific proteins and then kill the cancer cells that display those proteins. CAR-T cell therapies have shown great efficacy against blood cancers, but have been less successful against prostate cancer and other solid tumors. Aiming to successfully treat metastatic prostate cancers, we developed a CAR-T cell therapy targeting the "STEAP1" protein that is found on most prostate cancers. It has shown excellent activity against prostate cancer in laboratory models. However, mice treated with the CAR-T cells eventually relapse due to decreased STEAP1 protein in the cancer or loss of anti-cancer CAR-T cell activity.

I now propose to address these issues that prevent the cure of prostate cancer with STEAP1 CAR-T cell therapy. Regarding the loss of STEAP1 protein, I will design and test a CAR-T cell therapy that simultaneously targets two proteins on prostate cancer: STEAP1 and another known as PSMA, which are both expressed at high levels in the same cancer cells. To address the loss of CAR-T cell anti-cancer activity, I will investigate next-generation STEAP1-specific CAR-T cells that are less likely to become fatigued and more likely to continue attacking cancer. The results of these studies will advance novel CAR-T cell therapies that can halt prostate cancer progression and reduce patient mortality while also informing CAR-T cell therapy advances against other solid tumors.

Proposal Title:	COP1 Regulation of Prostate Cancer Biology
Log Number:	PC220441
Current PI Name:	Feng Yang
Award Number:	HT9425-23-1-0095
Current Contracting Organization:	Baylor College of Medicine
Current Performing Organization:	Baylor College of Medicine
Web Approval Date:	07-03-2023

Castration-resistant prostate cancer (CRPC) is lethal. Interestingly, most CRPCs remain dependent on Androgen Receptor (AR) activity, and inhibiting AR remains a major therapeutic approach for most CRPCs. A protein named GATA2 plays essential roles in governing AR expression/ activation to promote PCa and castration resistance. Clinically, high GATA2 expression in human PCa correlates with advanced stages, worse prognosis, and therapy resistance. Thus, GATA2 forms a central node of PCa and lethal CRPC. Unfortunately, previous efforts have not yet identified true GATA2 inhibitors for therapy. GATA2 protein is unstable, and enhancing GATA2 protein degradation is a promising therapeutic avenue. These highlight the significance of understanding the molecular mechanisms regulating GATA2 protein stability in PCa/CRPC. The E3 ubiquitin ligases are enzymes essential for protein degradation. So far, the E3 ligase for GATA2 degradation in PCa/CRPC is unknown.

Our current study will establish the novel concept that COP1 is the first bona fide E3 ubiquitin ligase for GATA2 degradation in PCa/CRPC; this was originally discovered by the Principal Investigator and has never been reported. The successful completion of our study will fully characterize the striking activities of COP1 on suppressing PCa cell/xenograft growth and castration resistance. Our study will establish GATA2 as the missing major functionally significant substrate of COP1 in human PCa and define the COP1-GATA2 axis as a direct novel mechanism for COP1 regulating AR expression/activation, PCa growth, and castration-resistance. Mechanistically, we will define how COP1 utilizes different mechanisms for binding and thus degradation of GATA2 vs. other canonical substrates and assess the roles of GATA2 in mediating COP1 biology in PCa/CRPC. This grant will address the FY22 PCRP Overarching Challenge of “Define the biology of prostate cancer progression to lethal prostate cancer to reduce death.”

How the COP1-GATA2 axis is regulated in PCa is unknown. Our pioneering studies revealed that MAPK4, a protein not well studied, promotes tumor progression and therapy resistance via non-canonical activation of a key tumor-promoting signaling pathway (Wang et al., J Clin Invest, 2019; Shen et al., J Clin Invest, 2021; Cai et al., Sci Adv, 2021; Wang et al., Nat Commun, 2022). We also discovered that MAPK4 enhances GATA2/AR signaling to promote PCa growth and castration resistance (Shen et al., J Clin Invest, 2021), and this is at least partially mediated by MAPK4 blocking COP1-GATA2 binding and thus COP1-mediated GATA2 degradation. Therefore, therapeutically enhancing COP1 degradation of GATA2 can be achieved by (1) MAPK4 inhibitors (we have identified two leading compounds to inhibit MAPK4) and (2) developing molecular glue, a new class of small molecules, to further enhance COP1-GATA2 binding and/or glue GATA2 and COP1 proteins together in the presence of COP1-GATA2 binding blocker(s), e.g., MAPK4.

Therefore, the short-term impact of our study includes the discovery of COP1 promoting GATA2 degradation as a direct and novel mechanism for repressing AR expression/activation, inhibiting PCa growth, and sensitizing PCa to androgen-deprivation therapy. These will identify/establish targeting our newly discovered COP1-GATA2 axis as a novel and effective therapeutic avenue for PCa and lethal CRPC. The long-term impact includes developing MAPK4 inhibitors (we have already identified two leading compounds) and/or molecular glue(s) to activate our newly discovered COP1-GATA2 axis, which will

produce novel and effective therapies for lethal CRPC. All in all, by pioneering the COP1-GATA2 axis and COP1 biology in repressing AR expression/activation, PCa growth, and castration resistance, our study is original, highly innovative, and will have a high impact on both the PCa research field and patient care.

Proposal Title:	Defining and Exploiting the Molecular and Cellular Responses to Supraphysiological Androgens for Prostate Cancer Treatment
Log Number:	PC220447
Current PI Name:	Reza Alizadeh Ghodsi
Award Number:	HT9425-23-1-0102
Current Contracting Organization:	Fred Hutchinson Cancer Center
Current Performing Organization:	Fred Hutchinson Cancer Center
Web Approval Date:	01-27-2023

Scientific Objective and Rationale: Prostate cancer (PC) is a major health issue in the U.S. Metastatic PC (mPC) that has spread beyond the prostate is essentially incurable. A notable feature of mPC is the activity of a signaling program comprising the hormone testosterone and the androgen receptor (AR) which is activated in tumor cells by testosterone. The AR drives PCs to survive and grow. Therapies designed to eliminate testosterone in the blood – androgen deprivation therapy (ADT) – or block the activity of the AR – AR signaling inhibitors, are first-line therapies for most men with mPC and these approaches produce excellent initial responses. However, ADT and ARSIs do not cure patients with mPC; these tumors essentially always recur to a clinical state termed castration resistant PC (CRPC). Though cancer control is initially achieved, ADT and ARSIs have substantial effects on quality of life and other aspects of health.

Surprisingly, a strategy for treating patients resisting ADT and ARSI therapy is to give back high doses of testosterone – termed supraphysiological androgen therapy (SPA). SPA can enigmatically inhibit the abnormal functions of AR, but not its critical normal activities. However, SPA is not effective in all patients (~50%), and the mechanisms responsible for SPA anti-tumor effects remain poorly understood. My doctoral work revealed that SPA can activate an innate anti-viral response in PC cells that is normally designed to attack viral pathogens within a cell. Notably, the anti-viral reaction in cells can cause tumor cell death; however, the full-extent of the anti-cancer effects of this process remain unanswered. With this background, the objectives of this research project are to: (1), rigorously evaluate anti-cancer effects of SPA-mediated anti-viral responses in clinically relevant models of PC (patient-derived tumors on dish and in mice) and determine whether potential abnormalities in other cellular material such as DNA (the cell's instruction manual) and proteins (the cell's action molecules) can affect this response; (2) fully characterize the SPA-mediated anti-viral response in PC cells, aiming to precisely understand the process involved in the activation of this reaction by cancer cells; and (3) investigate other drugs that can maximize anti-cancer activities of SPA.

Applicability of Research: The overall concept and strategy comprising this study is original, and it is based on research that I conducted. If successful, the results may enhance the depth and duration of responses to SPA in PC patients who are no longer responding to conventional therapies. The goal of this research is to further our understanding of SPA-mediated anti-cancer effects, with a focus on anti-viral responses, to develop novel therapeutic strategies such as new drug combinations that can avert the development of resistance to SPA in therapy responder patients and overcome resistance to SPA in patients who have failed SPA. Results of these studies will contribute vital information towards personalized therapeutic strategies for patients. Success in these aims could profoundly influence how SPA is administered to patients with advanced PC in the clinic.

Principal Investigator's Goals in Prostate Cancer Research: My overall career ambition is to become an independent scientist leading a world-class team to deliver impactful research for clinically relevant issues in the field of PC research, particularly in the area of molecular endocrinology. My ultimate research goal is to maximize the quantifiable aspects of this disease (e.g., overall survival and therapy response) and minimize

the qualitative side effects of current therapies (e.g., stress, fatigue), which significantly influence the quality of life. Clinical trials (e.g., NCT02090114, NCT02286921) have revealed that SPA-based therapy can produce substantial and durable responses in patients who are not responding to conventional therapies. Building on the expertise that I have acquired in the field of PC research, I am determined to understand how SPA elicits anticancer effects, which will enable me to develop more effective therapies for patients with very limited options. Additionally, the mentorship and training plans outlined in this research proposal will provide me with vital scientific knowledge, skillsets, and ample support to develop a very successful career. More specifically, this program will include interdisciplinary coursework such as advanced training in computational biology (which will allow me to analyze abnormalities in DNA samples of patients), hands-on experimentation and exposure to new lab technologies, and lifelong scientific and clinical collaborations that will have a lasting benefit on my research career.

Contributions to the Field of Prostate Cancer Research: This proposal has direct relevance to the overarching challenge to “develop treatments that improve outcomes for men with lethal prostate cancer.” The overall objective is to understand how SPA represses PC growth and to exploit this information to develop new approaches that enhance the duration and depth of responses to SPA as a treatment strategy for men with advanced prostate cancer.

Proposal Title:	Dissecting Translational Dynamics and Lineage Plasticity in Prostate Cancer
Log Number:	PC220450
Current PI Name:	Yeon Soo Kim
Award Number:	HT9425-23-1-0123
Current Contracting Organization:	Fred Hutchinson Cancer Center
Current Performing Organization:	Fred Hutchinson Cancer Center
Web Approval Date:	05-01-2023

Prostate cancer is the most prevalent malignancy and the second leading cause of cancer death in men in the United States. Over the last few decades, advances in our understanding of prostate cancer biology have led to major breakthroughs in the clinic including androgen deprivation therapy (ADT) and androgen receptor (AR) inhibitors. These treatments rely on the finding that prostate cancer cells depend prominently on AR for their survival and accelerated growth. During the initial treatment, prostate cancer is AR-sensitive and responds well to ADT. However, prostate cancer cells progressively evolve and adapt to low androgen conditions and ultimately become castration-resistant. Patients with castration-resistant prostate cancer (CRPC) have limited treatment options as prostate cancer cells become insensitive to AR inhibitors. Even with the tremendous efforts in the prostate cancer research community, treatment-induced CRPC remains the leading cause of death in men with advance staged disease.

My research proposal will address the 2022 PCRP Overarching Challenge of “defin[ing] the biology of prostate cancer progression to lethal prostate cancer to reduce death.” To this end, improving our understanding of the biology behind the transition of AR-sensitive prostate cancer to CRPC is critical for the development of new, effective treatments for patients with advanced stage disease. In particular, it will dramatically help patients if we can determine the processes and factors directly responsible for resistance to AR-directed therapies. In this proposal, I will study a new process I recently discovered that drives therapy resistance and will further demonstrate its potential as a therapeutic target. My research focuses on how prostate cancer cells exploit protein synthesis to meet their growth needs, and break their dependence on AR. I found that a molecule called tRNA is critical for driving these processes. It does so by hijacking the protein-making factors of a cell so that cancer cells can make new proteins that help them grow faster and resist ADT or AR inhibitors. My research will explore how prostate cancer selectively induces protein synthesis through tRNA and other elements to create dangerous and lethal forms of the disease. I will use multidisciplinary approaches, including new methods that I have developed, to study the process of protein synthesis in cells. Critical to this work, I will also study tRNA in human tissues from patients with lethal advanced stage disease.

It is important to note that my proposed study is exceedingly unique and has never been attempted in the field of prostate cancer before. Therefore, I believe my efforts within the next 3 years will have the great potential of culminating in the discovery of new targets that have not been considered in highly treatment resistant prostate cancer. For example, one of the goals in my proposed research is to define new hidden elements within cancer-causing RNAs that control their ability to make proteins. If I discover such prostate cancer-promoting elements and define how they work, I will be able to collaborate with drug companies to develop a new class of therapeutics that target these regions. The last 2 years of the pandemic have brought a significant investment and research interest in mRNA vaccines and RNA biology. Given the unprecedented advances in RNA-based therapeutics, I foresee that my proposed research will not only tackle important unanswered biological questions underlying therapy resistance in prostate cancer but also bring forth a new paradigm in prostate cancer treatment.

My ultimate career goal is to become an independent researcher with a focus on prostate cancer. I joined the Hsieh Lab for my postdoctoral training to expand my expertise in prostate cancer and mRNA biology and to discover new therapeutic approaches for CRPC. Through the co-mentorship of Drs. Hsieh and Pan (tRNA expert), I will learn the critical aspects of prostate cancer biology as well as new experimental techniques and skill sets for successful completion of this project. Dr. Hsieh's work as a prostate cancer oncologist will also inform me of the potential impact of my work in real world patients. Last, the collaborative environment of the Fred Hutch will help me build expertise in the prostate cancer field and advance my scientific career as an academic investigator.

Proposal Title:	The Role of Androgen Receptor and Chromatin Binding Proteins in Mediating the Testosterone Paradox
Log Number:	PC220451
Current PI Name:	Sushant Kachhap
Award Number:	HT9425-23-1-0105
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	05-02-2023

Androgen deprivation therapy (ADT) is the backbone of treatment for recurrent and metastatic prostate cancer. Although the initial response rate to ADT is high, progression inevitably develops leading to a clinical state of castration resistance. Through the pioneering preclinical and clinical work at Hopkins studying the paradoxical effects of supraphysiologic testosterone (SupraT) in prostate cancer, we have discovered a novel therapeutic strategy using high-dose (supraphysiological levels) testosterone as a treatment for metastatic castrate-resistant prostate cancer (mCRPC). To date, our group has treated 330 patients across four phase 2 studies using bipolar androgen therapy (BAT), documenting safety as well as significant clinical activity in a subset of men with mCRPC patients. Our goal is to understand and define the molecular features of the tumors that respond dramatically to BAT therapy. This is important as it will provide valuable clinical insights which can then be utilized for future selection of patients who would respond to the therapy and provide biomarkers for response to therapy. We have recently published a finding that suggest that BAT is able to activate immune system in patients who respond to BAT. Activation of immune cells is highly sought in prostate cancer, as prostate cancer is refractory to immunotherapy since immune cells do not infiltrate tumors and kill prostate cancer cells. We intend to find the molecular underpinnings of immune activation by BAT. This will allow us to find novel biomarkers that could predict which patients will respond to BAT. In our preliminary studies, we have found that androgen receptor (AR) activity in prostate tumors dictates response to BAT. Understanding how AR functions in response to BAT can provide insights about why certain tumors respond dramatically to BAT. We will employ an innovative labeling strategy to isolate and identify key regulators of AR function in prostate cancer. This strategy would explain how AR reprograms the genes in response to BAT to bring about growth inhibition and immune cell activation. Thus, enabling us to uncover novel protein markers of BAT response which can be used to stratify which patients will respond to BAT and also to combine BAT with immune therapeutics to achieve better therapeutic outcome. We have assembled a team of translational oncologists, prostate cancer molecular and cell biologist who are expert in animal cancer models, and tumor immunologists who would provide their complementary expertise in accomplishing our common goal. This is the first study to prospectively assess AR modulators as biomarkers of response to BAT therapy in prostate cancer. Most importantly, this proposal aims to study a never-before tested idea with potential transformative (rather than incremental) impact on the field. We hope that insights gained after successful completion of the project will help us offer BAT therapy in an informed manner and help us devise strategies to provide benefit of the therapy to a larger cohort of advanced metastatic prostate cancer patients.

Proposal Title:	Defining and Targeting Genomic Mechanisms Driving Resistance to AR Signaling Inhibitors
Log Number:	PC220456
Current PI Name:	Wanting Han
Award Number:	HT9425-23-1-0462
Current Contracting Organization:	Fred Hutchinson Cancer Center
Current Performing Organization:	Fred Hutchinson Cancer Center
Web Approval Date:	10-02-2023

Scientific Objective and Rationale: Prostate cancer (PC) is the most commonly diagnosed solid cancer in U. S. men and the second leading cause of cancer-related death. Men with primary PC often receive surgery or radiation therapy and cures are common. However, once PC spreads to distant sites, cures are very rare. However, systemic treatments, primary those that interfere with androgen receptor (AR) signaling, can extend survival by many years. However, the depth and duration of AR targeted treatments – including androgen deprivation therapy (ADT) and the use of AR signaling inhibitors (ARSIs) vary substantially from individual to individual. Recent studies detailing the molecular composition of metastatic PC has determined that these advanced tumors are driven by recurrent genomic alterations across of a spectrum of genes. A subset of these genomic alterations involve genes uniquely altered in PC such as TMPRSS2-ERG rearrangements and mutations in the AR, whereas others have well-described roles across many types of cancer. Importantly, a number of these well-known cancer-associated genes appear to functionally modify important aspects of AR signaling, and influence response rates to AR directed treatment.

This proposal is specifically focused on understanding and exploiting the role of the important tumor suppressor gene TP53 with respect to AR function. Alterations in TP53 occur in ~50% of all metastatic PCs, emphasizing the key role for this key “guardian of the genome” in PC biology and potentially in therapy resistance. In this proposal, I will test the hypothesis that genomic alterations commonly observed in PC – specifically alterations in TP53 – influence AR signaling and promote resistance to AR pathway-directed therapy. I further hypothesize that TP53 alterations exert effects via reprogramming the PC cell epigenome and that specific signaling pathways altered through this reprogramming can be targeted to overcome resistance to AR-directed treatment.

The specific aims of the project are designed to directly test the above hypotheses. In Aim 1, I will identify alterations in the PC AR cistrome resulting from inactivation of TP53. In Aim 2, I will determine how TP53 loss promotes PC cell survival in the context of AR pathway repression. In Aim 3, I will develop a therapeutic approach co-targeting AR signaling and TP53-regulated resistance pathways. The experimental strategy will use controlled experiments with novel PC model systems and patient-derived tumors with attention to rigor and reproducibility.

Applicability of the Research: This project directly relates to the overarching challenge – “define the biology of lethal prostate cancer to reduce death.” The research is applicable to patients diagnosed with PC that have progressed to a state where the cancer has spread to distant sites (metastases), but may also have relevance to high-risk localized disease. Understanding why and how PC behaves differently in different men is important for understanding prognosis and also provides key insights for developing new treatment strategies. In this context, TP53 is an important gene involving in suppressing tumor development, growth and spread. While it has been challenging to develop treatments that directly target/exploit TP53 itself, downstream pathways regulated by TP53, and particularly interactions with the AR pathway present new opportunities. Consequently, the anticipated results of this research project will contribute vital information towards personalized therapeutic strategies for patients, and potentially identify new drug combinations that

could improve responses and/or extend survival. The planned work utilizes preclinical models that reflect the biology of human prostate cancer. The near-term output will include biomarkers of resistance/response to ARSIs and the identification of combination therapeutics that demonstrate preclinical activity. As clinically approved compounds will be tested, it is possible that patients could be treated within 5 years to demonstrate benefit.

Principal Investigator Career Goals: My career aspiration is to continue my work in translational cancer research focusing on molecular mechanisms and preclinical therapeutics of late-stage PC as an independent investigator in an academic environment. I remain fascinated by the molecular mechanism responsible for the development of cancer, and the many safeguards built into complex vertebrates to repress/block carcinogenesis. I plan to lead an independent research group that exploits tumor suppressor mechanisms – ideally through the identification of new drugs and treatment strategies – possibly genome engineering – to improve outcomes. I also enjoy teaching/training young researchers and plan to devote a component of my career to mentoring the next generation of scientists in the cancer research field. The research and training plan embedded within this proposal will endow me with the skill set and expertise to reach my career goals.

Proposal Title:	Dissecting the Role of a Germline HOXB13 Variant Associated with Risk of Lethal Prostate Cancer in Men of African Ancestry
Log Number:	PC220481
Current PI Name:	Jun Luo
Award Number:	HT9425-23-1-0612
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	10-02-2023

The proposed studies will focus on a gene named HOXB13, and an altered protein, named X285K, made by an altered or mutated form of this gene, in some men of African descent when inherited from their parents. We assembled an expert team including investigators with a long history of studying prostate cancer in African American men to investigate the functional and treatment implications of this novel risk variant.

Historically, genetic variants associated with prostate cancer were notoriously challenging to validate. HOXB13 was the first bona fide prostate cancer gene implicated in risk for the disease following the discovery of the European ancestry specific G84E variant (published in the New England Journal of Medicine in 2012 by one of our co-investigators). However, the association between G84E and prostate cancer risk was equally strong in men with aggressive and non-aggressive disease. Recently, we and others investigated a novel stop-loss HOXB13 variant (X285K). One unique property of the X285K variant that sets it apart from all other HOXB13 variants is its association with risk of aggressive and potentially lethal prostate cancer specifically in men of African ancestry. In our humble opinion, X285K is quite possibly the most important marker identified to date for lethal prostate cancer risk in the African American population. Nevertheless, genetic association of X285K with aggressive prostate cancer is the only information currently available, limiting its clinical utility.

Our goal is to dissect the functional and treatment implications of this unique germline variant. We hypothesize that X285K mediates an aggressive phenotype associated with cell-cycle dysregulation, and this unique property manifests clinically as early diagnosis of potentially lethal prostate cancer and also hormonal insensitivity. We propose a series of detailed functional and mechanistic analyses focusing on the comparison of X285K, G84E, and their normal or wild type counterpart. The proposed studies will use unique tools and resources developed during the initial studies, and will be necessary in order to strategize the proper use of genetic testing results to benefit patients and their families.

This unique variant therefore presents opportunities to make tangible impact on patients and their families in the short term. Treatment implications of the X285K variant, if established in the study, will enable actionable interventions in men with self-identified African ancestry and will help to mitigate the disproportionate burden of prostate cancer by informing genetic testing strategies. We envision that adoption of genetic testing and early screening in high-risk populations can be facilitated by public dissemination of our findings on a rare variant, and early screening is one of the strategies that will result in early detection and early treatment of life-threatening prostate cancer. In addition, for men who are diagnosed with advanced prostate cancer, genetic testing is available but X285K is currently not reported in testing results. If our proposal studies confirm the treatment implications, the test result may inform treatment decisions, and optimal treatment selection will improve patient outcome and quality of life.

The proposed studies will also help to understand the roles of germline HOXB13 variants in prostate cancer. In the long term, knowledge on these heritable risk variants will benefit all men and their families especially given the recent interest in using HOXB13 as a therapeutic target.

In the United States, prostate cancer is a prevalent disease that is also well known to disproportionately affect African American men. Development of actionable interventions targeting ancestry-specific or ancestry- enriched mutations, germline or somatic, remains an important approach to mitigate prostate cancer disparity. This application will address three FY22 PCRP Overarching Challenges: (1) Develop treatments that improve outcomes for men with lethal prostate cancer; (2) Advance health equity and reduce disparities in prostate cancer; and (3) Define the biology of prostate cancer progression to lethal prostate cancer to reduce death.

Proposal Title:	Profiling and Eliminating Tumor Subclones Destined for Radioligand Resistance
Log Number:	PC220482
Current PI Name:	Rohit Bose
Award Number:	HT9425-23-1-0594
Current Contracting Organization:	California, University of, San Francisco
Current Performing Organization:	California, University of, San Francisco
Web Approval Date:	10-02-2023

Rationale, Objective, and Aims: Advanced drug-resistant prostate cancer that has spread to other organs, is the second-leading cause of cancer death in the United States. For a limited period, this type of disease can be controlled with targeted therapies that inhibit testosterone signaling, such as Xtandi, Erleada, Zytiga, while taking Lupron. Inevitably however, drug resistance develops, after which the disease becomes much more difficult to control, and requires much strong therapies with many side effects, including chemotherapies. Recently a new class of therapies is being tested in multiple cancers called radioligand therapy; in prostate cancer, the agent is called “Lu-PSMA,” i.e., radioactive Lutetium bound to a small molecule that targets PSMA on the surface of prostate cancer cells. This is a form of radiation therapy that is administered intravenously once a month. Initially, it is effective in most patients, but it only extends advanced patients’ lives by a median (or “middle time”) of 4 months.

To those ends, we are exploring why Lu-PSMA stops working in many patients after that short time. We have developed a new powerful system called “BRUTE,” which simulates the hormonal sensitivity of most prostate cancers and can be performed with an intact immune system, unlike many other experimental systems. We are using our BRUTE system and correlative de-identified patient data to identify the molecular pathways that lead to Lu-PSMA to stop working. Once we identify those, we will either use existing drugs to prevent Lu-PSMA resistance, or even develop future versions of Lu-PSMA that can get around such blocks.

Next, we use our BRUTE system and correlative de-identified patient data to identify why patients with prostate cancer that has spread to the bone cannot usually have their cancer eradicated. We will try to identify what changes occur within the cancer cells, in response to growing within bone that make it drug resistant. Ultimately, if we could effectively treat bone metastases, or block drug resistance from occurring there, we could prevent much of the suffering associated with prostate cancer.

Finally, we examine whether the mechanisms of resistance to drugs such as Xtandi and Lu-PSMA occur in rare prostate cells before exposure to those drugs or whether they predominantly occur after drug exposure. If there are rare pre-existing cells, this means that we could detect them much earlier, and potentially even eradicate them.

Applicability of the Research Contributions To FY21 PCRP Overarching Challenges: Directly addresses two challenges (1) by helping define the biology of lethal prostate cancer to reduce death and (2) developing treatments that improve outcomes for men with lethal prostate cancer.

Which Patients: Advanced prostate cancer patients who are progressing on targeted therapies, including radioligand such as Lu-PSMA. One part of the study focuses on why we cannot eradicate bone metastases.

Potential Clinical Applications, Benefits, Risks: Understanding resistance to radioligands will enable the development of combinatorial or sequential therapies to delay it from occurring. We will also be able to predict which patients may respond the best (or the worst) to it and select therapies accordingly.

Projected Time for Patient-Related Outcome: Three years for detection of resistance mechanisms in patients, 4-5 years to set up an investigator-initiated trial to delay resistance using existing FDA-approved drug, and >5 years to develop a novel compound that circumvents existing radioligand pathways.

Interim Outcomes: One to three years to identify validated cellular mechanisms of radioligand resistance in murine models, 1-3 years to identify how bone metastases develop resistance, and 1-3 years to identify whether rare drug-resistant cells exist before drug exposure.

Proposal Title:	Novel SDF-1 mRNA Therapy for Prostatic Radiation-Induced Erectile Dysfunction
Log Number:	PC220484
Current PI Name:	Johanna Hannan
Award Number:	HT9425-23-1-0227
Current Contracting Organization:	East Carolina University
Current Performing Organization:	East Carolina University
Web Approval Date:	07-03-2023

In U.S. military Veterans, prostate cancer is the most common cancer diagnosis. Annually among Veterans, there are approximately 500,000 prostate cancer patients with 15,000 new diagnoses. There are many options to treat prostate cancer, including surgical removal of the prostate, chemotherapy, radiation of the prostate, and hormone therapy. While the treatments are effective at curing prostate cancer with a 5-year 98% survival rate, these same treatments often lead to devastating side effects. Most notably, many prostate cancer survivors suffer from erectile dysfunction, which greatly impacts their overall quality of life and well-being. Erectile dysfunction due to radiation therapy is often the result of radiation-induced damage to healthy nerves and arteries supplying the penis. Unfortunately, when these penile structures are damaged, these patients do not respond to the current available erectile therapies such as Viagra, Cialis, Levitra, or penile injection therapy with vasodilators. All erectile dysfunction treatment options only treat the symptoms and not the underlying disease. A cost-effective therapy that can prevent or reverse the damage to the penile nerves, arteries, and tissues to recover erectile function and provide a long-lasting therapeutic benefit is greatly needed.

The goal of this proposal is to develop a long-lasting therapy for radiation-induced erectile dysfunction. Stromal cell derived factor 1 (SDF-1) is produced by various cells in response injury to initiate the repair pathway and recruit circulating stem cells. However, following radiation and hormone therapy, the amount of SDF-1 produced is insufficient to initiate mending. We believe that greatly increasing the production of SDF-1 proteins following radiation and hormone treatment for prostate cancer will repair the damage to the penile nerves, arteries, and tissues and recover erectile function. Using a similar technology to the Pfizer and Moderna COVID-19 vaccines, we propose to increase SDF-1 using a mRNA-based therapy administered by penile injections. To test our hypothesis, we propose to look at the effects of SDF-1 mRNA to increase Schwann cell and neuron survival and growth in pelvic nerves grown in cell culture after castration and radiation therapy. We also propose to examine the ability SDF-1 mRNA therapy to recover erections in animal models of radiation with and without hormone therapy. Finally, we will also administer SDF-1 mRNA therapy to a mouse model with active prostate cancer tumors to confirm that the treatment does not increase cancer progression or impair the effectiveness of radiation therapy. Over the last 30 years, only 20 studies have been published examining the pathological mechanisms leading to radiation-induced erectile dysfunction, and none have assessed the additive impact of castration. Our findings will significantly increase our understanding of radiation-induced erectile dysfunction and bring us closer to finding a treatment.

We believe that SDF-1 mRNA-based therapy will improve outcomes in prostate cancer survivors suffering from erectile dysfunction (FY22 PCRP Overarching Challenge) and provide a clear clinical benefit. These studies are in collaboration with the company, Evincis Bio, which is in the process of gathering data for an Investigational New Drug application with the FDA. These studies will help provide further preclinical evidence to support FDA approval to start clinical trials in patients. While this proposal is targeted to

radiation-induced erectile dysfunction, SDF-1 mRNA-based therapy has the potential benefit to improve erectile dysfunction in a wide variety of disease states and potentially provide a cure for millions of individuals worldwide.

Proposal Title:	Targeting Delta-Like Ligand 3 (DLL3) in Neuroendocrine Prostate Cancer
Log Number:	PC220515
Current PI Name:	Himisha Beltran
Award Number:	HT9425-23-1-0407
Current Contracting Organization:	Dana-Farber Cancer Institute
Current Performing Organization:	Dana-Farber Cancer Institute
Web Approval Date:	09-28-2023

Despite significant advances in prostate cancer therapy over the last decade, prostate cancer remains a leading cause of death for men worldwide. Treatment resistance is a major cause of prostate cancer mortality. Some prostate cancers are capable of evading prostate cancer therapies by transforming to a small cell /neuroendocrine prostate cancer (NEPC). The current diagnosis of NEPC relies on a tumor biopsy. They tend to look very similar to small cell lung cancer (SCLC). NEPC is very aggressive and there are no approved therapies for men with NEPC. Although NEPC occurs in up to 15% of late-stage prostate cancer, there have been very few clinical trials for men with NEPC.

Over the last 12 years, our group has been dedicated towards understanding the molecular mechanisms that drive NEPC in order to develop more effective therapies for patients. We recently discovered a protein called delta like ligand 3 (DLL3) is highly expressed in the majority of NEPC cancers and is not present in normal tissues or regular prostate adenocarcinoma. We have been developing novel ways to target DLL3. Based on this work, we are excited to collaborate with Harpoon Therapeutics on a clinical trial of the DLL3-targeted therapy HPN328. We have been enrolling NEPC as well as SCLC, another highly aggressive cancer that also expresses DLL3, and have experienced encouraging results so far. HPN328 is a T cell engager—the drug binds DLL3 on cancer cells as well as CD3 on the patient's own immune cells (T cells) and brings T cells in to kill the cancer. It's a new kind of targeted immunotherapy.

Based on encouraging results we have seen both in the lab and in the clinic as well as safety, we have developed this DOD Translational Science Award to deeply interrogate tissue and blood samples from patients enrolled on the clinical trial of HPN328. We will investigate how both cancer and immune features influence response and resistance to HPN328 and we will develop strategies to optimize patient selection. We will also use patient-derived preclinical models in the lab to investigate combination treatment strategies in order to improve the efficacy of HPN328 and bypass resistance. The clinical trial is already open, and we expect ongoing clinical results in parallel with the results from the tissue and blood analyses during the course of the Award period. This study will pave the way for further biomarker-driven expansion of this novel therapy for patients with NEPC.

If successful, this novel approach for targeting DLL3 with HPN328 will address a major clinical unmet need and significantly improve the lives of men with advanced prostate cancer. Based on their similarities, insights from NEPC may also help improve outcomes for patients with SCLC, another highly lethal cancer.

This proposal addresses the FY22 PCRP Overarching Challenge to develop treatments that improve outcomes for men with lethal prostate cancer.

Proposal Title:	Germline Influence on Prostate Cancer Metastasis and Treatment Response
Log Number:	PC220521
Current PI Name:	Tyler Seibert
Award Number:	HT9425-23-1-0532
Current Contracting Organization:	California, University of, San Diego
Current Performing Organization:	California, University of, San Diego
Web Approval Date:	10-03-2023

Rationale: Prostate cancer is a genetic disease, but most men with prostate cancer do not have a single “bad gene” or mutation that runs in the family. Instead, prostate cancer risk is inherited as the combination of numerous genetic factors. We have developed genetic scores that predict very well who will get prostate cancer. Unfortunately, current genetic scores do not distinguish between men at risk of lethal prostate cancer and men at risk of harmless prostate cancer. We recently looked at normal DNA and tumor specimens from the same people. We found that genetic markers in a man’s normal DNA (i.e., what he inherited at birth) predicted what kind of prostate cancer he had. The DNA a man is born with influences not only whether he will get prostate cancer, but also what kinds of mutations caused his cancer to develop and what kind of molecular abnormalities his cancer has. Therefore, we could use genetic tests to predict whether he might have—or develop—lethal cancer. If he does have an aggressive prostate cancer, we could combine results from tests of his birth DNA with molecular tests of his tumor to help doctors make better treatment decisions. We are proposing in the present study to investigate these concepts using data from a large clinical trial that compared moderate or high doses of radiation therapy for men with prostate cancer.

Objective: To determine whether inherited genetic features are predictive of clinically relevant tumor types and of risk of developing metastatic prostate cancer.

Aims: First, we will evaluate whether inherited genetic factors described in our previous work are able to predict which men from the clinical trial eventually developed metastatic prostate cancer after their initial treatment with radiation therapy. We will also test whether we can predict which men benefit from higher radiation therapy doses. Second, we will study how well genetic markers predict the pattern of tumor DNA methylation, which is a tool cells use to turn on or off certain genes. Thus, we will learn about how inherited DNA influences cancer cell behavior. Third, we will consider the influence of ancestry and race. Black men are more likely to die from prostate cancer. While race is defined in social terms, we have found that African genetic ancestry likely contributes to the higher risk of prostate cancer death. We will investigate how genetic drivers of aggressive prostate cancer might differ for Black men. Finally, we will explore some additional concepts, including genetic risk of side effects from radiation therapy.

What Are the Likely Contributions of this Study to the FY22 PCRP Overarching Challenges? We will develop genetic tests to predict who is at risk of progression to lethal prostate cancer. We will determine whether genetics can tell us which patients need a higher dose of radiation. We will explore whether genetic tests can help predict side effects of treatment so that they can be avoided, in an effort to improve quality of life. We will study genetic contributions to health disparities to help us eliminate the increased risk of prostate cancer death in Black men.

What Types of Patients Will Be Helped and How Will It Help Them? We could identify men who have a high genetic risk of aggressive prostate cancer so that we can invite them to get screened and find the cancer before it has spread. Patients who already have prostate cancer will be helped, too. We will use genetic tests to predict who needs more intense treatment and what treatment dose is safest for them. We will learn about

how genetics and race interact to contribute to prostate cancer risk. What are the potential clinical applications, benefits, and risks? The clinical applications are described in answer to the question above. There is no risk to any participants in this study because we are using specimens collected in a previous clinical trial.

What is the Projected Time It May Take to Achieve a Patient-Related Outcome? A strength of the proposed study is that we are using data from a major clinical trial that was already completed. That means we can test right now how well genetic tools predict the likelihood of developing metastatic disease. The same is true for predicting side effects. If we find useful prediction in our study, we plan to confirm the findings in another dataset (another completed clinical trial for which we have existing data and specimens). We are eager to pursue the confirmatory study and will secure funding for it while the current one is ongoing. This would allow us to immediately design a clinical trial to test the genetic test(s) in patients.

Proposal Title:	Pharmacologic Downregulation of SOX2 to Enhance Efficacy of Anti-AR Therapies in Prostate Cancer
Log Number:	PC220525
Current PI Name:	Donald Vander Griend
Award Number:	HT9425-23-1-0619
Current Contracting Organization:	Illinois, University of, at Chicago
Current Performing Organization:	Illinois, University of, at Chicago
Web Approval Date:	10-03-2023

As a son and son-in-law of prostate cancer survivors, I am keenly aware of the deficiencies in our ability to treat prostate cancer. Having walked through their cancer journeys with them, it is clear that we have a long way to go for us to overcome the scourge of lethal prostate cancer. The sole purpose of my research program is to develop new ways to combat prostate cancer, from early-stage tumors to advanced metastatic disease. In particular, there is a dire need to provide surgeons and oncologists with more options for patients to aid in: (1) whether to pursue surgery, radiation, or active surveillance after an initial diagnosis and (2) what to do when the cancer returns and progresses to metastatic disease. My lab has worked to accelerate the discovery of new biomarkers and drugs using bio-informatics, cell biology, publicly-available patient-derived gene array datasets, drug screening, and research collaboration to identify new signaling pathways in prostate cancer that can be translated into biomarkers and drug targets.

The SOX2 protein is an important driver of prostate development, and when expressed in tumors, it drives tumor growth and metastatic progression. In our ongoing work we have used bioinformatic, clinical, and molecular approaches to discover that SOX2 proteins mark aggressive, metastatic prostate tumors, and that blocking SOX2 protein expression blocks tumor growth, metastasis, and makes cells much more sensitive to enzalutamide. Further, we have identified a class of drugs targeting the Insulin Growth Factor Receptor 1 (IGF1R) that decrease SOX2 expression in prostate cancer cells. This prioritizes SOX2 as a critical determinant of lethal, drug-resistant metastatic disease. Thus, understanding how it functions has the high probability to identify new targets for therapy and to galvanize SOX2 expression as novel staging tools for men diagnosed with prostate cancer. The work proposed here has three main purposes:

1. To further understand how SOX2 promotes lethal prostate cancer and identify new strategies for preventing drug resistance and treating advanced prostate cancers.

2. To understand what regulates SOX2 in prostate tumors, and to develop new strategies for decreasing SOX2 expression and blocking its activity. Our recent drug screening efforts have identified IGF1R signaling (Insulin Growth Factor 1 Receptor) as a key driver of SOX2 expression. Importantly, there are many available inhibitors of IGF1R under clinical development, and we have found that these re-sensitize SOX2-positive prostate cancer cells to enzalutamide. Thus, work proposed here has the high potential to lead to clinical trials targeting IGF1R, SOX2, and the Androgen Receptor (AR) to prevent or overcome resistance to AR-targeted therapies.

3. To develop and characterize robust biomarker approaches that can be utilized in a clinical trial to identify patients with SOX2-positive tumors that may benefit from our potential new approaches.

It is my plan and sincere ambition that completion of the work proposed here represents a significant benefit to prostate cancer patients at both early and advanced stages of disease, and also benefits future prostate cancer researchers.

Proposal Title:	Triple AKR1C3/COX2/sEH Inhibition for the Treatment of Lethal Prostate Cancer
Log Number:	PC220527
Current PI Name:	Christopher Evans
Award Number:	HT9425-23-1-0324
Current Contracting Organization:	California, University of, Davis
Current Performing Organization:	California, University of, Davis
Web Approval Date:	09-13-2023

Clinical Significance: Prostate cancer will claim lives of over 30,000 American men in 2022 alone. The disease evolves from primary tumors to castration-resistant prostate cancer (CRPC), which only takes around 2-3 years. Metastasis is the primary cause of mortality in prostate cancer patients. Bone metastases occur in more than 90% of patients with advanced prostate cancer and are associated with poor survival. CRPC is still driven by androgens, such as testosterone, which is why androgen receptor signaling inhibitors (ARSI) are widely used to treat the disease. These drugs included enzalutamide (XTANDI®), abiraterone acetate (ZYTIGA®), apalutamide (ERLEADA™) and darolutamide (Nubeqa). Although these drugs are highly effective initially, patients often quickly develop resistance to these drugs. Therefore, there is an urgent need to identify strategies to control the emergence of ARSI-resistant prostate cancer. Our research team has shown that genes such as AKR1C3, which promotes the production of androgens, are upregulated in prostate cancer cells and xenograft tumors that have stopped responding to enzalutamide and abiraterone treatment. Clinical data from the literature confirm that inflammation promotes the development of prostate cancer and is accompanied by high-grade cancer. PTUPB, previously identified as an anti-inflammatory small molecule, has shown great potential to inhibit AKR1C3 enzymatic activity and synergize with enzalutamide treatment in ARSI-resistant prostate cancer. The overarching goal of this translational application is to further develop PTUPB and build a strong rationale for translating the drug to a clinical trial for the treatment of lethal CRPC. Notably, our assembled team has a great deal of experience in the design and implementation of clinical trials for the treatment of men with CRPC. Research strategies and major expected outcomes: We have developed a novel small-molecule, PTUPB, which is more effective than our previous reports on indomethacin in AKR1C3 enzymatic activity inhibition, as well as in suppression of prostate cancer growth. Preliminary data also demonstrated that PTUPB inhibited tumor inflammatory response and had a superior pharmacokinetic profile. In this project, we will evaluate the properties of PTUPB and test its efficacy in novel patient tumor-derived models of CRPC for future clinical trial initiation. We will determine whether PTUPB alone and in combination with enzalutamide suppresses prostate cancer progression using novel prostate cancer metastasis models. We will study PTUPB for its inhibition of steroidogenesis and the inflammatory network and discover novel targets for prostate cancer treatment.

Translational Implications: Our team has extensive expertise in translating therapeutics into the clinical setting underscoring the feasibility of our proposal. After the completion of the project, we expect to initiate a pre Investigational New Drug (IND) consultation with the FDA and perform the studies in the following year. We expect to conduct a single-center, open-label, single-arm phase I dose escalation study using PTUPB in CRPC patients subsequent to the IND report within 5 years. We will also consult with our prostate cancer patient’s advocate to ensure that patients’ voices or concerns are considered. PTUPB is based on the FDA approved celecoxib structure. Therefore, we do not anticipate the potential adverse effects of PTUPB but will validate its safety and tolerability. Importantly, our previous findings indicate that PTUPB may reduce cardiovascular risks compared with celecoxib. The outcomes of the proposed studies will provide a strong rationale for translating PTUPB into the clinical setting and addressing the major unmet need to overcome ARSI resistance in CRPC patients. We believe that PTUPB will ultimately increase the overall survival and improve the quality of life of men diagnosed with CRPC.

Proposal Title:	Triple AKR1C3/COX2/sEH Inhibition for the Treatment of Lethal Prostate Cancer
Log Number:	PC220527P1
Current PI Name:	Chengfei Liu
Award Number:	HT9425-23-1-0325
Current Contracting Organization:	California, University of, Davis
Current Performing Organization:	California, University of, Davis
Web Approval Date:	09-13-2023

Clinical Significance: Prostate cancer will claim lives of over 30,000 American men in 2022 alone. The disease evolves from primary tumors to castration-resistant prostate cancer (CRPC), which only takes around 2-3 years. Metastasis is the primary cause of mortality in prostate cancer patients. Bone metastases occur in more than 90% of patients with advanced prostate cancer and are associated with poor survival. CRPC is still driven by androgens, such as testosterone, which is why androgen receptor signaling inhibitors (ARSI) are widely used to treat the disease. These drugs included enzalutamide (XTANDI®), abiraterone acetate (ZYTIGA®), apalutamide (ERLEADA™) and darolutamide (Nubeqa). Although these drugs are highly effective initially, patients often quickly develop resistance to these drugs. Therefore, there is an urgent need to identify strategies to control the emergence of ARSI-resistant prostate cancer. Our research team has shown that genes such as AKR1C3, which promotes the production of androgens, are upregulated in prostate cancer cells and xenograft tumors that have stopped responding to enzalutamide and abiraterone treatment. Clinical data from the literature confirm that inflammation promotes the development of prostate cancer and is accompanied by high-grade cancer. PTUPB, previously identified as an anti-inflammatory small molecule, has shown great potential to inhibit AKR1C3 enzymatic activity and synergize with enzalutamide treatment in ARSI-resistant prostate cancer. The overarching goal of this translational application is to further develop PTUPB and build a strong rationale for translating the drug to a clinical trial for the treatment of lethal CRPC. Notably, our assembled team has a great deal of experience in the design and implementation of clinical trials for the treatment of men with CRPC. Research strategies and major expected outcomes: We have developed a novel small-molecule, PTUPB, which is more effective than our previous reports on indomethacin in AKR1C3 enzymatic activity inhibition, as well as in suppression of prostate cancer growth. Preliminary data also demonstrated that PTUPB inhibited tumor inflammatory response and had a superior pharmacokinetic profile. In this project, we will evaluate the properties of PTUPB and test its efficacy in novel patient tumor-derived models of CRPC for future clinical trial initiation. We will determine whether PTUPB alone and in combination with enzalutamide suppresses prostate cancer progression using novel prostate cancer metastasis models. We will study PTUPB for its inhibition of steroidogenesis and the inflammatory network and discover novel targets for prostate cancer treatment.

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Proposal Title:	Ultrasmall PSMA-Targeted Particle Radiotherapy for Regulating the Immunosuppressive Prostate Cancer Tumor Microenvironment and Enhancing Treatment Efficacy
Log Number:	PC220534
Current PI Name:	Michelle Bradbury
Award Number:	HT9425-23-1-0631
Current Contracting Organization:	Joan and Sanford I Weill Medical College of Cornell University
Current Performing Organization:	Joan and Sanford I Weill Medical College of Cornell University
Web Approval Date:	09-13-2023

Advanced prostate cancer (PCa) is the most common malignancy in Veterans and the fourth leading cause of cancer death in men worldwide. Despite advances in therapeutic options for metastatic and treatment-resistant disease, the average 5-year survival rate is less than 30%. Principal issues leading to treatment failure include resistance that often develops to standard-of-care therapy and an inherently “cold” tumor microenvironment (TME) that suppresses immune responses needed to fight the disease. The reversal of these suppressive activities remains a clinical challenge, as they are primarily driven by multiple inhibitory molecules and immune cells, as well as a relative lack of tumor-specific antigens and T cells needed to boost immune responses. As a result, most advanced PCa patients derive little benefit from immunotherapeutic (IT) agents. An important objective of this work is to therefore develop novel combination treatment strategies that not only enhance the activity of ITs, in this case, immune checkpoint blockade (ICB), but can substantially improve clinical outcomes.

In this proposal, we introduce an emerging treatment strategy utilizing ultrasmall (sub-8 nanometer [nm]) fluorescent silica nanoparticles, Cornell prime dots (or C’ dots) that encapsulate fluorescent dye molecules for optical target visualization. Earlier generation C’ dots have previously been translated to the clinic for fluorescence image-guided surgery and, more recently, as a drug delivery vehicle for cancer therapy. Recently, we discovered that the base particle itself, in the absence of a drug, exhibits multiple inherent anti-cancer properties that can be used to alter the TME in a manner that potentially boosts responsiveness to IT. In PCa models, C’ dots have been found to significantly (i) reduce immune cell populations within the TME that can limit treatment effectiveness; (ii) activate anti-tumor immune cells, such as cytotoxic T cells; and (iii) induce cancer cell death programs that may synergize with IT. On this basis, we develop and assess the therapeutic capabilities of a novel ultrasmall targeted C’ dot radiotherapy (RT) that, combined with IT, can favorably reprogram the TME in a mouse model of PCa, the Hi-Myc model, that shares molecular features with human PCa.

To realize these goals, we will first precision engineer C’ dots with varying numbers of molecules targeting prostate-specific membrane antigen (PSMA), a highly expressed marker on PCa cells. These newer generation PSMA-targeting C’ dots (or PSMAi-C’ dots) will be screened in Aim I to identify one (or more) lead probes that maximize cell kill, as well as biologic and immune-related properties relative to non-targeting C’ dots. In Aim II, lead probes will be adapted with an imaging radiolabel to identify candidates showing favorable whole-body distributions in the Hi-Myc model after intravenous injection using positron emission tomography (PET) imaging. In Aim III, we will identify novel combination treatment paradigms (i. e., lead PSMA-C’ dot RT plus ICB) that maximize anti-tumor immune responses and therapeutic efficacy in this same model over single-agent therapies.

The contributions of this work and our projected outcomes support the overarching challenges of the Prostate Cancer Research Program. First, we have developed a novel treatment that has the potential to ultimately improve outcomes in men with advanced/lethal PCa. Specifically, we aim to advance a clinically translatable first-in-class sub-8-nm particle platform that selectively targets and delivers a PSMA-targeting and beta-emitting RT, 177Lu PSMAi-C' dots to sites of disease. Second, we anticipate that radiation-induced tumor cell kill, augmented by the intrinsic therapeutic properties of the C' dots itself, will minimize inhibitory TME activities and lead to efficacious anti-cancer responses. We also predict that 177Lu-PSMAi-C' dots will further extend survival times relative to commercially available molecular RTs given its broader array of therapeutic capabilities localized only to the TME. Third, by converting a "cold" to a "hot" TME, we predict a more responsive TME to both conventional and newer generation ICB regimens; such regimens are expected to synergize with 177Lu-PSMAi-C' dots to yield improved treatment outcomes over molecular RTs. Upon meeting these milestones by the end of the post-award period (Year 2), we will execute regulatory steps towards the translation of 177Lu-PSMAi-C' dots to the clinic, including the completion of an investigational new drug (IND) application and an early-stage clinical trial protocol to assess product safety in patients with advanced/lethal PCa (e.g., metastatic and treatment-resistant disease). The projected time to reach a patient-related outcome will be about 2 years after study completion.

Potential clinical applications and their benefits include: (i) evaluation of novel combinatorial treatment paradigms aimed at significantly improving treatment outcomes and ultimately aiding disease eradication; (ii) treatment of immunologically "cold" TMEs beyond the primary site (i.e., lymph nodes) to further reduce systemic disease burden and improve patient outcomes; and (iii) validation of preclinical therapeutic endpoints in clinical trial settings for their potential future use as early-stage response indicators. While adverse events pose a potential risk, these can be addressed by readjusting the dose levels of 177Lu-PSMAi-C' dots administered.

Proposal Title:	Ultrasmall PSMA-Targeted Particle Radiotherapy for Regulating the Immunosuppressive Prostate Cancer Tumor Microenvironment and Enhancing Treatment Efficacy
Log Number:	PC220534P1
Current PI Name:	Ulrich Wiesner
Award Number:	HT9425-23-1-0632
Current Contracting Organization:	Cornell University, Ithaca
Current Performing Organization:	Cornell University, Ithaca
Web Approval Date:	09-13-2023

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In this proposal, we introduce an emerging treatment strategy utilizing ultrasmall (sub-8 nanometer [nm]) fluorescent silica nanoparticles, Cornell prime dots (or C’ dots) that encapsulate fluorescent dye molecules for optical target visualization. Earlier generation C’ dots have previously been translated to the clinic for fluorescence image-guided surgery and, more recently, as a drug delivery vehicle for cancer therapy. Recently, we discovered that the base particle itself, in the absence of a drug, exhibits multiple inherent anti-cancer properties that can be used to alter the TME in a manner that potentially boosts responsiveness to IT. In PCa models, C’ dots have been found to significantly (i) reduce immune cell populations within the TME that can limit treatment effectiveness; (ii) activate anti-tumor immune cells, such as cytotoxic T cells; and (iii) induce cancer cell death programs that may synergize with IT. On this basis, we develop and assess the therapeutic capabilities of a novel ultrasmall targeted C’ dot radiotherapy (RT) that, combined with IT, can favorably reprogram the TME in a mouse model of PCa, the Hi-Myc model, that shares molecular features with human PCa.

To realize these goals, we will first precision engineer C’ dots with varying numbers of molecules targeting prostate-specific membrane antigen (PSMA), a highly expressed marker on PCa cells. These newer generation PSMA-targeting C’ dots (or PSMAi-C’ dots) will be screened in Aim I to identify one (or more) lead probes that maximize cell kill, as well as biologic and immune-related properties relative to non-targeting C’ dots. In Aim II, lead probes will be adapted with an imaging radiolabel to identify candidates showing favorable whole-body distributions in the Hi-Myc model after intravenous injection using positron emission tomography (PET) imaging. In Aim III, we will identify novel combination treatment paradigms (i. e., lead PSMA-C’ dot RT plus ICB) that maximize anti-tumor immune responses and therapeutic efficacy in this same model over single-agent therapies.

The contributions of this work and our projected outcomes support the overarching challenges of the Prostate Cancer Research Program. First, we have developed a novel treatment that has the potential to ultimately improve outcomes in men with advanced/lethal PCa. Specifically, we aim to advance a clinically translatable first-in-class sub-8-nm particle platform that selectively targets and delivers a PSMA-targeting and beta-emitting RT, 177Lu PSMAi-C' dots to sites of disease. Second, we anticipate that radiation-induced tumor cell kill, augmented by the intrinsic therapeutic properties of the C' dots itself, will minimize inhibitory TME activities and lead to efficacious anti-cancer responses. We also predict that 177Lu-PSMAi-C' dots will further extend survival times relative to commercially available molecular RTs given its broader array of therapeutic capabilities localized only to the TME. Third, by converting a "cold" to a "hot" TME, we predict a more responsive TME to both conventional and newer generation ICB regimens; such regimens are expected to synergize with 177Lu-PSMAi-C' dots to yield improved treatment outcomes over molecular RTs. Upon meeting these milestones by the end of the post-award period (Year 2), we will execute regulatory steps towards the translation of 177Lu-PSMAi-C' dots to the clinic, including the completion of an investigational new drug (IND) application and an early-stage clinical trial protocol to assess product safety in patients with advanced/lethal PCa (e.g., metastatic and treatment-resistant disease). The projected time to reach a patient-related outcome will be about 2 years after study completion.

Potential clinical applications and their benefits include: (i) evaluation of novel combinatorial treatment paradigms aimed at significantly improving treatment outcomes and ultimately aiding disease eradication; (ii) treatment of immunologically "cold" TMEs beyond the primary site (i.e., lymph nodes) to further reduce systemic disease burden and improve patient outcomes; and (iii) validation of preclinical therapeutic endpoints in clinical trial settings for their potential future use as early-stage response indicators. While adverse events pose a potential risk, these can be addressed by readjusting the dose levels of 177Lu-PSMAi-C' dots administered.

Proposal Title:	A Primary Role for the Circulatory Microenvironment in African American Prostate Cancer Disparities?
Log Number:	PC220539
Current PI Name:	Norman Lee
Award Number:	HT9425-23-1-0409
Current Contracting Organization:	George Washington University
Current Performing Organization:	George Washington University
Web Approval Date:	10-02-2023

The incidence of prostate cancer (PCa) has increased markedly over the last decades and is the second most prevalent noncutaneous cancer in males in developed regions of the world. In the United States, PCa is the most frequently diagnosed cancer in men, with 268,490 estimated new cases in 2022. It is also the second most common cause of cancer deaths in men, exceeded only by lung cancer, with 34,500 estimated deaths in 2022. However, the risk factors associated with PCa remain to be elucidated. The only firmly established risk factors for PCa are age, family history, and race/ethnicity. Notably, African Americans have among the highest PCa incidence and mortality. The striking population disparities in cancer risk and survival outcomes are borne out of current health statistics data. Black U.S. males are 1.4 to 1.7 times more likely to develop PCa, and 1.7 to 2.2 times more likely to die from this disease compared to their White counterparts. Although PCa was originally thought to be uncommon in subequatorial Africa, it is increasingly apparent that, as the average age of central and west-African populations increases, PCa is becoming more common. As of 2020, sub-Saharan Africa and the Caribbean suffered some of the highest PCa mortality in the world with rates ranging from 22.0-27.9 deaths per 100,000, which is in line with the African American population of 37.9 deaths per 100,000.

Based on epidemiological studies, several hypotheses brought forward to explain these disparities include cultural, access to health care, diet, systemic racism, residential segregation leading to exposure of environmental pollutants and chemical carcinogens, and/or socioeconomic stressors, to name a few. In addition to these features, a growing body of biological and genetic evidence suggests that genetic polymorphisms and aberrant gene expression are important influences predisposing or contributing to the disparities of cancer incidence and mortality. While intriguing, these findings have been association-based (‘guilt by association’), and direct causal links of the candidate genes to cancer health disparities remain to be firmly established.

Another possible contributing factor to PCa disparities in the African American population is the immediate environment surrounding the primary tumor, known as the microenvironment. The primary tumor’s microenvironment contains a variety of components such as immune cells, fibroblasts, blood vessels and extracellular proteins that can interact with tumor cells to modify the behavior of the tumor cells. Research has demonstrated that these interactions may be different in African American’s with PCa, potentially leading to a more aggressive cancer.

In addition to the surrounding microenvironment of the primary tumor, there is another microenvironment that has received less attention, specifically the circulatory microenvironment comprised of platelets that encounter and communicate with circulating tumor cells. While platelets are primarily known for their cardiovascular role in blood clotting, increasing evidence points to their critical role in cancer progression and mortality. Our early findings indicate that the interactions of platelets and PCa cells are different in the African American population compared to the European American population. The objectives of this application is to study the interactions of platelets with PCa cells in the two populations, and how differences in these interactions may be contributing to PCa disparities in the African American population.

Proposal Title:	Increasing Access to Definitive Treatment for Prostate Cancer by Removing Transportation Barriers for Underserved Patients: A Multilevel Feasibility Study
Log Number:	PC220551
Current PI Name:	Quoc-Dien Trinh
Award Number:	HT9425-23-1-0784
Current Contracting Organization:	Brigham and Women's Hospital, Inc.
Current Performing Organization:	Brigham and Women's Hospital, Inc.
Web Approval Date:	10-03-2023

Scientific Objective and Rationale: In the United States, significant racial disparities persist for prostate cancer. This is especially true for Black men, who experience a twofold-higher risk of mortality compared to White men. In settings where Black and White men have equal access to care such as the military health system, there is very little racial difference in prostate cancer treatment and outcomes, suggesting that improving access to timely treatment for Black men can reduce disparities. While research on access to treatment typically focuses on insurance coverage, access is a multidimensional concept encompassing geographic, convenience, and cultural factors. Travel burden, reflected by the time and energy associated with travel to reach prostate cancer treatment, is associated with delays and worse outcomes. Residential segregation places Black men far from high quality care and reliable transportation networks, increasing their travel burden relative to White men. Therefore, reducing travel burden could improve equitable access to prostate cancer treatment, reducing Black-White disparities.

Our proposal aims to produce evidence that can inform facility-level interventions to reduce travel burden for high-risk, historically marginalized populations seeking prostate cancer treatment. Our study will address this gap using data from three sources. In Aim 1, we will conduct a quantitative cohort study of the impact of travel burden, estimated using a novel geospatial model, on treatment disparities. Mapping results will allow us to determine neighborhoods where reducing travel burden could lead to greatest increases in access to treatment in Black men. The mapped results will be made available online to inform future interventions aimed at addressing disparities in prostate cancer treatment. In Aim 2, we will conduct a pilot pre-post evaluation to estimate the impact of a rideshare intervention on missed appointments in Black men seeking prostate cancer treatment. Men who are eligible based on demographic and clinical criteria will be invited to participate through our Prostate Cancer Outreach Clinic, which serves primarily minoritized populations in the Greater Boston area. Historic controls will be identified and matched to participants based on geographic and clinical characteristics. In Aim 3, we will conduct qualitative research to identify facilitators and barriers of the intervention, seeking inputs from intervention recipients and implementers. Findings from this research will be reviewed by a diverse stakeholder board, with representation from patient advocacy groups, hospital officials, and urban planners. Using a Delphi process, we will synthesize feedback from our stakeholders to inform strategies for the refinement, sustainability, and scaling of successful components of our pilot to other hospital networks in Massachusetts. Evidence from this research will inform a randomized controlled trial of transportation-based interventions to reduce travel burden for high-risk and historically marginalized populations seeking prostate cancer treatment.

Applicability of Research: This research will improve our understanding of how hospital-based prostate cancer programs can address travel burden, an access barrier that disproportionately impacts Black men. Our study incorporates complementary methods (epidemiologic research, program evaluation, qualitative research) to generate evidence regarding the feasibility and impact of interventions to reduce travel burden

for high-risk, historically marginalized groups of men at risk of prostate cancer. Through continuous engagement with our diverse stakeholder board, we can ensure that policymakers at state and local levels can use these findings to implement programs to reduce barriers to treatment for their communities. This approach aligns with the Health Disparity Research Award's overarching challenge of advancing health equity and reducing disparities for men of African descent with prostate cancer. Ongoing discussions with patients and patient advocacy groups can reveal successes and pitfalls of the intervention, providing insight on how to refine the intervention to maximize effectiveness. Discussions with healthcare officials and urban planners will inform sustainability and scalability of the intervention. We anticipate that published findings can apply to other cancer programs serving similar populations, who are also seeking to increase access to care and reduce delays in treatment. Throughout the study, we will disseminate findings with our stakeholder groups, academic colleagues, and community partners through presentations, research articles, and newsletters.

Proposal Title:	Deconvolute Key Biological Drivers That Define Subsets of Lethal Prostate Cancer in African American Men
Log Number:	PC220557
Current PI Name:	Anders Berglund
Award Number:	HT9425-23-1-0650
Current Contracting Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Current Performing Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Web Approval Date:	09-13-2023

More African American men get and die from prostate cancer than other groups of men. We know that in addition to socio-economic and socio-cultural issues, biological factors such as gene expression (known as genomics) and environmental factors that impact gene function (epigenomics) all play a role in the observed prostate cancer health disparities. If we can learn more about the role of these genomic and epigenomic factors, then we may be able to give better care to African American men with prostate cancer and save more lives. A major setback in the field of prostate cancer disparities research has been the lack of an adequate tumor samples from African American patients that can be used to rigorously investigate and understand the genomic, and epigenomic factors that drive lethal prostate cancer in African American men. To overcome this obstacle, our group of scientists has established a collaborative team to create a database of prostate tumor samples with clinical and genomic information from over 1,000 African American men, all of whom have been de-identified to protect the individual patient health information privacy. Now that we have this large database resource in place, we are poised to begin careful curation of all the data elements and perform extensive quality checks using very sophisticated bio informatics tools. The next steps will include applying analytical tools to identify unique signals known as molecular signatures that are mostly enriched in tumor samples from Americans of African descent to help us to both understand why and know when patients develop an aggressive type of prostate cancer. Once we identify these unique signals at the time the patient is diagnosed, then it will help doctors to give these patients the right treatment in a timely manner. With this work, we will take important steps towards personalized medicine. This kind of medicine is tailored to the biology of the individual, making it more effective than a “one size fits all” approach. It can help to overcome cancer health disparities.

Proposal Title:	Deconvolute Key Biological Drivers That Define Subsets of Lethal Prostate Cancer in African American Men
Log Number:	PC220557P1
Current PI Name:	Kosj Yamoah
Award Number:	HT9425-23-1-0651
Current Contracting Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Current Performing Organization:	H. Lee Moffitt Cancer Center and Research Institute, at South Florida, University of
Web Approval Date:	09-13-2023

More African American men get and die from prostate cancer than other groups of men. We know that in addition to socio-economic and socio-cultural issues, biological factors such as gene expression (known as genomics) and environmental factors that impact gene function (epigenomics) all play a role in the observed prostate cancer health disparities. If we can learn more about the role of these genomic and epigenomic factors, then we may be able to give better care to African American men with prostate cancer and save more lives. A major setback in the field of prostate cancer disparities research has been the lack of an adequate tumor samples from African American patients that can be used to rigorously investigate and understand the genomic, and epigenomic factors that drive lethal prostate cancer in African American men. To overcome this obstacle, our group of scientists has established a collaborative team to create a database of prostate tumor samples with clinical and genomic information from over 1,000 African American men, all of whom have been de-identified to protect the individual patient health information privacy. Now that we have this large database resource in place, we are poised to begin careful curation of all the data elements and perform extensive quality checks using very sophisticated bio informatics tools. The next steps will include applying analytical tools to identify unique signals known as molecular signatures that are mostly enriched in tumor samples from Americans of African descent to help us to both understand why and know when patients develop an aggressive type of prostate cancer. Once we identify these unique signals at the time the patient is diagnosed, then it will help doctors to give these patients the right treatment in a timely manner. With this work, we will take important steps towards personalized medicine. This kind of medicine is tailored to the biology of the individual, making it more effective than a “one size fits all” approach. It can help to overcome cancer health disparities.

Proposal Title:	Utilizing B7-H3 Targeted Therapeutics to Prevent Lethal Prostate Cancer: The Help Elucidate & Attack Longitudinally (HEAL) Prostate Cancer Trial Cohort
Log Number:	PC220558
Current PI Name:	Eugene Shenderov
Award Number:	HT9425-23-1-0390
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	10-02-2023

Scientific Objective and Rationale: Prostate cancer is the most prevalent male cancer and the second most common cause of cancer-related death in men. Advances have been made in early detection and local therapy, but few treatments have been able to dramatically alter the course of disease once it becomes metastatic, i.e., spreads to other organs, such as bone.

Immunotherapy–the use of a person's own immune system—to target and kill melanoma, lung, and colon cancer has resulted in unprecedented advances in treatment. Unfortunately, in prostate cancer, current immunotherapy strategies have yielded minimal objective responses—possibly due to targeting of non-optimal immune mechanisms that are involved in prostate cancer, suggesting the need for alternative immunological approaches.

We are investigating B7-H3, a presumed immune checkpoint molecule (a protein that is thought to keep the immune system in check, and blocking it allows activation of the immune system in this case against a person's own cancer). It has been shown to modulate anti-tumor immune responses and its expression has been shown to correlate with worse clinical outcomes in men with prostate cancer. Specifically, B7-H3 expression appears to be associated with biochemical progression (PSA rise) and clinical progression (visible disease on imaging scans) following local treatment.

To determine the anti-tumor, immunological and biological effects of B7-H3 targeted immunotherapy in men with high-risk localized prostate cancer, a pre-surgical, neoadjuvant, study (NCT02923180) was completed and recently presented at ASCO 2022. Patients underwent a pre-treatment prostate biopsy and then received enoblituzumab, a humanized monoclonal antibody against B7-H3, prior to radical prostatectomy. They then undergo radical prostatectomy. The primary endpoints of this study are the safety and feasibility of enoblituzumab in the pre-surgical prostate cancer setting, and the PSA0 Response (undetectable PSA level.

We have now designed a phase 2, randomized, neoadjuvant clinical trial in high-risk local prostate cancer patients that is powered to properly assess whether enoblituzumab truly has clinical efficacy seen in the small, underpowered, 32-patient trial. Novel pathologic and immunologic analyses, which are the focus of this proposal, are included as secondary endpoints in order to allow a thorough interrogation of the mechanisms underlying enoblituzumab's putative antitumor effects and provide lethal prostate cancer evolution. We hypothesize that B7-H3 is central to prostate cancer development and metastatic potential and that the studies herein will help understand lethal prostate cancer as never before.

Ultimate Applicability of the Research and Impact: The proposed research utilizes human clinically annotated samples from patients treated with enoblituzumab as described above. If the clinical trial is

successful we will proceed to an FDA registrational trial and use the analyses herein to guide trial design. No neoadjuvant therapy is approved in prostate cancer and hence significant need exists. We believe that the outlined research can achieve a patient-related outcome within 3 years, as all insights will be done in parallel to patient accrual for this soon to be launched trial, and DOD support is crucial for the biomarker correlative studies proposed.

Proposal Title:	miRNA-Related Polygenic Risk Score and miRNA Expression on Prostate Cancer Survival in Whites and Blacks
Log Number:	PC220560
Current PI Name:	Hui-Yi Lin
Award Number:	HT9425-23-1-0438
Current Contracting Organization:	Louisiana State University Health Sciences Center
Current Performing Organization:	Louisiana State University Health Sciences Center
Web Approval Date:	10-03-2023

One out of seven American men will develop prostate cancer (PCa) during their lifetime. PCa is the second leading cause of cancer-related deaths in American men and has substantial clinical heterogeneity. The 5-year survival rate for PCa patients with the distant stage is only 31%, although the rate for overall PCa patients is 99%. At the time of diagnosis, physicians often have difficulty distinguishing between patients who will develop indolent tumors and those who will develop aggressive tumors. In addition, health inequities of PCa incidence and progression have been observed between Whites and Blacks. Blacks suffer a 60% higher PCa incidence rate and 2.2 times higher mortality rate than Whites. Although the racial disparity between Whites and Blacks remains for decades, the determinants of this high rate of incidence, aggression, and mortality in Blacks are still unknown. The existing clinical features (such as clinical stage and Gleason score) are useful but insufficient for classifying high- and low-risk PCa patients. With this inadequate risk classification, approximately 20% of low-risk PCa patients died due to conservative treatment, and many PCa patients suffered side effects of overtreatments. Thus, there is an urgent need to identify additional biomarkers to improve the prediction accuracy of lethal PCa. Most current genetic association studies focus on evaluating individual effects of single nucleotide polymorphisms (SNPs), inherited genetic variants, which are insufficient to explain the complexity of disease causality.

The objective of this study is to identify tumor miRNA expression and related SNP-SNP interaction pairs and build polygenic risk scores (PRS) for predicting PCa survival by searching for the miRNA genes for Whites and Blacks. miRNAs regulate 30-60% of human genes and are considered essential components of gene regulatory networks. Our published study supported that several SNPs located in a miRNA binding site can predict PCa aggressiveness. In Aim 1, we will identify a set of miRNA expression associated with both PCa survival/aggressiveness and SNP-SNP interaction pairs or SNPs in the KLK3 SNP-interaction network and the SNPs identified in genome-wide association studies. We will perform tumor miRNA expression for the ~850 PCa cases (including 308 Blacks). In Aim 2, we will evaluate SNP-SNP interaction pairs in this candidate gene set associated with PCa-specific survival/aggressiveness using the novel and powerful statistical methods proposed by our research team. In Aim 3, we will develop “race-specific” PRS systems and compare their performance with PCa clinical features [such as PSA, tumor stage, and Gleason score]. For Aims 2 and 3, the large-scale PCa consortium, a collection of 37,303 White and 4,888 Black PCa patients, will be applied.

Our study can make a great impact because it has many strengths. The biological mechanisms of lethal PCa may differ for race groups. To address this issue, this study will develop these risk classifiers of PCa survival /aggressiveness for Whites and Blacks separately. By doing so, better prediction of PCa survival can be achieved for each race group, so these race-specific risk classifiers based on SNPs and miRNA expression profiles can potentially reduce PCa progression and death overall and reduce disparities in PCa. In addition, this study will use data from the largest PCa consortium globally, and both Whites and Blacks will be evaluated. A majority of current studies focused on white men only. The findings will be based on solid validation for both SNP-SNP pairs and miRNA-based PRS associated with PCa survival/aggressiveness. This design will reduce false-positive findings and make results more generalizable to other PCa patients. In

addition, powerful and novel statistical methods will be used for SNP-SNP interactions and miRNA expression analyses. The identified PRS systems can predict future lethal PCa development. Our identified miRNA expression can be used for early detecting aggressive PCa. Our reliable study findings can provide valuable information toward risk model building, understanding the pathogenesis of PCa, and help to identify downstream genes. These results can guide future studies and may lead to the discovery of novel therapeutic targets. As for the long-term impact, the study results can be applied in developing effective "race-specific" screening tools to predict PCa aggressiveness and reduce PCa-related deaths. Thus, our findings will provide valuable information to precision medicine for PCa patients and reduce the racial disparity of PCa progression and mortality.

Proposal Title:	Signaling Interplay Underlying Prostate Cancer Racial Disparity
Log Number:	PC220561
Current PI Name:	Ajay Singh
Award Number:	HT9425-23-1-0452
Current Contracting Organization:	South Alabama, University of
Current Performing Organization:	South Alabama, University of
Web Approval Date:	10-02-2023

Prostate cancer (PCa) is the most commonly diagnosed non-cutaneous malignancy and second leading cause of cancer-related death in males in the United States. It also exhibit one of the most significant disparities in incidence and clinical outcomes. Especially, African American (AA) men are nearly at two-third higher risk of being diagnosed with PCa and two-times more likely to die because of it, compared to the Caucasian American (CA) men. Over the past several decades, we have made significant advancements in our understanding of PCa biology and therapeutic management, but a significant proportion of patients still continues to succumb to this disease, sooner or later, due to the therapy failure and disease recurrence. Androgen deprivation or castration therapy (ADT/CT) remains the mainstay for the treatment of advanced and metastatic PCa, but the tumors relapse in a castration- resistant (CR) form after an initial clinical response in most of the cases. CR PCa are highly aggressive and do not respond optimally to other alternative therapies as well, resulting in a poor prognosis. Thus, it is extremely imperative to characterize mechanisms underlying disease progression and therapy resistance, and use that knowledge to develop novel preventive and therapeutic interventions.

To address race-associated PCa health disparities, it is important to first understand how tumors from different races differ in their biology and molecular landscape. In that regard, we have made several preliminary observations that provide us clues for possible mechanistic underpinning of PCa racial disparities. We have observed that AA PCa has higher expression of MYB, a protein associated with disease aggressiveness and castration therapy resistance. Interestingly, high MYB expression is also detected in androgen receptor (AR) negative prostate cancer cells and in cell lines derived after prolonged exposure to antiandrogen (enzalutamide). These cells also tend to express higher levels of glucocorticoid receptor (GR), a protein linked with emergence of enzalutamide resistance. Furthermore, we observe that AA PCa patients have higher levels of cortisol (glucocorticoid, a stress hormone) and IL6 (an inflammatory cytokine) in their serum compared to CA PCa patients. Interestingly, we also observe that IL6 induces MYB expression and MYB forms a complex with ligand activated GR in PCa cells. Moreover, glucocorticoid/dexamethasone treatment promotes PCa cell survival and enzalutamide resistance, which is dependent on MYB overexpression. These compelling findings have led us to hypothesize that enhanced expression of MYB in AA PCa mediates the crosstalk of stress (Cortisol/GR) and inflammatory (IL6/STAT3) signaling pathways to drive PCa aggressiveness and therapy resistance. We will test this hypothesis in three specific aims by (i) investigating mechanisms of IL6-mediated MYB upregulation and gene regulatory consequences of MYB-GR crosstalk in PCa cells, (ii) establishing the pathobiological significance of MYB-mediated GR and IL6 signaling crosstalk in aggressive PCa progression and therapy resistance, and (iii) examining the correlative expression of MYB, GR, and IL6 and their racial association and collective significance with the clinicopathologic progression of PCa. Resulting data from proposed innovative studies will provide experimental, mechanistic, pre-clinical and clinical support for a novel signaling interplay driving PCa aggressiveness, therapy resistance, and racial disparities. In the longer term, this information can be useful for devising novel approaches for risk prediction and PCa management leading to reduction of disparity gaps.

Proposal Title:	Proprietary Arginine Vasopressin Receptor Type 1a (AVPR1a) Antagonists for Treatment of Lethal Prostate Cancer
Log Number:	PC220586
Current PI Name:	Kerry Burnstein
Award Number:	HT9425-23-1-0389
Current Contracting Organization:	Miami, University of, Coral Gables
Current Performing Organization:	Miami, University of, Coral Gables
Web Approval Date:	07-31-2023

Background and Rationale: Androgen deprivation therapies are initially effective for prostate cancer, but invariably tumors become resistant to this treatment. This disease stage, termed castration-resistant prostate cancer (CRPC), is incurable and new therapeutic approaches are critically needed particularly for metastatic (m)CRPC which has a dismal 30% 5-year survival rate. PC most frequently metastasizes to the skeleton and causes extensive bone damage that greatly contributes to patient morbidity and diminishes the effectiveness of chemotherapies to treat PC. We discovered that a protein, arginine vasopressin receptor type 1a (AVPR1A), is present in highest amounts in human CRPC and metastatic CRPC tumors compared to early-stage tumors. We showed that a safe and effective compound that blocks AVPR1A (tested in human clinical trials for non-cancer disorders) decreased CRPC and, of particular importance, decreased end-stage CRPC growth in the skeleton in mouse models. We published these results in the high impact journal Science Translational Medicine (Zhao et al. 2019). Follow up studies (Heidman et al. 2022 Molecular Cancer Research) extended these findings and also showed that CRPC cells can make the hormone arginine vasopressin (AVP) that activates AVPR1A. This latter finding is of clinical importance because AVP made by CRPC tumors may establish a vicious cycle of perpetual tumor growth (particularly bone mCRPC). Thus, drugs that block AVPR1A may be very effective for mCRPC. Unfortunately, there are no FDA approved AVPR1A blocker drugs and the manufacturer of an existing compound (balovaptan) will not authorize its use for CRPC. With no available FDA-approved antagonists, co investigator Dr. Yangbo Feng, a medicinal chemist, designed and synthesized seven new balovaptan-like compounds for which intellectual property can be established. (Achieving intellectual property rights will facilitate all partnerships necessary for clinical trials.) These compounds performed well as AVPR1A blockers in cell-based experiments. The top two compounds demonstrated excellent drug-like properties in animals.

Objective and Aims: Given the success we observed with known AVPR1A blockers in CRPC/mCRPC and the promising drug-like properties of our newly designed compounds, we hypothesize that blocking AVPR1A is a powerful approach for combatting CRPC/ mCRPC. We will test the most promising new AVPR1A blocker in a spectrum of pre-clinical CRPC models (from invasive to metastatic to late-stage growth in bone) to lay the foundation for Investigational New Drug approval and eventual clinical trials. Focusing the expertise of our multi-disciplinary team of scientists, our strategy consists of three highly achievable aims. First, we will complete our pharmacological analysis of the new AVPR1A blockers in animals, to identify the most selective and effective AVPR1A blocker and determine optimal dosing. Second, we will test the lead AVPR1A blocker in a continuum of CRPC models. Specifically, we will evaluate the anti-tumor effects of an AVPR1A blocker both in human CRPC cell lines growing in mouse prostates and in human tumors transplanted to mice (aka, patient-derived xenografts), collectively representing an array of PC features found in men. Third, co-investigator Dr. Conor Lynch’s highly specialized analysis of cancers that metastasize to the skeleton (in mice) will be leveraged to evaluate our top candidate in this devasting end stage of CRPC. These studies will include mice with fully functional immune systems.

PCRP Overarching Challenges: In targeting AVPR1A for drug treatment, we advance a therapeutic strategy for those with incurable CRPC/mCRPC, directly addressing the PCRP challenge, “Develop treatments that improve outcomes for men with lethal prostate cancer.”

Patient Applicability: This proposal focuses on treating CRPC/metastatic CRPC with a compound selected for ideal pharmacological properties. By directly targeting AVPR1A (known to block CRPC/mCRPC growth), successful discoveries will translate to clinical trials and near-future new therapies.

Clinical Applications, Benefits, and Risks: AVPR1A blockers have known safety in human clinical trials conducted for non-cancer disorders. While our studies are entirely pre-clinical, this work will lead to an investigational new drug application followed by clinical trials.

Projected Time to Patient-Related Outcomes: Existing AVPR1A blockers are known to be extremely safe in humans. Accordingly, a related compound, chosen for tolerability and efficacy in CRPC, may be effectively translated to patient-ready treatments for lethal PC in a few years. If an existing AVPR1A blocker becomes available for clinical trials in PC or is FDA-approved, our studies can even more rapidly be translated to the clinic. Nevertheless, we would also continue parallel development of our novel AVPR1A blocker (with minimal brain penetration) which is specifically designed and likely to be superior for PC.

Proposal Title:	Prostate Cancer Risk, Detection, and Outcomes in Transgender and Gender-Nonconforming Adults
Log Number:	PC220612
Current PI Name:	Jennifer Anger
Award Number:	HT9425-23-1-0419
Current Contracting Organization:	California, University of, San Diego
Current Performing Organization:	California, University of, San Diego
Web Approval Date:	09-28-2023

The number of transgender individuals in the U.S. has grown significantly in the past few decades. Specifically within the Veteran population, transgender-related diagnoses are 5 times more prevalent than in the general population. Because of widespread stigma associated with gender nonconformity, many transgender and gender non-binary individuals live on the margins of society and face discrimination, exclusion, and violence. They often have difficulties accessing appropriate health care, including both care specific to their gender needs and general medical care. They also face mistreatment in the health care system, leading them to avoid it entirely. As a result, they experience poor health compared to cisgender people.

One very common treatment option is feminization or masculinization of the body through hormone therapy, which is effective in alleviating gender dysphoria, the discomfort or distress that is caused by a discrepancy between a person's gender identity and that person's sex assigned at birth. Despite widespread hormonal use, it remains unknown whether hormone therapy in transgender individuals impacts the incidence of hormone-sensitive cancers, specifically prostate cancer in transgender women (assigned male at birth, living as women).

Prostate cancer can produce higher amounts of prostate-specific antigen (PSA) than normal prostate tissue. Therefore, elevated PSA levels in the blood are an indicator of possible prostate cancer and the need for referral to a urologist for further evaluation. Patients diagnosed with prostate cancer via PSA testing (vs. physical exam) have better survival due to the early detection of aggressive disease and improved therapies. Given the impact of hormonal therapy on PSA values in men with prostate cancer, it is likely that the levels to define "abnormal" PSA in transgender women on feminizing hormones are much lower than in cisgender men. Despite the growing transgender population, normal PSA values for transgender women remain unknown and there are no formal guidelines on optimal prostate cancer screening practices for transgender women. Survival after a prostate cancer diagnosis has been shown to be worse among transgender individuals. It is not known whether this disparity is due to biological differences in the disease that could be related to hormone therapy or due to barriers to care for transgender patients (i.e., ongoing stigma and discrimination, patient denial about undergoing screening or treatment for a "male" cancer, and patient /clinician awareness of prostate cancer risk).

We hypothesize that prostate cancer is underdiagnosed in this population, but this disparity can be reduced by establishing transgender-specific PSA screening guidelines and improving our understanding of the unique barriers to care and discrimination that transgender patients face. This study looks to improve the detection of prostate cancer in transgender women who suffer from worse health outcomes compared to their cisgender counterparts. Firstly, the study will quantify current disparities in cancer screening by looking at rates of PSA screening in transgender women compared to cisgender men. It will also improve detection by establishing normal PSA values for transgender women since current normal values are only based on cisgender men and determine the relationship between hormone levels and PSA values in transgender women. Lastly, we seek to understand attitudes about prostate cancer screening and care among transgender

Veterans, identify barriers to care and sources of discrimination, and propose potential solutions through interviews with transgender women with a history of prostate cancer.

Current guidelines for prostate cancer are based on studies of cisgender men and fail to fully encompass the nuanced biology and challenges of care of transgender women necessary to optimize health outcomes in this population. This study seeks to fill fundamental knowledge gaps about prostate cancer in transgender patients. The information gathered in this study will likely provide the necessary evidence to develop transgender-specific guidelines for prostate cancer screening and treatment to reduce disparities in this population.

Proposal Title:	Understanding Heterogeneity and Improving the Precision of Prostate Cancer Clinical Outcomes in the Modern Era
Log Number:	PC220615
Current PI Name:	Susan Halabi
Award Number:	HT9425-23-1-0393
Current Contracting Organization:	Duke University
Current Performing Organization:	Duke University
Web Approval Date:	07-31-2023

Currently, when a man develops metastatic prostate cancer and is commencing testosterone suppression, a decision needs to be made as to whether to add in docetaxel, a chemotherapy agent, or an agent that blocks the activity of the androgen receptor even further (abiraterone, enzalutamide, apalutalide, darolutamide). All of these drugs have been shown to prolong the survival of men in this setting. But not all men respond to these treatments, and we need to develop more therapies in an expeditious manner. Another major problem facing both physicians and men with metastatic hormone-sensitive prostate cancer is whether a specific therapy will work well for their individual needs and is a major unmet clinical need. Another unmet need is understanding the variability in response in men with hormone sensitive prostate cancer. In this Data Science Award (DSA), our team proposes to understand the factors that impacts a patient's survival.

Clinical Applications: By identifying the prognostic factors of OS, they can assist in identifying subgroups of men with particularly good or poor prognoses for whom therapy can be subsequently tailored. These factors can be incorporated into management of patients. We have assembled a team of internationally recognized medical oncologists, urologists, radiation oncologists, biostatisticians and clinical trialists in prostate cancer who have led the development and validation of models as well as clinical trials. We are uniquely positioned to leverage existing resources and conduct the proposed analysis that addresses the overarching challenges to optimize treatments of men with metastatic prostate cancer over this 3-year DOD DSA grant and will combine our institutional resources as leverage to ensure success. Successful development of prognostic models for survival and identification of patients who fail very quickly while on therapy will have short- and long-term impact for the prioritization of novel treatments, as well as the design and expeditious conduct (optimal sample size and duration of trial) and analysis of trials, and would speed the development of new treatments that will lead to faster access to effective care for men with advanced prostate cancer.

Proposal Title:	Understanding heterogeneity and Improving the Precision of Prostate Cancer Clinical Outcomes in the Modern Era
Log Number:	PC220615P3
Current PI Name:	Christopher Sweeney
Award Number:	HT9425-23-1-0394
Current Contracting Organization:	Adelaide, University of
Current Performing Organization:	Adelaide, University of
Web Approval Date:	07-31-2023

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Proposal Title:	Exploiting Real-World Clinical Genomic Data to Discover Biomarkers and Address Outcome Disparity for Prostate Cancer
Log Number:	PC220617
Current PI Name:	Gavin Ha
Award Number:	HT9425-23-1-0444
Current Contracting Organization:	Fred Hutchinson Cancer Center
Current Performing Organization:	Fred Hutchinson Cancer Center
Web Approval Date:	07-31-2023

All prostate cancer tumor cells will have the hallmark of mutated or altered DNA. Some of these DNA alterations can now be detected in the clinic using techniques of sequencing DNA of tumor cells from a biopsy. Tumor DNA sequencing generates data that requires computer software to analyze. As more of these tests are developed and applied in the clinic, tumors are profiled routinely for many patients in standard of care. These tests have generated data that represent a real-world accumulation of tumors from patients with various ancestry and ethnic backgrounds. However, despite these approved tests being offered routinely, there is still an under-representation of tumors profiled for some populations, such as those with African ancestry. Thus, it has been challenging to comprehensively study why prostate cancer mortality varies substantially between racial and ancestral backgrounds.

These clinical tests typically profile whether mutations exist in a certain panel of genes that are known to be associated with cancer. However, these panels usually represent less than 0.2% of the human genome, resulting in missing information that may be crucial to understanding how the tumor may respond to treatment. For example, inherited DNA differences or variants are rarely fully captured even though they are important for determining ancestral marks that may be linked to tumor risk and outcome; also missed by these panels are scars throughout the genome, which are patterns or footprints of disrupted biological processes in the cells.

To address these questions, we will analyze an unprecedented number of over 20,000 tumor samples profiled by DNA sequencing in the real-world clinical setting. Our objective is to develop innovative computer algorithms to analyze ancestry markers and DNA alterations throughout the entire genome, beyond the panel of selected genes. We aim to use these results to unravel the relationship between the DNA alteration patterns and ancestry markers to learn how they contribute to disparities in survival and to discover new biomarkers that predict how prostate cancers respond to treatment.

The new tools developed in this project can be readily adapted into current workflows for an immediate impact for patients. This project will also contribute to the on-going efforts to accelerate prospective studies of prostate cancer patients with diverse ancestral backgrounds and advance precision oncology for underserved populations. Therefore, this work will directly address the PCRP Overarching Challenges to define the biology of lethal prostate cancer to reduce death and to advance our understanding of health disparities in prostate cancer through innovative approaches to exploit real-world clinical data for biomarker discovery.

Proposal Title:	Exploiting Real-World Clinical Genomic Data to Discover Biomarkers and Address Outcome Disparity for Prostate Cancer
Log Number:	PC220617P2
Current PI Name:	Jian Carrot-Zhang
Award Number:	HT9425-23-1-0445
Current Contracting Organization:	Sloan Kettering Institute for Cancer Research
Current Performing Organization:	Sloan Kettering Institute for Cancer Research
Web Approval Date:	07-31-2023

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Proposal Title:	Preclinical Development of Novel Liver-Stage Active Antimalarials with Radical Cure Potential
Log Number:	PR210491P1
Current PI Name:	Alison Roth
Award Number:	CDMRPL-22-1-PR210491P1
Current Contracting Organization:	Walter Reed Army Institute of Research (WRAIR)
Current Performing Organization:	Walter Reed Army Institute of Research (WRAIR)
Web Approval Date:	06-23-2022

This application, titled “Preclinical Development of Novel Liver Stage Active Antimalarials with Radical Cure Potential,” supports studies that will make an important contribution toward research for disease control related to the following FY21 PRMRP topic area: Malaria. It specifically addresses the Area of Encouragement: Identification of novel and/or innovative malaria drug targets for blood- and liver-stage malaria parasites.

Malaria remains one of the deadliest diseases in the world today, as it has been so for thousands of years. The demoralizing impact of the disease extends beyond the annual body count to effects on family and social structure, on sustained poverty in endemic areas, and on creating untold suffering for nearly half of the world’s population. The situation is worsening due to the emergence and spread of strains of malaria parasites that harbor resistance to multiple drugs, including the front-line antimalarial combination therapy. If the global effort to eradicate malaria is to be successful and sustainable, both prevention and treatment must address the gaps and weaknesses in the armamentarium of available therapies. Ongoing needs include affordability, safety for the most vulnerable patients, single-dose treatment, aptitude for killing liver-stage parasites with relapse prevention, low susceptibility to drug resistance, and the ability to block transmission. Our novel acridone chemotype represents a broad-spectrum approach with the potential to vanquish those challenges.

In the previous project period, our approach was aggressive and rigorous, with extensive multi-stage evaluations and lead optimization processes. We have selected a preclinical candidate with promising attributes. The specific goal of this FY21 Expansion Award project is to conduct extensive and comprehensive preclinical evaluations of our late lead acridone candidate, and ultimately, we envision identifying and producing an antimalarial acridone candidate that warrants clinical development in phase 1 and phase 2 trials.

This research project builds upon a continuous collaboration between Portland VA Research Foundation and Walter Reed Army Institute of Research. The choice of a Partnering PI approach reflects the different sets of expertise that will be needed for successful completion of the project plan. The nature of the project will require the two laboratories to work together simultaneously and synergistically for extensive preclinical evaluations. Thriving on rapid information feedback and open communication between collaborators, our collaborative team has a long and fruitful history with great proven success.

Proposal Title:	Lead Discovery of Novel Chemotypes Effective Against Relapsing Malaria
Log Number:	PR210769
Current PI Name:	Alison Roth
Award Number:	CDMRPL-22-0-PR210769
Current Contracting Organization:	Walter Reed Army Institute of Research (WRAIR)
Current Performing Organization:	Walter Reed Army Institute of Research (WRAIR)
Web Approval Date:	06-15-2022

This proposal addresses the PRMRP Topic Area of Malaria and to two FY21 Areas of Encouragement: (1) identification of novel and/or innovative malaria drug targets for blood and liver stage malaria parasites; and (2) investigation of mechanisms of drug resistance in malaria, to include host, pathogen, and region-specific resistance against drugs used for treatment and prophylaxis.

Malaria ranks #4 on the infectious disease threat list created by the Military Infectious Disease Research Program. Every year, malaria infects about 229 million individuals and kills >400,000 worldwide. Plasmodium falciparum (one of the major malaria causing parasites) resistance to the first choice drug artemisinin has spread throughout Southeast Asia and emerged in Africa, the Western Pacific and South America, endangering the lives of U.S. military personnel deployed there. Additionally, there is a significant need for development of safe, potent drugs against Plasmodium liver stage infections, which is the first stage to occur after the mosquito takes a blood meal before transitioning into the disease-causing blood stages. P. vivax parasites can remain domant as “hypnozoites” in the liver for weeks, months, to years and then reactivate to cause numerous rounds of malaria. These relapses are problematic to diagnose and detect in U. S. Service Members and Veterans who were deployed in an endemic area. The current liver-stage drugs, primaquine and tafenoquine, are dependent the host for drug activation and have major safety issues including life-threatening toxicity in certain individuals.

This project seeks to identify novel compounds that may act as safe, effective protective drugs to prevent infection and/or disease or safely cure infection, which would enhance Soldier combat effectiveness and aid the global community in malaria eradication. Though collaborations between Walter Reed Army Institute of Research (WRAIR) with the National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH), and Medicines for Malaria Ventures (MMV), we have identified novel chemotypes with protective and curative activity against the dormant liver-stage form, enabling new approaches and potential targets for development of next-generation anti-relapse drugs. The proposed research project will allow us to optimize these novel validated candidates for selectivity, potency, in vivo efficacy, and drug-like properties to select lead series for preclinical studies.

The goal of this collaboration is to leverage the combined biological and chemistry expertise at WRAIR and NCATS (with in-kind support/consultation from MMV) to optimize compounds against the liver-stage forms of malaria. The two aims of this proposal are: (1) to identify and optimize potent, selective chemical compounds that inhibit the liver stages through an iterative medicinal chemistry program and (2) to characterize the efficacy, safety, and drug-like properties of these optimized compounds in vitro and in vivo.

Proposal Title:	A State-of-the-Art Microbur-Driven Non-Absorbable Epithelial SERT Antagonist to Treat Anxiety and Depression in Military Members and the General Public
Log Number:	PR220008
Current PI Name:	Kara Gross-Margolis
Award Number:	HT9425-23-1-0816
Current Contracting Organization:	New York University
Current Performing Organization:	New York University
Web Approval Date:	10-03-2023

Depression and anxiety are common disabling conditions that have a huge negative impact on the military and society at large. Major depression (MD) affects 13.1% and 12% military members previously and currently deployed, respectively. Anxiety disorders (AD), including posttraumatic stress disorder (PTSD), are also highly prevalent in military members. MD and AD are linked to serious negative outcomes in military personnel including an increased risk of suicide and suicide attempts. The high prevalence and increased suicide risk associated with MD/AD magnifies the critical need for adequate therapies.

Selective serotonin reuptake inhibitors (SSRIs) are the first line and most highly prescribed medications for MD and AD/PTSD. The SSRIs currently available, however, have key limitations that cause discontinuation or noncompliance, including: (1) low efficacy: SSRIs are partially or fully effective in 33% more likely to suffer from mental illness including depression, and SSRIs are the preferred therapy. There is thus a critical need for new ways to treat MD/AD that: (1) increase efficacy; (2) avoid common severe side effects; and (3) prevent the long-term harmful effects of in utero exposure.

Serotonin (5-HT) is a chemical produced by the body that is thought to help prevent and/or treat MD and AD. SSRIs work by increasing 5-HT through inhibition of its major inactivator, the 5-HT reuptake transporter (SERT). SERT is located in nerve cells in the (1) brain (central nervous system; CNS), (2) gut (enteric nervous system; ENS), as well as (3) cells lining the gut wall (gut epithelium). Current SSRIs are systemically absorbed through the intestine and thus increase 5-HT signaling in the CNS, ENS, and gut epithelium.

A key obstacle to improving upon SSRIs is a lack of understanding of precisely where they act to induce their beneficial mood effects. Although it has been thought that the major direct target of SSRIs is the CNS, recent data suggest that gut 5-HT could modulate mood. We thus sought to examine whether blocking SERT selectively in the intestine (gut epithelium versus ENS) contributes to the beneficial mood-altering effects and/or the negative side effects of SSRIs. To do this, we created mice where SERT was selectively eliminated from the gut epithelium or ENS. We made three major discoveries: (1) SSRIs, when taken chronically, act on a 5-HT receptor in the CNS (5-HT2C) to decrease their antidepressant and anxiolytic abilities (SSRI exposure to the CNS can have negative effects on mood); (2) Targeted SERT blockade in the ENS causes anxiety and depression, constipation, and abdominal pain (SSRI exposure to the ENS results in the negative side effects associated with currently available [systemic] SSRIs); and (3) Targeted SERT blockade in the intestinal epithelium (mimicking an SSRI targeted exclusively to the gut epithelium and not absorbed into the blood and thus not accessing the CNS/ENS) is anxiolytic and anti-depressive and does not induce GI problems. These findings support the idea that blockade of intestinal epithelial SERT, with limited

/no systemic absorption, will effectively treat anxiety and depression while avoiding negative side effects and placental SSRI transfer to a fetus. Development of a gut epithelium-specific non-absorbable SSRI may thus be more effective and safer.

We have created and validated a state-of-the-art drug delivery system (MB-SSRI) to selectively block SERT in the intestinal epithelium. Our preliminary data show that MB-SSRIs effectively remain on the intestinal epithelium long enough to release SSRI (fluoxetine; FLX) in a controlled-release fashion that simultaneously minimizes systemic absorption. Here, we postulate that MB-SSRIs will: (1) induce anti-anxiety and anti-depressive effects while avoiding negative GI and/or mood-associated effects; and (2) minimize fetal exposure and thus the adverse effects on mood, cognition and the gut that result from current (systemic) SSRI in utero exposure. Third, we will further enhance MB-SSRIs to refine their targeting to the small intestine (where beneficial mood effects may be greatest) and to create even greater dispersion control and sustained-release capacity to improve its ease of administration on the battlefield as well as therapeutic compliance.

The Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) "Direct Topic Area" and "Areas of Encouragement" addressed here are "suicide prevention": (1) "Develop and evaluate novel treatments, strategies or therapeutic targets, including research to repurpose existing drugs;" and (2) "Develop and test treatment strategies to manage symptoms and improve quality of life for those affected by associated psychological conditions." If successful, these studies will provide the foundation for clinical trials in people with MD and AD. Because SSRIs are already approved by the Food and Drug Administration, and our current MB-SSRI platform is safe and has minimal systemic absorption, it may also benefit from faster regulatory approval and translation to address the urgent needs of military personnel with MD/AD.

Proposal Title:	A State-of-the-Art Microbur-Driven Non-Absorbable Epithelial SERT Antagonist to Treat Anxiety and Depression in Military Members and the General Public
Log Number:	PR220008P1
Current PI Name:	Mark Ansorge
Award Number:	HT9425-23-1-0817
Current Contracting Organization:	Columbia University Medical Center
Current Performing Organization:	Columbia University Medical Center
Web Approval Date:	10-03-2023

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Proposal Title:	Development of Novel Cardiac Myocyte-Specific AAV Capsids
Log Number:	PR220047
Current PI Name:	Michael Kapiloff
Award Number:	HT9425-23-1-0057
Current Contracting Organization:	Leland Stanford Junior University, The
Current Performing Organization:	Leland Stanford Junior University, The
Web Approval Date:	12-23-2022

Gene therapies are biologic drugs that delivery a custom synthetic gene to cells of the body, fixing a genetic defect or introducing a new gene that confers benefit in disease. The development of gene therapy drugs is one of the most exciting areas of modern drug development. In particular, with the approval by the U.S. Food and Drug Administration of gene therapy drugs for the eye diseases Leber congenital amaurosis and retinitis pigmentosa and for the muscular disease spinal muscular atrophy, new gene therapies are actively being sought for many chronic diseases, including those affecting the heart. Gene therapies are being proposed for all types of heart diseases, including for inherited diseases like cardiomyopathy that occur in families and for common acquired diseases like heart attacks (myocardial infarction) and heart failure. These gene therapies typically take the form of synthetic adeno-associated viruses (AAV). AAV are small viruses that are useful as biologic drugs. AAV naturally present in the environment do not cause disease and are readily modified to deliver a synthetic “mini-gene” that might alter cells in a beneficial way in disease. In theory, AAV constitute an almost limitless approach to correcting diseased cells by altering their gene program.

In this project, we address within the Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Portfolio – Cardiovascular Health, the FY22 PRMRP Topic Area – Cardiomyopathy, and the FY22 PRMRP Strategic Goal – Develop novel therapeutics or advance treatment regimens for associated cardiovascular conditions that address sex/gender or ethnic/racial differences.

A major problem for the field of cardiac gene therapy is the lack of AAV that treats only the heart. Current AAV biologics will disperse throughout the body, delivering their genetic cargo to many different types of cells that are not intended to be treated and where the gene therapy can have deleterious side effects. The heart comprises about 1/200th of the body by weight, and as most AAV drugs end up elsewhere, much larger doses of drug must be used to treat the heart. Besides the obvious need to manufacture large amounts of drug to get at least some to the heart, infusion of large doses of AAV can make the patient ill through activation of the immune system. This has resulted in paused clinical trials. In this project, we propose to take a novel approach to the design of AAV gene therapy vectors to discover an AAV that would deliver its cargo uniquely to the heart.

In this PRMRP Discovery Award, we will identify novel AAV that may be used to design gene therapies for heart disease. An immediate application will be the rescue of genetic defects in familial cardiomyopathies. In mouse models for cardiomyopathy, it has been shown that a mutant gene causing disease can be replaced with a properly functioning gene by delivering the normal gene to the heart using AAV. For multiple technical reasons, however, AAV useful in mice for heart disease are not easily deployed in human patients. The key milestone to solving this problem for human patients will be to acquire an AAV that will efficiently carry the normal gene to the patient’s heart and nowhere else in the body. Successful completion of this project will result in the development of a new AAV that has the potential to transform gene therapy for human heart disease, including cardiomyopathy.

Proposal Title:	Gut Symbiont Lipid A Family: Structures and Immunomodulation in IBD
Log Number:	PR220071
Current PI Name:	Dennis Kasper
Award Number:	HT9425-23-1-0226
Current Contracting Organization:	Harvard University, Boston
Current Performing Organization:	Harvard University, Boston
Web Approval Date:	03-27-2023

Objectives and Rationale: Inflammatory bowel diseases (IBD) are a major disorder with significant disease burdens. Over the last three decades, the number of people with IBD has increased from 3.7 million (1990) to 6.8 million (2017) globally. The U.S. has the highest prevalence rate (464.5 per 100,000) and notably, among U.S. military Veterans, the increase in the prevalence of IBDs has been even more significant. Still, current therapeutic options for IBDs largely rely on non-specific immunosuppressive agents. The side effects of these drugs are particularly problematic in patients with an elevated risk of infection and trauma, such as active Service Members. Development of therapeutics with better specificity and less toxicity is imperative.

Expansion on Our Prior Department of Defense (DOD)-sponsored work (Gut Symbiotic Lipid A Family: Structures and Immunomodulation in IBD, W81XWH1910625): Many non-genetic factors are associated with IBD. It is clear that gut commensal microbiota make critical contributions to host immune development as well as modulation of inflammatory responses. Unfortunately, developing therapeutic leads from the microbiome has proven challenging because the work of discovery requires a unique combination of in-depth expertise in microbiology, immunology, and chemistry. Based on our extensive past studies, supported by the DOD, of gut symbiotic microbiota and modulation of the immune system, we now propose to investigate the unique therapeutic leads we have discovered that originate from human symbiotic microbes. We have performed discovery research studies using several prototypic synthetic molecules that are structural analogs of the Bacteroides lipid A. We have conducted these studies in cellular and animal models. We have successfully identified a specific analog (tetraacylated lipid A) as a dominant immunoregulatory structure. We are now proposing to expand our investigation to the preclinical level by studying the therapeutic efficacy of synthetic and biogenic analogs of tetraacylated lipid A in human cells as well as humanized animal models.

Relevance to Topic Area: Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Area—Inflammatory Bowel Disease; FY22 PRMRP- Strategic Goal—1) Foundational Study—This project is relevant to the therapeutic potential of the microbiome on immune-mediated disease (Inflammatory Bowel Disease); 2) Treatment—We plan to develop and test new treatments and/or refine existing treatment strategies to minimize toxicity, and to mitigate the inflammatory and/or allergic disease state. Our proposed program for preclinical development, level 3, precisely fits the Topic Area of IBD and the Strategic Goal of both a foundational study and treatment development. The foundation being built by our program is to identify and define naturally existing immunomodulatory molecules from the microbiome. There is now very good evidence that many molecules with immunomodulatory capacity exist in the microbiome, but few have yet been harnessed and mechanistically explored. The specific molecule of interest in our program offers significant potential for treatment of patients for IBD.

Ultimate Applicability of Work: Upon successful execution of the proposal, we expect to demonstrate potent protection by the target molecules against inflammation in human cells and humanized colitis model systems. These studies are required before moving onto level 4 clinical development. This therapy would be beneficial for patients with Crohn’s disease as well as those with ulcerative colitis. One major advantage of

using these lipid As for therapy is that they are analogs of the naturally occurring molecule found in the healthy intestine and therefore likely to be less toxic than current therapies. They only activate immune cells in the intestine and are not absorbed into the blood. In the current proposal, we will preclinically expand our discovery work and further explore the therapeutic potential of tetraacylated lipid A. In summary, our proposed work will expand our knowledge acquired from mouse cells and murine disease models to the preclinical level by examining the responses to these molecules in human white blood cells from patients with IBD and controls. We will also test the therapeutic efficacy of these molecules in mice bearing human genes and human microbiota. Successful execution of our project will reveal a clear path forward to human clinical trials.

Proposal Title:	Advancing the Pulmonary Assist System for Military Transport
Log Number:	PR220076
Current PI Name:	David Skoog
Award Number:	HT9425-23-1-0508
Current Contracting Organization:	Advanced Respiratory Technologies LLC
Current Performing Organization:	Advanced Respiratory Technologies LLC
Web Approval Date:	10-03-2023

The purpose of this proposal is to further develop a commercial Pulmonary Assist System (PAS). The PAS is designed to provide compact lightweight life support for wounded soldiers during military transport. Wounded Soldiers can develop a condition known as acute lung injury, or ALI, in which their lungs are not able to properly add oxygen to and remove carbon dioxide from their blood. The most advanced form of life support, "extracorporeal life support" or ECLS, can function to perform the work of the lungs, but it is currently difficult to deploy near the battlefield primarily due to the large size and weight of the equipment and the rapid blood clotting that occurs in system components. The clotting causes the devices to clog and fail, which necessitates the use of drugs known as anticoagulants to prevent clotting. However, these drugs cause patient bleeding. This results in a need to constantly monitor patients to balance clotting and bleeding risk. Due to these problems, ECLS is currently only used on a limited basis for transportation of wounded Soldiers. Currently, the military relies on a specialized team located in the United States to travel to the location of a wounded Soldier with ALI to start ECLS treatment. This results in 1-2 days passing before a wounded Soldier can be treated with ECLS. The PAS ECLS system that we are developing would enable the most advanced form of life support to be deployed closer to the battlefield, immediately after injury, rather than waiting for a transport team to reach the patient from the United States. The PAS would then be used to support wounded Soldiers as they are moved through progressively more advanced field hospitals until they are ultimately transported back to the United States or Europe. The PAS weighs only 2.3 kg, is highly mobile, has greater durability to avoid component failure and replacement, can be used without employing anticoagulant drugs until the patient is transported to more advanced care, and can be run on battery power to allow simpler transport of the injured Warfighter from remote combat operations to higher levels of care in the United States. Thus, this project addresses the Trauma Topic Area and the "Develop improved fieldable devices to treat traumatic/acute lung injury in far forward settings, including toolsets to enable correct airway placement, oxygenation in austere settings, or miniature and/or semi-automated ventilator" Focus Area.

We have demonstrated the PAS' performance using prototype systems, and we are now ready to work toward approval from the Food and Drug Administration (FDA) to enable support of injured Soldiers. The first objective of this proposal is to generate the procedures and documentation that are required by the FDA to gain approval for the PAS gas exchanger (PAS-GE). The PAS-GE is the component necessary to support the patient's lung function.

The second objective of this proposal is to generate a final commercial design of the PAS-GE that not only satisfies FDA requirements, but that is also durable and simple to manufacture in large quantities without manufacturing defects. A significant portion of the work will be designing the plastic parts so that they can be manufactured successfully. Part of ensuring this outcome will be performing computer simulations of the molding process to identify areas of the plastic parts that may result in defects and then adjusting the design to eliminate the defects.

The last objective of this proposal is to build and fine-tune a pilot manufacturing line to make the textile component of the PAS-GE. The PAS-GE uses a custom-coated fabric composed of hollow tube-like fibers that perform its oxygen and carbon dioxide transfer function. The previous supplier of this custom-coated fiber was bought by a larger corporation, 3M, who then stopped performing the final processing steps for

this fiber. We will purchase two pieces of custom machinery that will knit the fibers into a single layer of fabric and then convert this fabric into a two-layered fabric that can be used in the PAS-GE.

After fine-tuning the settings on this machinery, we will perform experiments that compare the two-layered fabric that we produced to similar commercially available fiber from 3M. These experiments will assess: (1) whether the custom coating of the fiber remains intact throughout manufacturing; and (2) whether the ends of the fiber are properly sealed during the production. Following completion of the above objectives, we will have: (1) successfully produced the key functional fabric component of the PAS-GE in-house in order to re-establish the supply chain for this component and protect the supply chain from future disruptions; and (2) generated a finalized PAS-GE design that is ready to be manufactured and tested in the experiments that are needed for FDA approval.

Once FDA approval is obtained, the PAS will enable rapid ECLS treatment of Warfighters who develop ALI in the combat theater without needing to give the patients anticoagulant drugs. The portability, simplicity, low rate of complications, and reduced monitoring needs will enable the system to be initiated close to the battlefield and during their transport to military hospitals in Europe or the United States. Thus, the PAS would make the world's most advanced form of respiratory support rapidly available to Soldiers close to the battlefield locations where they sustain their injuries.

Proposal Title:	Development of Innovative Therapeutics Against Pressure Ulcer-Associated Bacteria
Log Number:	PR220077
Current PI Name:	Alexander Serganov
Award Number:	HT9425-23-1-0422
Current Contracting Organization:	New York University School of Medicine
Current Performing Organization:	New York University School of Medicine
Web Approval Date:	10-03-2023

Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Area and Strategic Goal: We propose to develop a novel approach to treating the most problematic pressure ulcers, specifically addressing the Strategic Goal "Develop and test therapeutics or dressings that enhance wound healing" under the "Internal Medicine" Portfolio Category and "Pressure Ulcers" Topic Area.

Millions of Americans, including many military Veterans and active Service Members, suffer from pressure ulcers annually. Pressure ulcers, or bedsores, are surface wounds caused by the weight of the body in a person unable to move normally, such as a wounded Soldier or a Veteran using a wheelchair. Most pressure ulcers heal rapidly once the pressure is relieved, but one in four pressure ulcers takes a problematic course. Despite using the best available treatment, they can take as long as a year to heal, and some never heal at all. These lesions contribute disproportionately to the billions of dollars in annual medical costs of treating pressure ulcers. Some ulcers get so deep that they can spread infection to the blood, causing "blood poisoning" (sepsis), a very serious condition causing thousands of deaths annually in the United States.

The project aims to develop new medications for healing problematic pressure ulcers and for preventing early-stage pressure ulcers from becoming problematic. Our approach is based on the recent realization that bacteria colonize most pressure ulcers and can grow, sticking to each other and to the wound surface, to form a "biofilm." The biofilm prevents the normal healing process of pressure ulcers, and the worst-affected ulcers heal poorly and become chronic wounds. Bacteria in the biofilm are protected from the immune system and survive even the most aggressive antibiotic treatment. Therefore, the biofilms are almost impossible to eradicate with currently available medications.

We propose to develop a new kind of medication to address this serious problem. In our prior studies, we have discovered a universal "defense system" that protects bacteria from being killed by antibiotics, and we found safe chemical compounds compromising its activity. These "potentiator" compounds do not kill bacteria by themselves, but they make bacteria sensitive to the killing effect of many different antibiotics. Moreover, this defense system is required for forming biofilm, and when treated with our compounds, the bacteria have difficulty growing in biofilms. Thus, these compounds are promising starting points for innovative therapeutics, combining new potentiators with approved antibacterials, to effectively suppress biofilm-forming bacteria in pressure ulcers.

Within the 4-year timeline of the project, we propose to: (1) design and characterize improved versions of our potentiator compounds; (2) test the results against typical bacterial biofilms formed in pressure ulcers and ulcers-associated chronic wounds by antibiotic-sensitive and -resistant strains of golden staph (Staphylococcus aureus), Pseudomonas aeruginosa, and other bacteria; (3) identify their best combinations with clinical antibacterials (antibiotics and antiseptics); and (4) evaluate the most promising combinations in animal models of pressure ulcers and sepsis. As the result, we anticipate obtaining efficacious candidate therapeutics of pressure ulcers ready for advancement toward clinical studies.

Pressure ulcers and the associated complications are a serious risk for wounded U.S. Service Members and Veterans because they often follow combat injuries or other medical conditions that even temporarily preclude normal mobility or normal sensation in the skin. Over 50% casualties from Operation Iraqi Freedom had pressure ulcers. Patients confined to bed or requiring the use of a wheelchair are especially vulnerable: approximately half of Veterans with spinal cord injuries develop serious pressure ulcers during their lives.

Our innovative therapy will help the patients with advanced-stage pressure ulcers to heal. Moreover, we anticipate that timely administration of our therapy will preclude deterioration of early-stage pressure ulcers, keep healing on the optimal course, and help more patients to recover quickly. Our therapeutics will thus address the most problematic pressure ulcers that currently lack effective treatment options and disproportionately contribute to the cost of care, pain, suffering, and mortality. We also anticipate that our therapy could be effective in other types of chronic wounds, such as poorly healing surgical and post-burn wounds that complicate the treatment of combat-related injuries, as well as in other types of biofilm-based bacterial infections, such as those associated with implanted medical devices and prosthetics that frequently affect military Veterans. Importantly, our antibiotic potentiator compounds can be combined with many different antimicrobials (antibiotics and antiseptics), offering treatment options to most patients, even if their pressure ulcers become infected by bacteria resistant to a particular antimicrobial.

Proposal Title:	Development of Innovative Therapeutics Against Pressure Ulcer-Associated Bacteria
Log Number:	PR220077P1
Current PI Name:	Evgeny Nudler
Award Number:	HT9425-23-1-0423
Current Contracting Organization:	New York University School of Medicine
Current Performing Organization:	New York University School of Medicine
Web Approval Date:	10-03-2023

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Proposal Title:	A Novel High-Throughput Lipid Nanoparticle Barcode Surveying Method for Developing Nanoparticles for Friedreich's Ataxia Genome Editing
Log Number:	PR220080
Current PI Name:	Baisong Lu
Award Number:	HT9425-23-1-0050
Current Contracting Organization:	Wake Forest University Health Sciences
Current Performing Organization:	Wake Forest University Health Sciences
Web Approval Date:	01-20-2023

This proposal addresses the Peer Reviewed Medical Research Program (PRMRP) Topic Area Portfolio of Neuroscience, Topic Area Friedreich's Ataxia, and the Strategic Goal of Treatment, focusing on developing novel treatment strategies for Friedreich's ataxia. Friedreich's ataxia is the most frequent hereditary ataxia caused by mutations in both copies of the frataxin gene. The disease affects multiple organs including the nervous system, the heart, and the metabolism system. Over 90% of the patients are caused by the expansion of the tri-nucleotide repeat in the non-coding region of the frataxin gene on chromosome 9. The gene products of frataxin are present in the heart, spinal cord, liver, skeletal muscle, and pancreas, and this explains why mutation of this gene affects multiple organs. The expanded tri-nucleotide repeat decreases the gene products of frataxin, leading to decreased enzymatic activities of mitochondria, the powerhouse of human cells.

There is currently no cure or effective treatments for Friedreich ataxia. Providing a normal copy of the gene by viral vectors in disease model animals was observed to improve the phenotypes for a short time. However, too much frataxin protein expressed from the viral vectors was found to be toxic to mammalian cells. Using CRISPR/Cas9, the designer nuclease, to remove the expanded tri-nucleotide repeat, a process called genome editing, has been shown as a promising strategy. However, the lack of efficient strategy to send the nuclease into human cells hinders the clinical application of this strategy in Friedreich ataxia treatment.

Nanoparticles are a promising tool for sending CRISPR/Cas9 nucleases into human cells. However, currently they are only efficient for sending nucleases to the liver but not to most of the other deep organs. To treat Friedreich ataxia effectively, the nuclease has to be sent to all organs affected by this disease, including the brain, the heart, and the pancreas. Current nanoparticles are inefficient in doing so, especially in sending to the brain due to the presence of the blood-brain barrier. This project aims to develop nanoparticles for sending CRISPR/Cas9 nuclease into multiple organs affected by Friedreich ataxia.

By changing the compositions of the nanoparticles, the nanoparticles can be sent to one organ more efficiently than the other. This means that when screening enough types of different nanoparticles, one may find nanoparticles efficient in sending CRISPR/Cas9 nuclease to organs affected by Friedreich ataxia. However, current screening methods are not designed for this purpose. In this project, we will develop a novel screening method that can directly compare the gene-editing activities of hundreds types of nanoparticles in one experiment. Our method uses a unique barcode to label each type of nanoparticles. If the nanoparticles are efficient in sending the nuclease into the target organs, the barcode will be efficiently inserted into the genome of the cells. By identifying all barcodes inserted into the genome, which is easily achievable by next-generation sequencing, we can find the most efficient nanoparticles for a specific target organ. We will use this method to find the most efficient nanoparticles for sending the CRISPR/Cas9

nuclease into the organs affected by Friedreich ataxia and will test the application of these nanoparticles in removing the tri-nucleotide repeat in Friedreich ataxia model mice that show phenotypes similar to human Friedreich ataxia.

This project is novel in that it will develop a new method to directly compare the genome-editing activities of hundreds types of nanoparticles in one experiment. This enables the development of nanoparticles that can efficiently send CRISPR/Cas9 nuclease into multiple organs affected by Friedreich ataxia.

Our future plan is to use the nanoparticles found in this project to send CRISPR/Cas9 nuclease into Friedreich ataxia animal models and examine the improvement of phenotypes. If positive results are obtained, we will generate more preclinical data for Investigational New Drug Application. The nanoparticles developed in this project will be useful not only for Friedreich ataxia genome editing, but also for genome editing of other diseases. The project may promote the development of a new effective treatment for Friedreich ataxia and other diseases that affect multiple organs.

Proposal Title:	The Intestinal Mucosal Microbiome and Circulating Microbial Extracellular Vesicles in Rheumatoid Arthritis
Log Number:	PR220113
Current PI Name:	Michelle Ormseth
Award Number:	HT9425-23-1-0044
Current Contracting Organization:	Vanderbilt University Medical Center
Current Performing Organization:	Vanderbilt University Medical Center
Web Approval Date:	01-24-2023

Rheumatoid arthritis (RA) is disease characterized by an autoimmune attack on the joints and can lead to long term pain and disability and shorten lifespan by nearly 10 years. RA affects Veterans disproportionately, possibly due to environmental factors. One of these factors may be bacteria in the intestine. Researchers have studied bacteria in the stool rather than the bacteria on or in the lining of the intestine. However, these intestinal lining bacteria are likely the ones important for health and disease because they are so close to human immune cells and blood vessels. Thus, the bacteria themselves and certain bacterial products could enter the circulation more easily. Some of these products that bacteria make can affect inflammation in people and thus affect RA.

One product that bacteria make are structures called extracellular vesicles (ECVs). Bacteria and other microbes pinch off part of their cell wall to make ECVs so that the ECVs contain some of the same substances on their membrane which the bacteria have. Some of these substances can cause inflammation. Also, the bacteria package ECVs with short segments of nucleic acids (small RNAs or sRNAs). Bacteria use the sRNAs to regulate human genes, particularly genes affecting how the immune system works. The bacteria likely do this to promote their own survival.

We studied the sRNAs (not by isolating the ECVs, but by looking at all sRNAs) in the blood of patients with RA and people who do not have RA and found that RA patients had different bacterial sRNAs compared to people without RA and that the higher the abundance of some sRNAs, the lower the disease activity and the better patients responded to a new drug. We also found that one of these bacterial sRNAs decreases an immune response that also causes pain. So, some of the bacterial sRNAs are associated with good outcomes in RA patients. The problem is that we have not yet studied the whole package – the ECV with the sRNAs inside. This is important because the membrane of the ECV as well as the sRNA inside can alter inflammation, potentially in conflicting ways.

Our central hypothesis is that intestinal lining bacteria are different in RA patients and via ECVs transfer bacterial sRNAs to the circulation and thus affect disease. To examine this, we propose an innovative study in which we will (1) collect intestinal biopsies and compare the bacteria present in these specimens from patients with and without RA who are getting a routine screening colonoscopy. (2) We will collect blood from the patients undergoing colonoscopy and from additional Veterans with and without RA and purify the bacterial ECVs from the blood. (3) We will treat cells with the isolated ECVs, measure changes in cellular inflammatory proteins, and determine if these differ in cells treated with ECVs from RA versus non-RA patients. (4) We will measure bacterial sRNAs that the ECVs transfer to cells and determine if these differ in RA versus non-RA patients. (5) We will test the function of sRNAs that are most abundant and transferred from RA ECVs to cells. (6) We will statistically measure what percentage of the variability in the bacterial sRNAs in the blood can be explained by the bacteria present in the intestinal biopsies.

These studies will lay the important foundations necessary before applying for additional funding to broaden these experiments and incorporate manipulations of the intestinal wall bacteria, ECVs and sRNAs to change RA in animal models and eventually human patients.

This pilot project directly addresses the Fiscal Year 2022 Peer Reviewed Medical Research Program Strategic Goal to determine the impact of microbiome on the Portfolio Category Autoimmune Disorders and Immunology in the Topic Area Rheumatoid Arthritis. Studying the bacteria of the intestinal wall and the circulating bacterial ECVs which can transport sRNAs to human cells, and their impact on inflammation is critical to understanding how the bacteria in the body can affect RA. This may provide strategies for RA prevention and treatment.

Proposal Title:	Deep Phenotyping FSGS Using Artificial Intelligence and Special Transcriptome
Log Number:	PR220141
Current PI Name:	Haichun Yang
Award Number:	HT9425-23-1-0003
Current Contracting Organization:	Vanderbilt University Medical Center
Current Performing Organization:	Vanderbilt University Medical Center
Web Approval Date:	02-06-2023

Focal segmental glomerulosclerosis (FSGS) is a heterogeneous clinicopathological condition that can be classified into four categories according to etiology: (1) primary FSGS attributed to circulating permeability factor, (2) genetic FSGS caused by gene mutations, (3) secondary FSGS associated with, e.g., viral infections, medication or maladaptive changes, and (4) FSGS occurring without an identified cause. Some FSGS patients respond promptly to steroids; however, approximately 50% of individuals progress to end-stage kidney disease within 5-10 years despite therapy. FSGS is diagnosed by the morphologic changes observed in the renal biopsy sample. Currently, the Columbia classification used in renal pathology does not match well with the etiology and is not sufficiently robust to guide treatment and predict the clinical prognosis. The development of a precise and prognosis-relevant FSGS classification is limited by two factors: empirical morphometric features without quantitative molecular evidence, and biased and less reproducible semi-quantitative assessment of these features. Artificial intelligence (AI) has been applied to medical images, including histopathology, from which AI could extract more prognostic information than an experienced pathologist. Our approach is to develop a quantitative deep phenotyping algorithm for etiology-related signatures identification and quantification in FSGS.

Spatial transcriptomics allows us to measure all the gene activity and map their locations in the histological sections. By using this spatial transcriptome-supervised machine learning and quantitative AI algorithms, we will detect and quantify those etiology-related signatures in the FSGS biopsy samples. We will train the AI in mouse FSGS models, and transfer and apply this AI algorithm to human samples. This strategy will significantly reduce the requirement of a human training set. The quantitation of these morphology signatures will be carried on in a fraction of serial sections of renal biopsy, which show a more accurate prediction of FSGS lesions than 2D-based quantification.

This approach will help to generate a reference FSGS atlas by combining regular staining, spatial transcriptome, and computer vision in the feature. A new classification based on these etiology and transcriptomic related spatial signatures will not only improve the precision and reproducibility of diagnosis but also facilitate the identification of specific drug targets and ultimately enable individualized care in FSGS.

Proposal Title:	Incretin Mimetics and Thiazolidinediones as Therapy Against Excessive Airway Mucus to Improve Lung Function in COPD Patients
Log Number:	PR220150
Current PI Name:	Gee Lau
Award Number:	HT9425-23-1-0372
Current Contracting Organization:	Illinois, University of, Champaign/Urbana
Current Performing Organization:	Illinois, University of, Champaign/Urbana
Web Approval Date:	10-03-2023

COPD epidemic: This Fiscal Year 2022 (FY22) Peer Review Medical Research Program (PRMRP) application focuses on the Topic Area of Respiratory Health. Especially, we address the Chronic Obstructive Pulmonary Disease (COPD), which affects large numbers of Veterans and their beneficiaries. The prospects for individuals diagnosed with COPD are grim. It is a major cause of disability and the third leading cause of death in the U.S. According to the National Institute of Heart, Lung and Blood, approximately 15 million people in the U.S. have COPD, and another 10 million are undiagnosed. In 2011, there were approximately 730,000 hospitalizations for COPD, costing $5.7 billion. Tobacco smoking is the most common cause of COPD. Other important factors include air pollutants, such as toxic desert dusts inhaled by soldiers in the Middle East theaters, biomass smoke, and genetics. The odds of COPD in cigarette smokers are 318% greater than in nonsmokers. Unfortunately, the prevalence of tobacco use among the Veterans (39% in 2007) is staggeringly higher than the general U.S. population (19.8%). Long-term exposure to these irritants exacerbates inflammatory response, resulting in narrowing of the small airways and emphysema. The most common symptoms of COPD are excessive mucus and sputum production, microbial infection, shortness of breath, productive cough, chest tightness, and wheezing. Advanced COPD may lead to high pressure on the lung arteries, which cause leg swelling and bulging neck veins. A feeling of always being tired is common. COPD often occurs along with diabetes mellitus, ischemic heart disease, high blood pressure, muscle wasting, osteoporosis, lung cancer, anxiety disorder, and depression. An acute exacerbation of COPD is defined as increased cough, shortness of breath, increased sputum production, and a change in the sputum color from clear to green and yellow due to infection.

No specific therapies for COPD: Current therapeutic efforts target symptoms and quality of life and include smoking cessation, oxygen infusion, vaccination against pathogens, PDE4 inhibitors, bronchodilators, inhaled corticosteroids, and antibiotics to manage bacterial-mediated acute exacerbations. There is no cure for COPD other than a lung transplant, and none of the U.S. Food and Drug Administration (FDA)-approved therapies have been approved for attenuating disease progression or reducing mortality rates. Thus, new lines of therapeutics for COPD are sorely needed, which is the premise of our proposal.

FOXA2 as a therapeutic COPD target: Through multi-year, laborious, time-consuming, and expensive preclinical investigations in human lung cell culture models and in mice, we have shown that important COPD bacterial pathogens such as Pseudomonas aeruginosa cause mucus hypersecretion and emphysema by inactivating FOXA2, a critical lung protein that is needed to maintain healthy levels of mucus and for regulating emphysema development. And this focused effort has paid off: We have identified two classes of FDA-approved drugs for diabetes mellitus 2 - incretin mimetics and thiazolidinediones - that can effectively augment and preserve the function of FOXA2 in various human airway cell culture and mouse models of COPD even when infected by P. aeruginosa or exposed to its toxin pyocyanin. There is considerable data suggesting tremendous potential of FOXA2 as a drug target for COPD. The loss of FOXA2 function in mouse lung causes spontaneous proliferation of goblet cells that produce excessive mucus clogging the airways, as well as emphysema. These observations are supported with studies by us and others showing that FOXA2 expression level is inversely correlated to goblet cell hyperplasia in lung tissues of COPD,

bronchiectasis, and asthmatic patients. Regrettably, current COPD treatments do not target FOXA2. Based on our data, there is an opportunity to dramatically improve lives of Veterans stricken with COPD by augmenting the function of FOXA2.

Our goal, therefore, is to address the Overarching Challenges and Reduce the morbidity and mortality associated with COPD. We want to advance COPD care by replacing ineffective interventions with ones that are safe and effective. But how do we get there from here? We will use stepwise approaches: (i) Select the most efficacious FDA-approved incretin mimetics and thiazolidinediones by measuring their ability to restore FOXA2 expression, inhibit mucus production, and improve clearance of COPD exacerbating pathogen P. aeruginosa in human lung cells culture and lung explants; (ii) Decipher the underlying molecular mechanisms by which these drugs rescue FOXA2 function in the COPD lungs; and (iii) Examine if COPD patients with diabetes mellitus 2 and treated with incretin mimetics and thiazolidinediones have reduced mucus burden, improved lung function, and reduced exacerbations than those patients who do not receive these drugs.

Additional benefits of restoring FOXA2 function: We anticipate that repurposing these pro-FOXA2 drugs will also revolutionize treatments for: (1) active-duty military personnel deployed in the Middle East who are constantly bombarded with toxic desert dust; (2) those personnel exposed to burn pit smoke; and (3) the U.S. general public exposed to wildfire and wood stove smoke. By augmenting FOXA2's ability to regulate mucus homeostasis, lungs will be able to restore mucociliary clearance of inhaled pathogens and toxic particles. In turn, this will reduce excessive reliance on antibiotics and lighten the burden of epidemic antibiotic resistance in U.S. Department of Veterans Affairs (VA) hospitals.

Proposal Title:	Incretin Mimetics and Thiazolidinediones as Therapy Against Excessive Airway Mucus to Improve Lung Function in COPD Patients
Log Number:	PR220150P1
Current PI Name:	Russell Bowler
Award Number:	HT9425-23-1-0373
Current Contracting Organization:	National Jewish Medical and Research Center
Current Performing Organization:	National Jewish Medical and Research Center
Web Approval Date:	10-03-2023

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bronchiectasis, and asthmatic patients. Regrettably, current COPD treatments do not target FOXA2. Based on our data, there is an opportunity to dramatically improve lives of Veterans stricken with COPD by augmenting the function of FOXA2.

Our goal, therefore, is to address the Overarching Challenges and Reduce the morbidity and mortality associated with COPD. We want to advance COPD care by replacing ineffective interventions with ones that are safe and effective. But how do we get there from here? We will use stepwise approaches: (i) Select the most efficacious FDA-approved incretin mimetics and thiazolidinediones by measuring their ability to restore FOXA2 expression, inhibit mucus production, and improve clearance of COPD exacerbating pathogen P. aeruginosa in human lung cells culture and lung explants; (ii) Decipher the underlying molecular mechanisms by which these drugs rescue FOXA2 function in the COPD lungs; and (iii) Examine if COPD patients with diabetes mellitus 2 and treated with incretin mimetics and thiazolidinediones have reduced mucus burden, improved lung function, and reduced exacerbations than those patients who do not receive these drugs.

Additional benefits of restoring FOXA2 function: We anticipate that repurposing these pro-FOXA2 drugs will also revolutionize treatments for: (1) active-duty military personnel deployed in the Middle East who are constantly bombarded with toxic desert dust; (2) those personnel exposed to burn pit smoke; and (3) the U.S. general public exposed to wildfire and wood stove smoke. By augmenting FOXA2's ability to regulate mucus homeostasis, lungs will be able to restore mucociliary clearance of inhaled pathogens and toxic particles. In turn, this will reduce excessive reliance on antibiotics and lighten the burden of epidemic antibiotic resistance in U.S. Department of Veterans Affairs (VA) hospitals.

Proposal Title:	Inhibiting cGAS-STING Pathway as a Therapeutic Target in Inflammatory Bowel Disease
Log Number:	PR220157
Current PI Name:	Rizia Bardhan
Award Number:	HT9425-23-1-0071
Current Contracting Organization:	Iowa State University
Current Performing Organization:	Iowa State University
Web Approval Date:	02-21-2023

This proposal will address the Peer Reviewed Medical Research Program's (PRMRP's) Topic Area on "Inflammatory Bowel Diseases (IBDs)" and PRMRP's strategic goal to "Develop and test new treatments to minimize toxicity and mitigate the inflammatory disease state." Currently >3 million Americans are living with IBDs, a relapsing condition with no cure. Symptomatic patients have acute inflammation of the colon and rectum that gives rise to recurring diarrhea, severe stomachache, blood and mucus in stools, and extreme fatigue. Nearly 40% of patients develop resistance to current clinical therapies, and those with long-term symptoms develop other chronic conditions including cancer, arthritis, and liver disease. Many clinically approved drugs are also limited in their ability to target the site of inflammation resulting in severe off-target toxicities. Further, prolonged use of immunomodulators gives rise to side effects such as osteoporosis and increased risk of lymphoma. Therefore, an unmet clinical need exists for new therapeutic strategies that will specifically target the colon and rectum in IBDs, reduce inflammation with minimal toxicities, and improve the abundance of beneficial gut microorganisms to enable long-term protection against IBD flare-ups.

Our objective is to address this clinical challenge with a new paradigm in treatment for IBDs where hyaluronic acid (HA)-based therapeutic micelles will be designed and loaded with a novel drug that inhibits the stimulator of interferon genes (STING) protein. Recent studies in mouse models and clinical studies in patients now strongly support that activation of the STING protein (through gut bacterial DNA) worsens IBD symptoms. Yet not a single drug inhibiting the STING protein is available to IBD patients. This is in part due to poor bioavailability of the drugs after oral delivery, resulting in rapid clearance before therapeutic benefit is achieved. These drugs also have poor targeting ability, often failing to reach the site of inflammation and instead accumulating in other organs that leads to severe toxicities. The innovation of this work lies in the smart design of the STING-inhibiting micelles (SIMs), which overcomes the current clinical challenges by encapsulating the STING inhibitor in the micelles such that the drug is retained in the body for hours. HA is a natural biopolymer already in human use, and micelles are drug carriers currently in clinical trials (trial # NCT03168061, etc.). The micelles also reach the site of inflammation as HA naturally targets CD44 receptors expressed in the inflammatory colon. Further, our micelles also include a biocompatible linker that is designed to trigger drug release primarily at the site of inflammation substantially minimizing off-target toxicities, and drug encapsulation in micelles also ensures dose-controlled delivery in patients, saving high costs of treatment.

As IBD is contributed to by multiple risk factors in a genetically predisposed patient, in Aim 1, we will model environmental risk factor in a dextran sulfate sodium (DSS)-induced colitis mouse model, and in Aim 2, we will model microbial risk factor in a bacterial-induced colitis model. Here we will develop therapeutic SIMs and both benchmark against current clinical drugs 5-ASA, prednisone, and VSL#3, as well as study combination of SIMs with these drugs. By integrating robust mouse models and multiple preclinical analysis, we will understand the therapeutic impact of SIMs providing the "missing" causal link between patient's immune response and gut microorganisms. The near-term impact of our project will allow a safe micellar formulation of a potent drug for immediate reduction in IBDs symptoms with low doses and minimal side effects and enable a healthy gut environment. Our hypothesis is that by combining SIMs with

current clinical drugs, we will enable a potential “cure” for patients that will improve the diversity and stability of beneficial gut microbiota for sustained protection against IBDs and decrease risks of relapse. In the long term, this technology will impact the design of drug delivery vehicles for universal encapsulation of a number of drugs with improved bioavailability and efficacy, and the ability to target the CD44 proteins also correlated to Chron’s disease, irritable bowel syndrome, gastrointestinal disorders, and colorectal cancer. The straightforward large-scale synthesis of the micelles, high drug encapsulation capacity, and our proposed efficacy and biosafety studies in vivo will ultimately allow rapid clinical translation of this potentially revolutionary platform and streamline the pharmaceutical drug development pipeline. Upon successful achievement of the proposed preclinical studies, we expect SIMs will achieve a clinically relevant outcome in 5-10 years, allowing treatment in the convenient form of a “pill” (instead of intravenous delivery that is current clinical standard) and reduce the time spent in the hospital by both civilians and active-duty members. SIMs will also be applicable in diseases beyond IBDs such as kidney injury, sepsis, and Parkinson’ s where an increase in the STING protein has been linked to advanced disease.

Proposal Title:	Cardiomyocyte Autonomous Contractility Defects in Hypoplastic Left Heart Syndrome (HLHS)
Log Number:	PR220160
Current PI Name:	Stephanie Nakano
Award Number:	HT9425-23-1-0165
Current Contracting Organization:	Colorado, University of, at Denver
Current Performing Organization:	Colorado, University of, at Denver
Web Approval Date:	03-23-2023

Infants can be born with a severe heart malformation (congenital heart disease) called hypoplastic left heart syndrome (HLHS), where the left half of the heart is underdeveloped and non-functional. Without any intervention, HLHS is fatal. Significant medical and surgical advances over the past several decades have allowed many infants with HLHS to now survive into childhood or even adulthood. The most common treatment for infants with HLHS is a series of open-heart surgeries, which modify the patterns of blood flow so that patients can survive with just one pumping chamber instead of two: the right ventricle becomes the main pump, supplying blood to the body. However, sometimes early during childhood and other times during the second or third decade of life, the right ventricle can become weak, with a decreased ability to squeeze, resulting in heart failure. It is challenging to predict which HLHS patients will develop heart failure, and when. In the majority of HLHS patients, we do not understand the factors that cause the heart muscle to fail.

Traditional heart failure medications are used in HLHS patients with heart failure; however, these medications do not improve outcomes nor reverse heart failure. Specifically, many medications that improve outcomes in adults with heart failure are not effective in children with HLHS, likely because the underlying cause of heart failure is different between these two populations and the body's response to heart failure may change with age. Unfortunately, the only option for long-term survival for HLHS patients with end-stage heart failure is heart transplantation. While heart transplantation can be life-sustaining, we do not consider heart transplantation a "cure," since complications such as rejection, coronary artery disease, infection, and cancer can arise following heart transplantation. Additionally, subsequent heart transplants may be needed, as transplanted hearts last 15 years on average. Identifying the mechanisms underlying heart failure in HLHS is needed to develop novel medical therapies specifically for HLHS patients, which may delay or even decrease the need for heart transplantation. As such, this proposal is well-aligned with the Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Strategic Goal to "develop less-invasive treatment technologies for associated cardiovascular conditions" within the FY22 PRMRP Topic Area of Congenital Heart Disease.

The overall goal of the proposed research project is to better understand the changes in HLHS heart muscle cells that predispose them to fail. In addition to HLHS being a disease where the heart is structurally abnormal, there is suggestion that the heart muscle cells themselves have intrinsic defects that may contribute to the development of heart failure. This proposal will generate heart muscle cells from circulating white blood cells from HLHS patients, known as human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM). This hiPSC-CM model will be used to study the proteins and contractile function of HLHS heart cells, without having to directly take a heart tissue biopsy from a HLHS patient. Additionally, we will utilize these patient- derived HLHS hiPSC-CM to perform an unbiased screen of thousands of potential compounds to see if any have efficacy in improving heart muscle cell contraction. By increasing our understanding of the causes of heart failure in HLHS hearts, we hope to identify targets for novel and effective medical therapies specifically for this vulnerable population. Ultimately, information gained from this proposal will help improve outcomes for children and adults with HLHS.

Proposal Title:	Using High-Density EEG to Identify Sleep-Dependent Cortical Replay of Laboratory Analog Trauma and Effects on Distressing Dreams and Intrusive Memories
Log Number:	PR220162
Current PI Name:	Anne Richards
Award Number:	HT9425-23-1-0024
Current Contracting Organization:	Northern California Institute for Research and Education
Current Performing Organization:	VA Medical Center, San Francisco, CA
Web Approval Date:	12-23-2022

The proposed research project addresses the Peer Reviewed Medical Research Program Portfolio Neuroscience, the Topic Areas Sleep Disorders and Restriction and Trauma, and the Strategic Goal Foundational Studies.

Posttraumatic stress disorder (PTSD) occurs in 8% to 10% of civilians, and 20% to 30% of combat Veterans. Core features of PTSD are distressing memories of traumatic experiences during wake, and trauma-related nightmares at night. Nightmares occur in the majority of individuals with PTSD and are associated with severe subjective distress and numerous negative outcomes, including suicide. Similar to distressing memories during wake, nightmares are considered an intrusive, re-experiencing symptom of the disorder because they manifest as vivid memories of lived trauma experiences, ranging from brief fragments to extensive, complex distressing replays of traumatic memories. There is now strong evidence that wake distressing memories impact sleep symptoms, and vice versa, indicating a vicious cycle through which unwanted daytime and nighttime trauma symptoms reinforce each other and perpetuate PTSD.

Despite the distress and negative outcomes associated with nightmares, and their bidirectional relationship with classic wake intrusive memories in PTSD, extremely little is understood about their neurobiology. However, a growing scientific understanding of learning and memory indicates that sleep plays a fundamental role in “off-line” memory consolidation through networks of neurons (brain cells) that fire during sleep in patterns that replay the neuronal firing patterns of wake experiences. This sleep replay results in consolidation of the neuronal traces of daytime experiences into memories. Innovative science also demonstrates that electroencephalography (EEG), a non-invasive strategy for measuring brain activity, can provide information about this process. For example, EEG collected during wake learning and subsequent sleep can capture brain activity patterns that are repeated across wake and sleep states and can predict how well information is learned and/or recalled. Similar methods have shown that patterns of EEG activity during sleep can also be used to predict content of dreams that is described when subjects are awakened from sleep.

Research has also shown that dreaming about something learned before sleep can enhance memory for that information. This fascinating research indicates that sleep, dreams, and memory are intricately linked. This is of critical relevance to PTSD, since PTSD revolves around the memory of a traumatic event, and recurrent trauma dreams and wake trauma memories are a core feature of the disorder. Cracking the code that creates and reinforces trauma memories during both sleep and wake would result in a major breakthrough in the science and would provide critical information relevant to PTSD prevention and treatment.

Our Discovery Award proposal comprises an initial step in cracking the code, using EEG in a laboratory experiment involving medically healthy male and female Veterans along with sophisticated EEG data analysis strategies. The main part of the study involves one laboratory visit with EEG data collection during

wake tasks followed by an early afternoon nap. During the visit, participants will initially view a series of brief trauma movie clips (established by Veterans to be highly distressing in a preparatory phase of our project), along with control (non-trauma) clips. Trauma clips will serve as laboratory “analogs” of true trauma experiences, enabling us to study how distressing experiences are registered and imprinted in the brain. After viewing, participants will also go through a series of trials during which they will briefly imagine the most distressing scenes from the trauma clips. They will subsequently have a 2-hour nap opportunity. EEG measurement will take place continuously throughout the wake and sleep procedures. After the laboratory visit, participants will complete a sleep diary mobile app daily to report on lab-trauma memories and dreams and will also report sudden memories of the trauma clips as they occur (in real time) on a wristband device.

The main objectives of the study are (1) to identify the neural engram, or the pattern of brain activity, that forms a signature of a trauma experience in the laboratory while the study participants are awake and viewing or imagining the film clips; (2) to identify replay of the neural engram during subsequent sleep in the laboratory and to examine whether replay happens at the same time as brain rhythms known to be important for memory; and (3) to examine the relationship between replay and later memories and dreams of the distressing laboratory trauma content. Sophisticated analytic strategies, including state-of-the-art machine learning strategies, will be utilized to carry out our aims. Our team has the skill and expertise in PTSD, sleep biology, and advanced signal processing to carry out the work. We expect to contribute to breakthroughs in understanding the neurobiology of trauma memory, and the neurobiology of sleep’s role in trauma memory.

Proposal Title:	Novel Drug/Receptor Codelivery to the Liver-Lung Axis to Overcome Glucocorticoid Resistance and Acute Respiratory Distress Syndrome in Sepsis
Log Number:	PR220163
Current PI Name:	Hong Lu
Award Number:	HT9425-23-1-0073
Current Contracting Organization:	New York, State University of, Upstate Medical University
Current Performing Organization:	New York, State University of, Upstate Medical University
Web Approval Date:	01-31-2023

Peer Reviewed Medical Research Program Topic Areas: Respiratory Health, Sustained Release Drug Delivery

1: What is the critical problem?

Acute respiratory distress syndrome (ARDS) is a life-threatening condition caused by combat injuries and lung infections like pneumonia and COVID-19. Pseudomonas aeruginosa (PA) is a World Health Organization (WHO) “Priority 1: Critical” pathogen that frequently causes ventilator-associated pneumonia, particularly in patients with cystic fibrosis or combat casualties. Due to their potent anti-inflammatory and lung-protective effects, glucocorticoids (GCs) are widely used to treat respiratory diseases, e.g., neonatal respiratory distress syndrome, chronic obstructive pulmonary disease, and asthma. In a large clinical trial, dexamethasone (DEX), a highly potent GC significantly reduced deaths in COVID-19 patients who need oxygenation/ventilation but tended to slightly increase mortality in patients who don’t need oxygenation /ventilation, likely due to the immunosuppressive effects of GCs. Additionally, many patients do not respond to GC therapy due to genetic mutations and/or environmental modifications of the GC receptor (GR). Thus, there is a critical need to develop a targeted GC therapy to achieve tissue-specific drug delivery and overcome the GC non-responsiveness. Such a targeted GC therapy will protect the lung and reduce inflammation in patients with ARDS caused by severe COVID-19 or PA infection. This is important because in combat casualties, PA infection causes higher morbidity and mortality than other infections.

2: Why is it important?

Severe COVID-19 and pneumonia cause sepsis that results in hyperinflammation and multi-organ dysfunction syndrome (MODS). MODS is a life-threatening condition that causes the body’s organs to shut down. In addition to lung injury, liver dysfunction and injury is an early event and independent risk factor for sepsis-induced MODS and death. Severe COVID-19 causes a pulmonary GR deficiency. Hepatic GRs are also reduced in septic patients. Experimentally lowering GRs in the liver causes liver failure and increased mortality in septic mice. GR activation has many beneficial effects on the liver and lungs, but it can also cause many side effects on the neuromuscular, adipose, and immune systems. New treatments for ARDS and sepsis should overcome GC non-responsiveness to reduce hyperinflammation and restore organ function without impairing the body’s ability to fight against infections. The liver is the metabolic center essential for survival, and the lung is the first organ to fail in ARDS and MODS. Thus, we believe that developing a novel “two-in-one” targeted GC therapy that not only overcomes GC non-responsiveness but also specifically targets the liver-lung axis will be safer and more effective for ARDS and sepsis patients compared to the current systemic GC treatment.

3: What are the proposed experiments?

As a proof of concept, our preliminary study demonstrated better GC responsiveness of a modified GR (fortified GR) than the wild-type GR. We also created a novel drug/receptor codelivery system in which we can co-deliver the drug (DEX) and the fortified GR protein into both the liver and lung to study how specifically activating GR in the liver and lung affects ARDS and sepsis outcomes. We will: (1) develop a novel "two-in-one" system of drug/receptor codelivery of DEX and a fortified GR to test its effects on GC non-responsiveness in human lung and liver cells and (2) determine effects of codelivery of DEX and the fortified GR on GC non-responsiveness and MODS in a mouse model of PA-induced ARDS and sepsis. Our central hypothesis is that our innovative "two-in-one" drug/receptor codelivery system will not only overcome GC non-responsiveness but maximize GC exposure to the liver and lung. By maximizing the beneficial effects of GCs on the liver and lung and minimizing GC's side effects on other tissues and the immune system, our innovative "two-in-one" drug/receptor codelivery system will significantly improve lung health and survival for patients with ARDS and sepsis.

4: What is the ultimate applicability and impact of the project?

Our novel "two-in-one" drug/receptor codelivery GC therapy will overcome GCR, maximize the protection of GCs in the liver and lung, and minimize GCs' side effects on other organs. The codelivery of DEX and a fortified GR will provide precision medicine for many patients who otherwise would not respond to GCs due to genetic mutations or environmental modifications of GR. This "two-in-one" codelivery system will result in safer and more effective GC treatments for patients with viral (COVID-19) or bacterial (PA) pneumonia, sepsis-induced ARDS, and other diseases driven by hyperinflammation. Importantly, our novel drug/receptor codelivery system is based on a U.S. Food and Drug Administration (FDA)-approved drug delivery platform and thus can be rapidly transformed into an innovative therapy for injured Warfighters and their family members who suffer from ARDS and sepsis.

Proposal Title:	Preclinical Development of Gene Replacement Therapy Using Novel AV Vectors in Familial Cerebral Cavernous Malformations
Log Number:	PR220166
Current PI Name:	Douglas Marchuk
Award Number:	HT9425-23-1-0644
Current Contracting Organization:	Duke University
Current Performing Organization:	Duke University
Web Approval Date:	10-03-2023

Peer Reviewed Medical Research Program (PRMRP) Strategic Goal: Treatment. Develop less-invasive treatment technologies for associated cardiovascular conditions.

PRMRP Topic Area: Cardiovascular Health. Vascular Malformations.

Cerebral Cavernous Malformations (CCMs) are a type of vascular malformation; abnormal clusters of blood vessels that develop in the brain and spinal cord of affected patients. The CCMs are prone to bleed causing headaches, stroke and even death. In the inherited form of the disease the patients develop dozens or more of these CCMs in the brain. There is no effective treatment except for highly invasive brain surgery, but for the patients with inherited disease it is impossible to remove more than one or two malformations, and a non-surgical therapy is critically needed. We will address CCM therapy at the root cause by adding back a copy of the normal gene – so-called gene addition therapy. Our proposal involves a test of this approach to therapy in animals, using new strategies and tools developed by the three investigative teams. These include a new mouse model of CCM disease that highly resembles the human disease, a novel gene delivery system using engineered viruses, and innovative imaging and biochemical techniques to determine the effectiveness of the treatment. Upon study completion, we will have identified a gene delivery strategy and blood tests for its monitoring, to be used for a subsequent clinical trial in CCM patients.

Proposal Title:	Skin Immunoengineering to Induce Long-Lasting Systemic Immune Tolerance to Food Allergens
Log Number:	PR220169
Current PI Name:	Emrullah Korkmaz
Award Number:	HT9425-23-1-0168
Current Contracting Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Current Performing Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Web Approval Date:	03-02-2023

Food allergy (FA) is a highly prevalent, potentially life-threatening health condition that poses a tremendous burden on affected people, their families, health care providers, and the medical system. Despite the significant challenges associated with FA, there is still no effective therapy for FA, with durable clinical benefits, and the standard of care for FA remains strict avoidance of food allergens and uninterrupted access to epinephrine auto-injectors at the risk of severe adverse effects, including anaphylaxis. These mainstays of FA management are unsatisfactory for affected people, and thus, FA patients are prone to high morbidity rates, and the emergency room visits associated with FA have significantly increased over the past decade. Further, constant vigilance due to strict dietary restrictions causes a high level of stress and anxiety for affected individuals and for their families, leading to poor quality of their lives. Due to the lack of effective therapies for FA, there are other critical impacts of FA beyond affected individuals and their families: (1) FA places pressure on local neighborhoods and schools, industry practices, and government policy; and (2) FA has national security implications because a diagnosis of FA renders people ineligible to join the military, posing challenges to recruit and maintain the operationally ready Service Members. As such, the increasing prevalence of FA over the past decade and the lack of safe and effective therapies for FA highlight the ongoing considerable challenges associated with the management of FA.

The aforementioned challenges associated with FA translate into a pressing need for the development of safe, well-tolerated, inexpensive, highly effective, and broadly applicable therapies for FA. Over the past decade, there has been increasing research dedicated to the investigation of food allergen-specific immunotherapy (ASIT) as a disease-modifying strategy that has the potential to lead to desensitization and protection against accidental food allergen exposure. Despite great advances in the development of ASIT for FA, the existing ASIT methods result in low and transient efficacy, bear the risk of severe adverse effects, including anaphylactic reaction, because of systemic allergen exposure, and suffer from poor patient adherence and high cost due to a large number of clinical visits over several years. The goal of our project is to develop an entirely novel immunoengineering strategy for FA to obviate the shortcomings of existing ASIT methods, thereby leading to a safer, more effective, lower-cost, and more convenient ASIT for FA. Specifically, we propose to leverage our experience in skin immunobiology, biomaterials science, controlled release drug delivery, and immunology to devise a cutaneous immunoengineering platform based on microneedle patches (MnPs) that will exploit the emerging immune communication between the skin and other tissues (e.g., gastrointestinal tract) to induce long-term systemic tolerance to food allergens. As such, this project will directly address the Peer Reviewed Medical Research Program (PRMRP) Fiscal Year 2022 (FY22) Portfolio Strategic Goal of developing and testing a new treatment strategy to mitigate the inflammatory and/or allergic disease state under the PRMRP Topic Area of Food Allergies for the FY22 Portfolio of autoimmune disorders and immunology.

The hypothesis of this proposal is that skin immunoengineering using multicomponent MnPs incorporating food allergens, tolerogenic immunomodulators, and lymph node-targeting nanoparticles (NPs) will generate long-lived allergen-specific systemic tolerogenic immune responses that will reverse the clinical symptoms of FA. Our strategy will rationally modulate the cutaneous immune system (1) at the time of food allergen

delivery (in the immunoresponsive skin layers) and (2) at the time of allergen presentation to lymphocytes (in the skin-draining lymph nodes) to exploit the remarkable plasticity of cutaneous antigen-presenting cells (APCs) and lymphocyte trafficking of T cells to different tissues. Our innovative immunoengineering platform will (1) reproducibly target food allergen into the immunoresponsive skin microenvironment while imprinting cues to render skin APCs into a tolerogenic phenotype at time of food allergen delivery and (2) provide an ideal environment for these APCs in the skin-draining lymph nodes at the time of antigen presentation to lymphocytes to mount food allergen-specific systemic tolerogenic immune responses for the development of a novel therapeutic strategy for FA. Our rapidly translatable immunoengineering platform will be compatible with a variety of allergens and tolerogenic adjuvants, and will be shelf-stable without refrigeration, offering an economically feasible and broadly deployable ASIT for FA. Cutaneous immunoengineering using multicomponent MnPs will represent an entirely novel ASIT for FA, which will dramatically improve the efficacy, durability, safety, and convenience compared to existing ASITs. The proposed studies will focus on (1) development of a cutaneous immunoengineering platform based on MnPs; (2) validation of spatiotemporal controlled release performance of these MnPs; and (3) evaluation of the therapeutic efficacy, safety, and functional durability of MnP-based immunoengineering in established murine models of peanut and milk allergies. Collectively, our MnP-based cutaneous immunoengineering strategy will be effective, easy-to-administer, systemic side effect-free, low-cost, and patient-friendly, thereby leading to a next-generation FA immunotherapy that will address the shortcomings of existing ASIT methods and will benefit both the civilian population and military members.

Proposal Title:	Activation of Brain Lymphatic Drainage Through Piezo1-Controlled Mechanotransduction as a Novel Hydrocephalus Therapy
Log Number:	PR220238
Current PI Name:	Young-Kwon Hong
Award Number:	HT9425-23-1-0822
Current Contracting Organization:	University of Southern California
Current Performing Organization:	University of Southern California
Web Approval Date:	10-03-2023

Hydrocephalus, also called water in the brain, is an abnormal accumulation of fluids in the brain, which causes headaches, weakness, problems balancing and walking, vision difficulties, memory loss, or even death when left untreated. Head injury, bleeding in the brain, infections, and brain cancers are a few causes of hydrocephalus. Current treatment for patients with hydrocephalus is the surgical insertion of a tube (shunt) as a bypass that can drain this fluid to other parts of the body. Although this surgical treatment has been performed for decades, the shunts get blocked and break down over time, requiring additional invasive surgeries. Although ~40,000 shunts are surgically placed in the brain annually in the United States, these types of surgeries are costly procedures with significant morbidities. Considering all these complications, it is critical to develop new treatment options that can effectively treat patients with hydrocephalus.

The cerebrospinal fluid (CSF) in the brain is crucial for removing the brain waste products into the bloodstream. The lymphatic system has a network of vessels to drain fluid from tissues and back into the blood system. The brain has long been thought to be devoid of the lymphatic system. However, the brain lymphatic system was newly discovered. Since then, studies showed that the brain lymphatic system is responsible for the CSF fluid drainage from the brain and, by doing so, dispose of the brain cellular and metabolic wastes. Recently, our studies uncovered that a small molecule, Yoda1, can increase lymphatic drainage of the CSF. Yoda1 is a compound that activates a protein called Piezo1 residing on the surface of lymphatic cells. Piezo1 is a newly identified receptor that senses any physical forces applied to the cells, including fluid flow. The key finding is that Yoda1 is capable of activating Piezo1 in the absence of physical force. We exploited this principle and took advantage of Yoda1 as a Piezo1 activator in lymphatic cells, stimulating the lymphatic function. Indeed, we found that Yoda1 could enhance the lymphatic drainage of the CSF and thus effectively prevent brain swelling in animal models of hydrocephalus. However, Yoda1 turned out to be a poor drug candidate because it is metabolically instable and rapidly decayed in water. Thus, we initiated an extensive screening program and identified two Yoda1-like molecules with better medicinal properties and comparable therapeutic efficacies.

The goal of this proposal is to test these two new molecules in rodents and large animals. Specifically, we will implement a human hydrocephalus-like injury in mice, rats, ferrets, and sheep. We will then treat these animal models with our new compounds and evaluate their efficacies, toxicities, and all drug-like properties. Our research group is an outstanding multidisciplinary team consisting of lymphatic biologists, pharmaceutical scientists, biomedical engineers, neurosurgeons, neuroscientists, animal geneticists, molecular biologists, neuroradiologists, brain-imaging specialists, and biostatisticians. If successful, we will have a novel drug for both active-duty warfighters sustaining brain trauma on the battlefields and the war Veterans who developed chronic hydrocephalus long after head injuries experienced during military service. After this proposal, we will be in a position to advance Yoda1 into an Investigational New Drug (IND)-enabling studies and set the stage for human testing.

We believe that our experimental evidence of the effectiveness of two new molecules against hydrocephalus deserves thorough testing to determine the promise of this discovery. With the support of this proposal, we

are confident that the project can be rapidly advanced into human studies. This approach is a much-needed therapeutic for patients who currently have limited treatment options. The ability to effectively treat hydrocephalus can improve the chance of regaining normal life for our Warfighters and Veterans.

Proposal Title:	Mitochondria-Derived Extracellular Vesicles in Friedreich's Ataxia
Log Number:	PR220244
Current PI Name:	Katia Aquilano
Award Number:	HT9425-23-1-0005
Current Contracting Organization:	Rome, University of, Tor Vergata
Current Performing Organization:	Rome, University of, Tor Vergata
Web Approval Date:	02-28-2023

Friedreich’s ataxia (FA) is a rare neurodegenerative disease characterized by mitochondrial dysfunction caused by deficiency of the mitochondrial protein frataxin (FXN). Low amount of FXN leads to accumulation of iron within mitochondria, which are the powerhouse of the cells. Patients affected by FA display loss of neurons and inflammation in the spinal cord, cerebellum, and peripheral nerves, and this leads to neurological manifestations and motor disability. To date, no cure exists for FA, and the cause of neuronal death and inflammation are still obscure.

When mitochondrial are damaged, they can be removed inside the cell by a process called mitophagy. An alternate way to eliminate damaged mitochondria in physiological conditions is their ejection outside the cell via the so-called mitochondrial extracellular vesicles (mitoEVs) that can be removed by specialized cells of the immune system by phagocytosis. In the brain, the predominant phagocytic cells are microglia that eliminate microbes, dead cells, and other particulate that may endanger neurons.

In this project, we want to test whether in FA there is an abnormal release of mitoEVs and an impaired or aberrant phagocytic and inflammatory response of microglia. To this end, we will use mouse models of FA affected by mild and severe form of the disease. In the first phase of the research, we will characterize mitoEVs of cerebellum and cultured cerebellar neurons in terms of morphology, abundance, and cargo to highlight possible differences between healthy and FA affected mice. Microglia differences will be also investigated through high-throughput techniques. Next, we will carry out a series of in vitro experiments on neurons and microglia cells isolated from cerebellum to test whether in FA the communication between such cell types is altered due to the presence of abnormal mitoEVs in terms of abundancy or cargo and this could be responsible for the setting of an inflammatory phenotype of microglia.

As mitoEVs can spread in circulating fluids, we will also search for cerebellum-derived mitoEVs in plasma and cerebrospinal fluid to test whether their abundancy could reflect the stage of the disease. Performing this research, we will hopefully lay the foundation for future studies not only for expanding our knowledge on the implication of mitoEVs in the neurodegeneration but also for suggesting cerebellar-derived mitoEVs as low-invasive biomarkers for assessing FA progression and response to therapy in preclinical models.

Proposal Title:	Detecting Cartilage Surface Degeneration Using Photon Counting CT and Solute Transport Modeling
Log Number:	PR220272
Current PI Name:	Marc Levenston
Award Number:	HT9425-23-1-0331
Current Contracting Organization:	Leland Stanford Junior University, The
Current Performing Organization:	Leland Stanford Junior University, The
Web Approval Date:	04-25-2023

Osteoarthritis (OA), a joint disease that currently has no cure, is characterized by progressive and largely irreversible damage to the soft tissues such as the articular cartilage that normally provides a smooth, durable surface to support forces produced during daily activity. OA is a major cause of disability and pain affecting 12% of the U.S. adult population and is a substantial burden on the health care system, with estimated annual costs upwards of $40 billion. While OA is not always associated with known factors, joint injuries such as tears to the meniscus or anterior cruciate ligament (ACL) in the knee dramatically increase the likelihood of developing OA, often rapidly and at a young age. OA in general, and knee OA following joint injury specifically, are more common among military Service Members and Veterans than among the general population. Efforts to understand early disease progress and develop and test interventions to slow OA progress are hampered by the relative insensitivity of existing medical imaging tests to the earliest signs of degeneration. This proposal aims to address this critical roadblock by developing a new imaging approach to detect early physical changes to the cartilage surface characteristic of early degeneration. This proposal addresses the Diagnosis Strategic Goal and the Arthritis Topic Area within the Orthopaedic Medicine Portfolio Category of the Fiscal Year 2022 Peer Reviewed Medical Research Program.

The overall goal of the proposed research is to develop techniques to characterize changes in contrast agent diffusivity, a measure of how easily a substance in the joint fluid can penetrate the cartilage surface, as a marker for early degenerative changes indicative of early-stage OA. Our approach uses computed tomography (CT), a three-dimensional imaging method based on x-rays, to detect the progressive penetration into the articular cartilage of a clinical CT contrast agent injected into the knee joint. Specifically, we will use a CT imaging approach based on a new type of x-ray detector (photon counting CT, PCCT) and will develop specific technical advances to produce even finer detail at high accuracy. We will analyze the sequential image data using mathematical models that account for confounding factors that occur in real patient scans, allowing us to determine maps of the diffusivity over the joint surface. Because diffusivity (ease of contrast penetration) increases as the tissue near the surface degenerates, we expect to be able to identify regions where early changes occur before these changes propagate through the entire tissue.

Aim 1 of the proposed project will focus on developing and validating specific technical advances of the CT imaging approach to increase the spatial resolution of the images and allow more accurate detection of the cartilage surface location. Aim 2 will focus on improving methods for estimating the contrast agent diffusivity from the CT images by accounting for realistic, complex patterns that occur in clinical images but are not often factors in the laboratory. We will validate these approaches by comparing diffusivity values determined from scans of patients prior to total knee replacement surgeries to values determined for isolated samples of their tissue removed during the procedure. Aim 3 will test the ability of the combined approach to detect degenerative changes in patients after anterior cruciate ligament repair surgery, as this patient group is known to have a greatly increased risk of developing OA.

We expect this new imaging strategy to be beneficial for research on developing new interventions and for clinical diagnosis. In the near term, the ability to sensitively detect early degenerative changes will provide a

powerful research platform for investigating early disease processes, risk factors for rapid progression, and effectiveness of mechanical or pharmaceutical interventions to modify OA progression. In the longer term as PCCT imaging systems become more widely available, this could lead to a clinical diagnostic tool to detect early changes in at-risk patients and identify appropriate interventions based on patterns of altered diffusivity.

Proposal Title:	Detecting Cartilage Surface Degeneration Using Photon Counting CT and Solute Transport Modeling
Log Number:	PR220272P1
Current PI Name:	Adam Wang
Award Number:	HT9425-23-1-0332
Current Contracting Organization:	Leland Stanford Junior University, The
Current Performing Organization:	Leland Stanford Junior University, The
Web Approval Date:	04-25-2023

Osteoarthritis (OA), a joint disease that currently has no cure, is characterized by progressive and largely irreversible damage to the soft tissues such as the articular cartilage that normally provides a smooth, durable surface to support forces produced during daily activity. OA is a major cause of disability and pain affecting 12% of the U.S. adult population and is a substantial burden on the health care system, with estimated annual costs upwards of $40 billion. While OA is not always associated with known factors, joint injuries such as tears to the meniscus or anterior cruciate ligament (ACL) in the knee dramatically increase the likelihood of developing OA, often rapidly and at a young age. OA in general, and knee OA following joint injury specifically, are more common among military Service Members and Veterans than among the general population. Efforts to understand early disease progress and develop and test interventions to slow OA progress are hampered by the relative insensitivity of existing medical imaging tests to the earliest signs of degeneration. This proposal aims to address this critical roadblock by developing a new imaging approach to detect early physical changes to the cartilage surface characteristic of early degeneration. This proposal addresses the Diagnosis Strategic Goal and the Arthritis Topic Area within the Orthopaedic Medicine Portfolio Category of the Fiscal Year 2022 Peer Reviewed Medical Research Program.

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powerful research platform for investigating early disease processes, risk factors for rapid progression, and effectiveness of mechanical or pharmaceutical interventions to modify OA progression. In the longer term as PCCT imaging systems become more widely available, this could lead to a clinical diagnostic tool to detect early changes in at-risk patients and identify appropriate interventions based on patterns of altered diffusivity.

Proposal Title:	IND-Enabling Studies for Targeted Phase Shift Microbubbles for Sonothrombolysis
Log Number:	PR220274
Current PI Name:	Emmanuelle Meuillet
Award Number:	HT9425-23-1-0893
Current Contracting Organization:	MICROVASCULAR THERAPEUTICS, LLC
Current Performing Organization:	MICROVASCULAR THERAPEUTICS, LLC
Web Approval Date:	10-03-2023

One person dies every 36 seconds in the United States from cardiovascular disease. About 659,000 people in the United States die from heart disease each year, that is 1 in every 4 deaths. Although these numbers have decreased somewhat, people are still dying from heart attacks or acute myocardial infarction (AMI).

Treatment for MI ranges from lifestyle changes and cardiac rehabilitation to medications, stents, and bypass surgery. Often stents, angioplasty or a minimally invasive procedure such as percutaneous coronary intervention, (PCI) are performed. The risks of PCI are the complications that may be consequent to the procedure and induce the detachment and generation of small blood clots lodging themselves in the arteries and creating an obstruction called microvascular obstruction of microvascular obstruction (MVO). MVO leads to worse clinical outcomes. There is no consistent therapy, and MVO occurs in up to 60% of patients post-PCI, despite successfully restoring blood flow in the major arteries.

We have developed ultrasound targeted microbubbles that are condensed in smaller size bubbles (also herein referred as phase shift microbubbles [PSMB], in the size of the nanometer, 1/10th the size of microbubbles) that are able, when given in an intravenous injection, to travel directly to the clot, and upon entry in the ultrasound field applied externally, disrupt the clot and restore the blood flow and eliminate the clot. In addition, we have engineered these agents to carry a material that causes these small bubbles to bind tightly and directly to the clot so that very low doses are needed and allow clots to be dissolved very quickly when ultrasound is applied. We have obtained proof-of-concept results in animals (rats and pigs) for this technology. This innovative treatment is being explored as a potentially exciting and promising treatment option for patients who have AMI with MVO.

This project will support the development of a novel “less-invasive treatment technology for associated cardiovascular conditions” in the primary Topic Area of “Cardiovascular Health.” Veterans are at greater risk of heart disease than the general population. There is great potential of this technology to improve outcomes in Veterans with coronary artery disease to effectively treat MVO. Also relevant to the military in duty, the technology may be used remotely where other treatments are not available.

The major goals of the proposal are the following: (1) test the new product in animals and determine the best conditions (concentration and parameter of ultrasound); (2) to produce in high amounts the product so that it would be ready to be tested in agreement with the regulations for the clinic in humans, (3) test the product to make sure it is safe and doesn’t exhibit side effects, (4) request an early meeting with the U.S. Food and Drug Administration (FDA) to obtain their guidance as to what other studies are needed to have this product accepted to be tested in humans and start the clinical trial, and (5) perform these recommended toxicology studies and file the entire package at the FDA to request the authorization to test the product in humans with their required supplementary animal data.

Our team has the expertise in the field of ultrasound contrast agent as well as in cardiovascular research and clinical trials. The project is poised to be successful considering the preliminary results acquired to date, the

team of experts assembled for this proposal, the proposed experiments, and the realistic timeline. The ultimate goal is to move the product through the required regulatory procedures and obtain the authorization from the FDA for a clinical trial for patients afflicted with AMI.

Proposal Title:	Systematic Pipeline for ADAR-Mediated Transcript Therapeutics in Rett Syndrome
Log Number:	PR220302
Current PI Name:	Ronald Emeson
Award Number:	HT9425-23-1-0111
Current Contracting Organization:	Vanderbilt University
Current Performing Organization:	Vanderbilt University
Web Approval Date:	01-31-2023

The studies proposed in this application were developed in response to the Fiscal Year 2022 Peer Reviewed Medical Research Program Funding Opportunity for the Topic Area “Rett syndrome” and the strategic goals to develop and validate a novel and innovative therapeutic intervention for the treatment of this severe disorder that places a heavy emotional and financial burden on parents and caretakers.

Rett syndrome (RTT) is an early-onset neurodevelopmental disorder that affects approximately 1 in 10,000 girls. This disorder is characterized by normal development but is then interrupted by the loss of previously acquired social and motor skills (such as speaking and walking) that begins at 6-30 months of age. Additionally, respiratory and gastrointestinal problems, seizures, anxiety, and repetitive behaviors may subsequently emerge that persist throughout adulthood. Advances in molecular genetics have identified mutations in several genes associated with this syndrome, although 90%-95% of patients diagnosed with classic Rett syndrome have been shown to carry spontaneous mutations in the MECP2 gene. The MECP2 gene encodes a protein that is expressed throughout the body, yet it is most highly expressed in neurons and is essential for nervous system function. While several therapeutic strategies for Rett syndrome are being pursued, currently there are no effective treatments for this disorder and no approaches to repair the underlying mutations in the MECP2 gene.

Recent advances in CRISPR-mediated gene targeting technologies have provided an opportunity for to correct genetic mutations identified in the DNA of patients diagnosed with numerous genetic disorders, including Rett syndrome. Despite the promise of such approaches however, CRISPR-mediated gene editing has several limitations that include the potential to make changes at inappropriate places in the genome, the potential for immune responses to large bacterial proteins that are used for this approach, the requirement for cellular delivery of multiple therapeutic components, the potential imprecision of this approach even when the modification occurs in the correct region of the DNA, and inefficient DNA modification in several cell types including, most notably, neurons in the brain.

In recent years, the use or targeting of RNA molecules, rather than DNA, has become a promising strategy for therapeutic intervention and many RNA-based therapeutics have been approved for indications as diverse as spinal muscular atrophy, the reduction of LDL-cholesterol, and immunization against SARS-CoV-2, the virus responsible for COVID-19. As an alternative approach to CRISPR-mediated engineering of genetic mutations, several lines of investigation have focused upon the “repair” of mutant RNA transcripts by taking advantage of a normal cellular process in which specific adenosines are converted to inosine residues in RNA to increase the diversity of encoded protein expression and function. Being able to selectively modify an adenosine of one’s choice in MECP2 RNA, and convert it to inosine, provides a powerful therapeutic approach by which to correct a subset of MECP2 mutations for Rett syndrome patients. Advantages to targeted RNA editing include the fact that RNA modifications are transient, eliminating concerns associated with permanent DNA alterations, that it can be directed by the delivery of a single, small therapeutic RNA, and that it takes advantage of normal cellular enzymes to efficiently modify MECP2 transcripts in neurons.

We have developed a strategy to select short, engineered RNAs that will promote the efficient repair of specific adenosine residues in MECP2 transcripts, thereby eliminating the mutations in the encoded protein products. For these studies, we have chosen to focus upon the repair of two distinct MECP2 mutations using a test tube-based system with MECP2 transcripts, the enzymes that catalyze the conversion of adenosine to inosine, and a large population of small therapeutic RNAs. Once we have employed our novel approach to select the small RNAs capable of promoting MECP2 repair in a tube, we will advance our studies to cultured human neuroblastoma cell lines that have been modified to carry the MECP2 mutations. The short therapeutic RNAs will be introduced into these neuronal cells and assessed for their ability to promote the repair of endogenous MECP2 transcripts using normal cellular machinery. Future studies will employ mouse models of Rett syndrome, not only to assess the repair of MECP2 transcripts in mutant animals, but also to assess the reversal of symptoms associated with this disorder. We anticipate that using these small, synthetic RNAs to recruit the endogenous RNA editing enzymes holds promise as a transformative approach for treating not only Rett syndrome, but also a wide array of other human disorders resulting from pathogenic mutations in the genome.

Proposal Title:	IDO1 and Dysregulated Tryptophan Metabolism in Polycystic Kidney Disease
Log Number:	PR220321
Current PI Name:	Katharina Hopp
Award Number:	HT9425-23-1-0255
Current Contracting Organization:	Colorado, University of, at Denver
Current Performing Organization:	Colorado, University of, at Denver
Web Approval Date:	04-02-2023

This proposal addresses the Fiscal Year 2022 Peer Reviewed Medical Research Program portfolio category Internal Medicine with the focus on the Topic Area Polycystic Kidney Disease (PKD). PKD is one of the most common genetic disorders. The adult-onset form of PKD, autosomal dominant PKD (ADPKD), affects ~1 in 800 people in the world and is considered the most frequent, life-threatening genetic kidney disease. Given our country's ~67.5 million military personnel, Veterans, and military family members, an estimated 135,000 have ADPKD, representing ~27% of the total ADPKD population within the United States. ADPKD is characterized by progressive enlargement of the kidney, caused by growth of fluid-filled cysts, leading to crowding of normal kidney tissue and eventual kidney failure, which is the key long-term complication and comorbidity of the disease. In 50% of patients, loss of kidney function requiring dialysis or transplantation occurs by 60 years of age.

To date, there is only one approved therapy, tolvaptan, to slow the progression of ADPKD. However, it is only approved for patients with very rapid cyst growth, and it impairs quality of life. Therefore, understanding the processes that drives kidney cyst growth better, to then identify and test new treatment options, is of high priority in PKD research.

Critical insight into the mechanisms that drive cyst growth could be gained by understanding why at times cyst grow rapidly and kidney failure happens at a young age or why at times cysts grow slowly and patients don't face dialysis until old age. In the past few years, research has identified changes in metabolism and immune cell function to be critical modifiers of cysts growth. However, the cellular and molecular mechanisms mediating these effects are not well understood.

In our preliminary studies using a mouse model of ADPKD that mimics the disease characteristics found in patients, we identified tryptophan metabolism as one metabolic pathway that is highly dysregulated as disease progresses. Breakdown of tryptophan produces molecules called kynurenines that have been shown to block the immune system and promote cell proliferation. In cancer, a disease that has many pathological parallels with PKD, this pathway is associated with tumor growth, and inhibitors of the pathway have been shown to slow cancer progression and reduce metastases. We found that products of tryptophan breakdown are highly elevated in our model, and we confirmed the importance of the pathway to kidney cyst growth by genetically deleting as well as pharmacologically inhibiting the key enzyme metabolizing tryptophan (IDO1) in two different ADPKD models. These manipulations significantly slowed kidney cyst growth. Our studies also found that the function of different types of immune cells is compromised in the setting of PKD. The immune cells affected are known to be critical players in cancer progression. Interestingly, inhibition of tryptophan metabolism corrected functional immune cell defects in our mice, potentially contributing to the slowed cyst growth.

In our proposal, we will utilize a multitude of highly sophisticated tools to understand how dysregulated tryptophan metabolism regulates kidney cyst growth at a single cell level. We will then confirm our findings by manipulating various aspects of the process, e.g., IDO1 expression in different cell types, or functions of

various immune cells or kidney epithelial cells, within different ADPKD models. Most importantly, we will test the therapeutic efficacy of an IDO1 inhibitor that is approved in the United States to treat various forms of cancer in our ADPKD models. Therefore, our proposal follows the strategic goal to perform foundational studies that improve the understanding of a long-term complication and comorbidity of PKD – end-stage kidney disease – due to cyst growth.

This project is innovative as it focuses on a metabolic pathway that has not been mechanistically or therapeutically studied in PKD, analyzes a novel connection between metabolism, immune and epithelial cell function, and cyst growth, and utilizes state-of-the-art single cell methodologies to gain new insights into the mechanisms driving ADPKD. The impacts of our studies are to (1) delineate molecular/cellular changes within the cystic kidney that are modulated by tryptophan metabolites, (2) provide preclinical data using a clinically approved IDO1 inhibitor as a new treatment option, (3) strengthen the rationale to utilize tryptophan metabolites as disease progression biomarkers, and (4) generate a catalog of changes occurring during PKD progression using a disease relevant model, which can be used by the scientific PKD community to clarify mechanisms driving disease and test additional/different treatment approaches.

Proposal Title:	Understanding Cardiovascular Disease in Mental Health/Stress Disorder
Log Number:	PR220330
Current PI Name:	Prasanna Krishnamurthy
Award Number:	HT9425-23-1-0278
Current Contracting Organization:	Alabama, University of, at Birmingham
Current Performing Organization:	Alabama, University of, at Birmingham
Web Approval Date:	04-29-2023

This proposed study is in response to Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Portfolio (Cardiovascular Health); FY22 PRMRP Topic Area (Cardiomyopathy); FY22 PRMRP Strategic Goal [Prevention (Predict and prevent potential impact of extreme environments, posttraumatic stress disorder, and/or infections on cardiovascular health)].

Mental health disorder is of public health significance. One in five U.S. adults experience mental illness each year. Reports have shown that a significant percentage of Veterans deployed to Afghanistan and Iraq suffer from depression and/or posttraumatic stress disorder (PTSD). Mental disorders such as major depressive disorder, psychosis, and PTSD have been shown to increase risk for developing cardiovascular diseases. However, the underlying mechanism for such risk is poorly understood and is understudied. In this study, we will investigate the cause-effect relationship between depression and cardiovascular disease (CVD).

In this regard, our preliminary studies from our laboratory have shown that secretion of brain-derived neurotrophic factor (BDNF) is low in mice subjected to experimental depression studies. Similar data has been observed in clinical patients with depression. Low blood levels of BDNF have been negative effects in depression, bipolar disorder, and mania. Interestingly, neurotrophins (NTs) are also reported to influence hypertension, atherosclerosis, and myocardial disease. BDNF is synthesized in the body by conversion from pro-BDNF. BDNF and pro-BDNF have opposite effects on blood vessels, heart cells, etc. We speculate that the reduced BDNF or higher pro-BDNF during depression affects cells in the heart such as survival and function of cardiomyocytes and vascular endothelial cells. Our proposed study will systematically investigate whether heart dysfunction during depression is due to cardiomyocyte cell death or vascular cell malfunction. We will use models of depression, pharmacology, and molecular biology tools to investigate the proposed hypotheses.

Short-term and long-term impact of our study: In the short term, we hope our proposed study will help understand the molecular mechanism of CVD during depression. Also, our long-term goal is that our studies might identify new molecular signals that could targeted to inhibit development of CVD in patients with depression.

Proposal Title:	Management of Mitochondrial Disorders Through Increased Intestinal Fermentation and Reduced Metabolic Burden
Log Number:	PR220334
Current PI Name:	Alessandro Bitto
Award Number:	HT9425-23-1-0016
Current Contracting Organization:	Washington, University of
Current Performing Organization:	Washington, University of
Web Approval Date:	12-15-2022

This proposal addresses the Peer Reviewed Medical Research Program (PRMRP) portfolio category of nutrition and metabolism, topic area concerning Mitochondrial Disease, with the strategic goal of understanding correlations between nutrition and disease susceptibility. Mitochondrial disease is a term describing several illnesses characterized by loss of function of mitochondria, cellular organelles that oversee multiple aspects of energy and nutrient metabolism. Examples of mitochondrial diseases are severe conditions such as MELAS (Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like episodes) syndrome and Leigh syndrome, one of the most common pediatric mitochondrial disorders that is often fatal in the first 3 years of life. These diseases are caused by mutations in genes involved in multiple aspects of mitochondrial function.

Mutations in mitochondrial genes can also alter the composition of the intestinal flora, the diverse set of bacteria residing inside the intestines, also known as the microbiome. Certain species of bacteria in the microbiome can ferment dietary fibers and other nutrient into small metabolites known as short-chain fatty acids (SCFAs.) These SCFAs can in turn improve mitochondrial function and energy metabolism both in the intestine and in other organs, including the brain, with known benefits in metabolic pathologies such as obesity and diabetes.

Despite the obvious connection between mitochondrial function and the microbiome, to date no study has determined whether mitochondrial disease can alter the composition of the intestinal flora or whether increasing the abundance of intestinal bacteria that produce SCFAs can improve the symptoms and progression of mitochondrial disease.

To test this hypothesis, we plan to determine which bacteria and in what amounts are present in the intestines of mutant mice at different stages of the disease and whether they produce higher levels of SCFAs. To do this, we will sequence a unique portion of the bacterial genome that will allow us to identify different bacterial species. In parallel, we will measure the abundance of SCFAs in ceca, sera, and brains of mutant mice via mass spectrometry. In the second aim, we will transfer the bacteria from the intestines of acarbose-treated animals into germ-free mutant mice via feces transplantation. We will follow survival and disease progression in these mutant animals. In addition, we will promote proliferation of a fermenting microbiome and its activity via dietary interventions.

Our work will be the first to establish a role for the intestinal microbiome in the onset, treatment, and progression of severe mitochondrial disease and create the opportunity to further study the relationship between the two in greater detail. We will also potentially determine the mechanism of action of a well-characterized, U.S. Food and Drug Administration (FDA)-approved drug in mitochondrial disease, as well as identify novel diagnostic tools and therapies for these life-threatening disorders.

Proposal Title:	A Blood Test for Diastolic Dysfunction
Log Number:	PR220340
Current PI Name:	Samuel Dudley
Award Number:	HT9425-23-1-0042
Current Contracting Organization:	Minnesota, University of, Twin Cities
Current Performing Organization:	Minnesota, University of, Twin Cities
Web Approval Date:	12-15-2022

Portfolio Category: Cardiovascular Health

Topic Area: Cardiomyopathy

Strategic Goals: Develop strategies to enable detection of associated cardiovascular conditions before clinical symptoms are apparent.

There are two types of heart failure (HF). One where the heart fails to contract sufficiently, known as systolic HF, and one where the heart fails to relax properly, known as diastolic HF. When the heart does not relax properly, it does not fill to the proper extent, so that even when contraction is adequate, there is insufficient blood in the chamber to eject. In both systolic and diastolic HF, the heart does not eject enough blood. When this happens, a patient experiences the symptoms of blood building up in the lungs and periphery that include shortness of breath and peripheral fluid accumulation.

Systolic and diastolic HF are approximately equal in prevalence in the worldwide community, and they both have a similar mortality, which is roughly equivalent to locally invasive lung cancer (30% survival at 5 years). There are many medical and surgical options for systolic HF, but what has become clear is that these therapies do not work for diastolic HF. Therefore, it is important to distinguish these two types of HF. This can be done by imaging the heart, but it is expensive and time-consuming, usually only done after symptoms occur.

Diastolic HF is preceded by a period of asymptomatic diastolic relaxation dysfunction. This period allows an ideal time to intervene in the disease process. Patients are at increased risk of HF but have not yet developed the disease. This application sets out to develop a simple, inexpensive blood test to identify diastolic dysfunction (DD), a prerequisite of diastolic HF.

Through a decade of research, we have established that hypertension and diabetes mellitus induced DD is associated with a chemical modification of a cardiac-specific contractile protein, cardiac myosin binding protein C (cMyBP-C). This modified protein can also be found in blood, and we have preliminary evidence that its presence in blood is highly associated with cardiac DD.

Hypothesis: This application sets out to validate a simple, inexpensive blood test to identify DD. Currently, diagnosis depends on costly, time-consuming imaging procedures that are only undertaken after symptoms develop. We have shown in animals (mice and monkeys) and humans that a modified contractile protein cMyBP-C in blood may serve as a marker of DD. We propose to do a non-interventional human clinical study to validate our animal and preliminary human data.

Specific Aim: We will test if our proposed marker is increased in the blood of DD patients without HF when compared with age-matched control patients without DD or HF. Further, we will test if in plasma, the marker correlates with severity of DD as measured by cardiac imaging.

Impact: A blood test for DD could revolutionize HF care by providing definitive diagnosis of DD patients in an expedient, cost-effective manner. It could allow for determining the prognosis and eligibility of novel therapies, and it could improve diagnostic accuracy and save time for correct treatment of HF patients. A blood test detecting asymptomatic DD could allow for therapy to prevent HF. While therapies are limited for DD or diastolic HF, the Principal Investigator is developing orally bioavailable compounds for use in DD, and there are other promising avenues, suggesting specific therapies will be available for this prevalent condition soon.

Proposal Title:	Defining Cell Populations and Cell Type-Specific Transcriptional Dysregulation in Pediatric Coarctation of the Aorta
Log Number:	PR220351
Current PI Name:	Benjamin Landis
Award Number:	HT9425-23-1-0009
Current Contracting Organization:	Indiana University
Current Performing Organization:	Indiana University
Web Approval Date:	01-10-2023

The aorta is the largest blood vessel in the body and is responsible for delivering the blood that has been ejected from the heart to the body's organs. Coarctation of the aorta (CoA) is a common form of congenital heart disease, which is a Peer Reviewed Medical Research Program (PRMRP) Topic Area. CoA is defined by an abnormal narrowing of the aorta that is due to abnormal in utero development. After birth, CoA obstructs blood flow from the aorta to the rest of the body, resulting in organ injury including heart failure. Currently, infants with severe CoA require invasive cardiac surgery. In such operations, the area of coarctation is resected and removed from the body along with portions of the adjacent aortic tissues that do not have CoA. Surgery has significant costs and associated risks including death and organ injury. Even when surgery is uncomplicated and repair is excellent, many patients remain at long-term risk to develop other cardiovascular problems including increased blood pressure and early-onset coronary artery disease. Therefore, surgery does not fundamentally cure CoA. There are currently no medications to prevent CoA development, cure CoA postnatally, or specifically prevent or treat the long-term associated cardiovascular diseases. In order to develop such medical treatments, which are urgently needed, the precise disease mechanisms that cause CoA must be more clearly understood. The objective of this proposal is to precisely define the types of cells that are abnormally located in the diseased aortic tissues of neonates with CoA and determine how specific types of cells are abnormally functioning. We will pursue this objective using a cutting-edge technique called single-cell RNA sequencing (sc-RNAseq). In sc-RNAseq, every cell in a tissue is analyzed individually for how its genes are being expressed. The aorta has a mixture of cells including endothelial cells, smooth muscle cells, and others. From the data that is generated by scRNA-seq, in a single test each individual cell can be labeled according to what type of cell it is. This allows for a complete understanding of the types of cells and their relative amounts, as well as identifying the cells that are not normally present. The data can also be used to select the populations of specific types of cells and then perform a cell type-specific analysis of gene expression. Our central hypothesis is that CoA tissues contain distinctive cell populations and that comprehensive analysis of specific types of cells will provide a precise understanding of cell abnormalities that are driving the disease.

We propose to study the blood vessels that are removed surgically in infants with CoA in the course of routine clinical care. We have a fully developed infrastructure for the recruitment of study participants and collection of aortic biospecimens at our high-volume pediatric cardiac center. For each participant, we will perform sc-RNAseq of several tissue pieces, including the area of CoA and the adjacent blood vessels. These include the adjacent parts of the aorta that do not have CoA. In our first aim, we will define the types of cells that are located in each piece of resected tissue, hypothesizing that the CoA tissue will contain distinct cell population(s) and different amounts and proportions of cell types compared with the adjacent aortic tissues that do not have CoA. In our second aim, we will select specific types of cells in order to perform a cell type-specific analysis of gene expression. For each selected cell type, we will compare gene expression levels between different aortic tissue segments. We expect to identify genes that have abnormal expression levels in CoA and understand which specific types of cells display the abnormalities. This research is innovative because it will utilize sc-RNAseq for the first time in CoA. This research is conceptually innovative because it seeks to overcome the critical lack of understanding of the disease processes that are responsible for CoA.

The novel application of scRNA-seq technology will facilitate a robust cell type-specific analysis of gene expression that has been impossible to accomplish using the previous traditional methods to study CoA tissues. Indeed, there is a massive gulf between the capabilities of scRNA-seq and the prior approaches in terms of sensitivity and specificity. The expected discoveries will provide new insights into the disease processes of CoA, generating new lines of investigation. Ultimately, this work will advance our understanding of the cell abnormalities in CoA so that we may identify new medical targets and develop new medical treatments that avoid the need for invasive surgery. This pursuit aligns with the PRMRP Strategic Goal to develop less-invasive treatment technologies for cardiovascular conditions. Developing effective medical treatments would completely transform the therapeutic approach to CoA.

Proposal Title:	Why Dietary Lipids Influence Intestinal Endotoxin Transport and Systemic Inflammation
Log Number:	PR220359
Current PI Name:	Jonathan Kaunitz
Award Number:	HT9425-23-1-0180
Current Contracting Organization:	California, University of, Los Angeles
Current Performing Organization:	California, University of, Los Angeles
Web Approval Date:	02-21-2023

Peer Reviewed Medical Research Program (PRMRP) Portfolio Category: Nutrition and Metabolism

Fiscal Year 2022 (FY22) PRMRP Topic Area: Nutrition Optimization

FY22 PRMRP Strategic Goal: Foundational Studies – Understand correlations between nutrition and disease susceptibility (e.g., infectious, autoimmune, neurological, metabolic, cardiac)

The diet that most people living in Western countries is high in saturated fats that come from animals and some vegetables. These fats are associated with an increased amount of heart disease, diabetes, high blood pressure, and other diseases common among the military, in Veterans, and in the developed world in general.

The pathophysiology of many diseases that are common in the military and Veteran population such as diabetes, multiple sclerosis, chronic fatigue syndrome, fibromyalgia, arthritis, and asthma, is thought to be due in part to a “leaky gut,” in which toxic substances are thought to get into the body through gaps between the cells lining the intestine. Our laboratory has discovered that bacterial toxins that are believed to help cause many of these diseases enter the body by specific mechanisms that pass through, not around the cells lining the intestine.

In the studies proposed in this grant application, my laboratory plans to clarify the specific ways by which these toxins are absorbed. We hope to translate these studies into the discovery of new treatments that will help “tighten” the “leaky gut” with the hope of preventing or treating many of these diseases mentioned before that commonly afflict Veterans and people living in the developed world who consume too much fat in their diet.

Proposal Title:	Targeting the NAPE-PLD Pathway for Treatment of Pressure Ulcers
Log Number:	PR220405
Current PI Name:	Sean Davies
Award Number:	HT9425-23-1-0064
Current Contracting Organization:	Vanderbilt University
Current Performing Organization:	Vanderbilt University
Web Approval Date:	01-10-2023

Our proposed studies address the Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Internal Medicine Topic Area of Pressure Ulcers and the FY22 PRMRP Strategic Goals of Treatment – Develop and test therapeutics or dressings that enhance wound healing, as well as Foundational Studies – Improve understanding of long-term complications and comorbidities of associated diseases and conditions.

Pressure ulcers, also known as bed sores, can rapidly develop when an individual is unable to frequently shift positions to relieve pressure on the skin and underlying tissue when sitting or lying down. Pressure on the skin reduces the amount of blood that reaches the skin, damaging the underlying tissue. Patients that are particularly vulnerable to pressure ulcers include those with injuries that restrict their ability to move (for example, spinal cord injuries and hip fractures) or those with medical conditions that make them less sensitive to skin sensations that normally cause individuals to frequently shift position (for example, individuals with diabetes). Pressure ulcers are especially of concern in the military health care setting because of the increased frequency of immobilizing injuries such a spinal cord injury compared to the civilian population. Military Veterans also have an increased frequency of diabetes compared to the civilian population.

While pressure ulcers may be initially mild, they can rapidly worsen to where the top layers of the skin are broken and further progression can lead to exposure of the fat layer beneath the skin and also muscles, tendons, and bones. Not surprisingly, such deep wounds can easily become infected, greatly exacerbating any already existing health conditions. About one-sixth of patients with chronic foot ulcers require amputation, and about one-third of patients with these amputations will still die within 2 years. Despite the potentially dire consequences of skin ulceration, no new therapeutic medications for skin ulcers have been approved by the U.S. Food and Drug Administration (FDA) for more than 20 years. There is therefore a significant need to develop better therapeutic treatments for pressure ulcers.

Here, we present a novel and innovative approach for treatment of pressure ulcers. The rationale for our novel approach comes from a recent bioinformatics analysis examining the relationship between genes and various medical conditions. This analysis showed that individuals predicted to have lower levels of a specific protein, called NAPE-PLD, from their genetic data were much more likely to have been diagnosed with pressure ulcers and chronic foot and leg ulcers. NAPE-PLD had not previously been known to have any role in skin ulceration, so an important goal of our study is to test if mice genetically engineered to lack the ability to make NAPE-PLD are more sensitive to developing pressure ulcers than mice with a normal amount of NAPE-PLD. We will also test if mice that lack the ability to make NAPE-PLD take much longer to heal when their skin is wounded than mice that can make NAPE-PLD. If we find that mice lacking NAPE-PLD are indeed more sensitive to developing pressure ulcers and that their wounds take longer to heal, this result would support the need to study how NAPE-PLD protects the skin and helps wounds to heal. Such studies could also help us understand how diabetes worsens pressure ulcers because there is evidence that diabetes decreases the levels of NAPE-PLD is some tissues, although this has not been examined in the skin or in the immune cells that might be important for the development of pressure ulcers.

We have previously studied NAPE-PLD and have recently identified some drug-like chemicals that make the protein work better (NAPE-PLD activators) at least in the test tube and with isolated cells. NAPE-PLD in the body makes a fat-like molecule called palmitoylethanolamide (PEA), and PEA has previously been shown to have certain effects on immune cells and nerves that by extrapolation might help prevent pressure ulcers. For this reason, a second important goal of our study will be to test if applying these NAPE-PLD activators or PEA to the skin of diabetic mice will slow the development of pressure ulcers and help the ulcers that do develop heal faster. If our studies show that these compounds do help prevent the most severe forms of pressure ulcers and help the ulcers to heal faster, this would lay the foundation justifying performing additional studies to test whether such compounds could be used in humans to prevent and treat pressure ulcers. The eventual forms of treatment might vary, but a conceptually straightforward approach would be to apply ointments containing the NAPE-PLD activators or PEA to the skin at pressure points where pressure ulcers are beginning to develop, or in the case where they are already present, to apply them as an ointment to the wound and in bandaging.

Importantly, because NAPE-PLD has not been previously suspected to be important in preventing the development of pressure ulcers or in wound healing, our studies could potentially establish an entirely novel treatment approach to pressure ulcers. Especially appealing is that this pharmaceutical approach could readily be combined with other innovative approaches such a new wound scaffolds to achieve the goal of eliminating pressure ulcers and chronic foot ulcers for individuals served by the military health care system.

Proposal Title:	Preclinical Testing of the Effects of Diet and FMRP on Gut and Brain Barrier Integrity in a Mouse Model of Fragile X
Log Number:	PR220416
Current PI Name:	Cara Westmark
Award Number:	HT9425-23-1-0076
Current Contracting Organization:	Wisconsin, University of, Madison
Current Performing Organization:	Wisconsin, University of, Madison
Web Approval Date:	01-12-2023

The proposed research project addresses the Fiscal Year 2022 Peer Reviewed Medical Research Program Topic Areas of Fragile X Syndrome (FXS) and Nutrition Optimization. FXS is a developmental disability caused by a mutation in the FMR1 gene on the X-chromosome, which results in loss of expression of fragile X messenger ribonucleoprotein (FMRP). FMRP is a messenger RNA (mRNA) binding protein that plays pivotal roles in the transport, localization, and expression of hundreds of mRNAs. FXS is characterized by low IQ, autistic-like behavior, and seizures. A critical gap in medical care for persons with FXS is a viable, mechanistic-based therapy that translates between preclinical and clinical research. Recent literature indicates worse neurological and metabolic outcomes (seizures, autism, increased body weight) in mouse and /or human models of FXS as a function of diet. Thus, early-life exposures, such as diet, may exacerbate disease outcomes. This Department of Defense (DOD) Discovery Award application is innovative in proposing to test for an FMRP- and tight junction-mediated mechanism underlying gastrointestinal (GI) and brain barrier function in FXS model mice as well as testing for diet-induced effects on gut barrier integrity. The goal is to determine the effects of pro- and anti-convulsive diets, i.e., soy- and casein-based single-source diets, on gut barrier structure and function in wild-type and Fmr1KO littermate mice. The rationale is that loss of FMRP contributes to leaky gut syndrome through a tight junction-mediated mechanism affected by diet and that leaky gut contributes to brain dysfunction. This research has potential to provide a novel therapeutic target, tight junctions, and repurposing of experimental Celiac disease drugs that targe tight junctions for the treatment of FXS.

Proposal Title:	3D-Printed Antithrombogenic Sutureless Device for Vascular Anastomosis
Log Number:	PR220426
Current PI Name:	Xiaowei Li
Award Number:	HT9425-23-1-0022
Current Contracting Organization:	Washington University in St Louis
Current Performing Organization:	Washington University in St Louis
Web Approval Date:	12-02-2022

Vascular anastomosis is hand-sewing or suturing together of blood vessels. It is a foundational surgical skill critical for all kinds of surgeries, including plastic and reconstructive surgery, transplant surgery, and vascular surgery. However, a surgeon needs a decade of training to perform this procedure. Even with skilled surgeons, 27% of cases result in complications and 25% require reoperation. Procedures are long, expensive, and require specialized operating rooms, equipment, and personnel, thus making the highly in-demand procedure prohibitive in the majority of combat hospitals. A more efficient, cost-effective, and safer alternative for vascular anastomoses is desperately needed.

We have created a unique anastomotic device, Vaso-Lock, as a sutureless coupler to hold free vascular ends together with traction by anchors. The anchors do not penetrate the vessel wall; rather, they exert force against the vessel walls, utilizing the elasticity of the vessels to hold the vessel in place with a tight seal. We utilize 3D-printing to prototype these couplers, which allows for quick adjustments in designs with customization freedom, cost-effective prototyping, and fast production. Importantly, the Vaso-Lock device can be deployed in arteries within 1 minute, while handsewn anastomosis can take around 1 hour by a proficiently trained surgeon.

In terms of clinical application of a permanent implantable anastomotic device, our next step is to devise methods to provide long-term patency. Here, we will develop a surface-modification approach to facilitate endothelial cell affinity and anticoagulant ability of the device and test its efficacy in a swine arteriovenous loop model. Our long-term goal is to apply our devices to change the paradigm of surgical training and practice and improve technical capabilities for vascular anastomosis. Eventually, our device can be scaled to dramatically enhance the efficiency and reduce the risk of complications associated with vascular injuries, such as tissue flaps for treatment of traumatic injuries in a wartime scenario.

Proposal Title:	Immunoprofiling of Diabetic and Nondiabetic Lumbosacral Radiculoplexus Neuropathy
Log Number:	PR220430
Current PI Name:	Divyanshu Dubey
Award Number:	HT9425-23-1-0100
Current Contracting Organization:	Mayo Clinic
Current Performing Organization:	Mayo Clinic
Web Approval Date:	01-24-2023

Lumbosacral radiculoplexus neuropathy (LRPN) is a form of non-systemic vasculitis more commonly encountered among patients with type 2 diabetes mellitus. Among patients with diabetes mellitus, it is also referred to as diabetic lumbosacral radiculoplexus neuropathy (DLRPN) or diabetic amyotrophy. Epidemiology studies have demonstrated that incidence of diabetic and non-diabetic LRPN is more than twice the incidence of Guillain-Barré syndrome or chronic immune polyradiculoneuropathy, highlighting importance of early identification and management of this disease. The diagnosis of these neuropathies continues to be based on clinical evaluation, and many cases require nerve biopsy. As most patients initially presents with abrupt severe unilateral leg pain, many are misdiagnosed as nerve root compression due to disc herniation or degenerative spine disease, leading to unnecessary back surgeries. Even among patients who are correctly diagnosed, it is difficult to predict the disease course and identify a subset of individuals who are going to have disease relapse. There exists, therefore, a critical need to identify blood biomarkers that can be utilized for diagnosis as well as long-term outcome prediction. Without such biomarkers, LRPN /DLRPN diagnostics will continue to challenge many physicians, and many cases may end being misdiagnosed.

We will use a library of bacteriophages expressing all known human proteins (phage display) to evaluate presence of disease-specific antibodies in patients. Careful analysis of autoantibody repertoires with this novel technique provides an opportunity to identify an antibody signature for LRPN/DLRPN detection (diagnosis) and a distinct antibody signature to inform disease outcome or treatment response (prognosis). We will compare these readouts across different neuropathy types in addition to comparisons with healthy individuals. The antibodies identified through phage display will be carefully evaluated through other techniques as well, to confirm their sensitivity and specificity. Based on the analysis of LRPN/DLRPN nerve biopsies, this autoimmune neuropathy appears to be mediated by inflammatory cells. Therefore, we will also evaluate autoantigen-specific cell-mediated immune responses among a subset of patients, by culturing the patients' lymphocytes and antigen-presenting cells in the presence of the proteins targeted by their autoantibodies, as identified through phage display. Our studies of autoantibody signature identification and /or autoantigen specific T-cell response should also yield immediately translatable data with therapeutic significance. For example, data from our T-cell studies could also support the development of targeted therapeutic approaches such as CAR-T cells directed against specific autoreactive T-cell receptors.

Our laboratory has a strong track record in antibody biomarker discovery over the past two decades including paraneoplastic neuropathy biomarkers such as CRMP5. We have utilized technologies such as phage display to identify novel antibody biomarkers associated with unique autoimmune or paraneoplastic syndromes such as kelch-like protein 11 [KLHL11], leucine zipper 4 [LUZP4], and neurofilament-light chain IgG. We have also successfully demonstrated autoantigen specific T-cell responses among a subset of these conditions such as KLHL11 and LUZP4. We have the necessary infrastructure, expertise, and patient samples to carry out this unique project. Our long-term goal is to develop an understanding of immune mechanisms that contribute to the development and progression of LRPN/DLRPN.

Proposal Title:	Mitochondrial Metabolism and FRDA Vulnerability
Log Number:	PR220440
Current PI Name:	Joriene de Nooij
Award Number:	HT9425-23-1-0092
Current Contracting Organization:	Columbia University Medical Center
Current Performing Organization:	Columbia University Medical Center
Web Approval Date:	01-12-2023

Friedreich ataxia (FRDA) is a prevalent genetic disorder characterized by cardiomyopathy and progressive neurological disease and is associated with an increased incidence of diabetes mellitus. The genetic perturbation is a trinucleotide GAA expansion in the FRATAXIN (FXN) gene, which encodes a mitochondrial protein (also called FXN). Mitochondria are the main producers of cellular energy, and when they fail to work, cells are no longer able to function properly and will die. The precise function of FXN in mitochondria and why its loss leads to disease remain unclear, however, making it difficult to identify an effective target for therapeutic intervention.

One very curious aspect of FRDA disease is that, although mitochondria are required in all cells in the body, some cells are much more affected by the loss of FXN protein than others. A very striking example of the cell-type-selective FRDA vulnerability can be seen in the peripheral somatosensory nervous system. This sensory system comprises three main classes of neurons, including neurons that cause us to feel pain when we are hurt (pain neurons), neurons that allow us to feel touch (touch neurons), as well as neurons that allow us to feel where our limbs are relative to the rest of the body (proprioceptor neurons). While touch and proprioceptive neurons are among the earliest and most severely affected in FRDA disease, pain neurons are largely unaffected in FRDA. We believe that by studying why some tissues are more vulnerable to the loss of FXN (heart, touch, and proprioceptor neurons) and what these tissues may have in common, we may better understand the role of FXN in causing FRDA.

In our lab, we study differences in FRDA vulnerability between pain and touch sensory neurons using induced pluripotent stem cells (iPSCs). Pluripotent stem cells can be made to differentiate into all cell types in the body. For our studies, we developed methods to generate pain and touch neurons from iPSCs that were derived from cells from FRDA patients. FRDA iPSC cells in which the FXN mutation was corrected served as control cell lines. We also manipulated these FRDA and control iPSCs genetically, to mark the pain or touch neurons (after they differentiate) with a fluorescent protein (allowing us to visually identify the neurons). In our preliminary phenotypic analysis of these neurons, we obtained three major and exciting results. First, when differentiating FRDA and control iPSCs into pain or touch neurons, we found that their differential vulnerability is recapitulated in vitro: FRDA touch neurons exhibit reduced growth compared to control touch neurons, but we observed no difference between FRDA and control pain neurons. Second, we found a profound divergence in mitochondrial metabolism between these two sensory neurons: mitochondria from pain neurons mainly rely on the use of pyruvate as their fuel to sustain their main function (ATP production), while mitochondria from touch neurons – similar to cardiomyocytes – primarily use fatty acids (FAs) and/or amino acids (AAs). Third, mitochondrial metabolism in FRDA pain neurons is minimally affected, if at all, by the loss of FXN. In contrast, in FRDA touch neurons pyruvate use is unaltered, but the use of FAs or AAs as mitochondrial fuels is impaired.

Based on these observations, our central hypothesis is that sensory neurons that rely on FAs as a mitochondrial fuel are disproportionally vulnerable to a loss of FXN compared to neurons that rely on pyruvate as their mitochondrial energy source. In this proposal, we will investigate this idea by (a) comparing the mitochondrial metabolic pathways between touch and pain neurons and by (b) defining the requirement of FXN in sensory neuron FA and pyruvate metabolism. Thus, these studies will delineate the metabolic consequences of FXN loss in the context of neuronal subtypes that rely on different energy

substrates and may help identify commonalities in FRDA disease mechanisms in heart and neural tissue. Ultimately, these studies will inform therapeutic strategies aimed at halting FRDA disease in all affected tissues.

Proposal Title:	Hedgehog Pathway in Chronic Pancreatitis
Log Number:	PR220456
Current PI Name:	Vikas Dudeja
Award Number:	HT9425-23-1-0306
Current Contracting Organization:	Alabama, University of, at Birmingham
Current Performing Organization:	Alabama, University of, at Birmingham
Web Approval Date:	05-03-2023

Fiscal Year 2022 Peer Reviewed Medical Research Program Topic Area: Pancreatitis

Pancreatitis is an inflammatory disease of the pancreas that results in significant morbidity, mortality, and hospitalizations. A single bout of pancreatic injury induces acute pancreatitis. Fortunately, the pancreas has the potential to recover from this injury and regain its normal form and function. However, ongoing injury to pancreas, e.g., ongoing alcohol abuse, results in continued inflammation of the pancreas and damage to the pancreatic tissue. This leads to a healing response that results in excessive fibrosis akin to a chronic wound. Unlike in acute pancreatitis (AP), for unknown reasons, the pancreas is not able to recover and gain normal form and function once chronic pancreatitis (CP) sets in. Chronic pancreatitis results in insufficient pancreatic function, i.e., insufficient digestive enzymes as well as insufficient insulin production, leading to diabetes. Patients with chronic pancreatitis also have unbearable pain, which can lead to spiral of drug abuse and alcoholism with accompanying social and economic consequences. Unfortunately, due to high prevalence of the etiologic factors including alcoholism, smoking, and drug abuse, our military personnel and Veteran population bear significant brunt of this disease. Despite the evident need, owing to our incomplete understanding of the pathophysiology of the disease, there is no specific therapy for CP.

In our preliminary studies, we have observed that the Hedgehog pathway, an evolutionarily conserved signaling pathway, is overactive in animal models as well as in human CP specimens. Furthermore, in our preliminary studies, inhibition of Hh pathway by drugs led to marked improvement in outcomes of chronic pancreatitis. These results are very promising as these suggest that Hh pathway inhibition could emerge as novel therapy for the treatment of CP, a disease for which no treatment is current available. The overarching goal of the proposed studies is to develop preclinical data that will support a clinical trial of Hh inhibition for the treatment of CP, and for preventing development of CP in patients who have recurrent attacks of AP in near future. These studies have high translational value as “multiple” drugs to inhibit Hh are in clinical use for non-pancreatic indications, for instance, for treatment of basal cell cancer and leukemia. These drugs also have been shown to be fairly tolerable. Our proposed studies will also elucidate the mechanism by which Hh pathway activation leads to worse outcomes in CP. Specifically, we will evaluate the effect of Hh pathway activation on pancreatic stellate cells, which are resident cells of pancreas and are known to orchestrate pancreatic fibrosis and injury in CP. Furthermore, we will elucidate how Hh pathway affect immune response in CP leading to further worsening of CP outcomes. As part of the current project, we are also analyzing over 100 pancreas specimens obtained from patients who have undergone surgery for CP. This is unique as such a large number of CP pancreas specimens have never been evaluated before. This will generate data that will not only help us study our area of interest but will be of benefit to current and future researchers who are trying to find novel therapies for CP. Thus, in short term, these studies will lead to development of inhibition of Hh pathway as novel therapy for the treatment of CP. In the long term, these studies will help us understand the pathogenesis of CP, which lead to development of additional drugs. This together will help improve the outcomes of our military personnel and civilians suffering from CP.

Proposal Title:	Hedgehog Pathway in Chronic Pancreatitis
Log Number:	PR220456P1
Current PI Name:	Melena Bellin
Award Number:	HT9425-23-1-0307
Current Contracting Organization:	Minnesota, University of, Twin Cities
Current Performing Organization:	Minnesota, University of, Twin Cities
Web Approval Date:	05-03-2023

Fiscal Year 2022 Peer Reviewed Medical Research Program Topic Area: Pancreatitis

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Proposal Title:	A New Treatment for Acute Pancreatitis Injury and Pain
Log Number:	PR220457
Current PI Name:	Fred Gorelick
Award Number:	HT9425-23-1-0329
Current Contracting Organization:	Yale University
Current Performing Organization:	Yale University
Web Approval Date:	04-27-2023

The proposed research project addresses the Fiscal Year 2022 Peer Reviewed Medical Research Program Topic Area of “Pancreatitis” and will pursue the Strategic Goal of establishing a new "Treatment."

Our overarching goal is to develop a novel new treatment that effectively decreases the pain and severity of acute pancreatitis. This disease is not uncommon in our Armed Forces populations and has become over 25% more common over the past 15 years. It is most often caused by cigarette smoking, alcohol abuse, and gallstones. There is often prominent morbidity in those with severe acute pancreatitis; mortality occurs in up to 25%—long-term complications including diabetes and an increased risk for pancreatic cancer. One of the hallmarks of this disease and one of its three diagnostic criteria is severe debilitating abdominal pain. There are no specific treatments for acute pancreatitis, and therapy is primarily limited to intravenous fluids. The most frequently used pain medications are opiates, but they often cause unwanted side effects such as nausea and vomiting, can worsen pancreatitis, and carry a risk of opiate addiction.

An ideal therapy for acute pancreatitis would decrease disease severity and reduce the associated pain and not be an opiate. Our preliminary and published work suggests that renalase, a protein found in blood, may have such properties. We have shown that the whole renalase protein has potent anti-inflammatory and prosurvival properties and that it dramatically reduces injury in several preclinical (mouse) models, including acute pancreatitis. Such models are used to determine whether a treatment has the potential to work in humans. We found renalase's anti-inflammatory and survival effects in a renalase peptide that we developed into a drug. Our preliminary data shows the drug dramatically reduces pancreatic injury and pain in mice with severe pancreatitis. The reduced pain responses are seen in intact animals and nerves.

We propose to use several mouse models of severe acute pancreatitis to understand whether the treatment could help treat acute pancreatitis injury caused by different etiologies. We will also examine if it can be effective if given before or after disease onset and assess its ability to reduce pain-related responses in whole animals using clinically relevant animal models in vivo and nerves isolated from those animals in vitro.

If our hypothesis is proven and subsequent findings in human studies show similar results, the treatments have the potential to reduce acute morbidity and mortality from this disease. By not activating opiate pathways, it avoids the risk of opioid addiction. Furthermore, by decreasing the initial severity of acute pancreatitis, the therapy could reduce its long-term complications such as diabetes and pancreatitis cancer and reduce the otherwise added significant healthcare costs. This renalase-agonist and the scientific understanding pursuant to the proposed studies potential to reduce the impact of acute pancreatitis, an unpredictable disease, on the readiness of our Armed Forces and to improve the health of those in service, their dependents, and the citizens of the United States.

Proposal Title:	A New Treatment for Acute Pancreatitis Injury and Pain
Log Number:	PR220457P1
Current PI Name:	Karin Westlund
Award Number:	HT9425-23-1-0330
Current Contracting Organization:	New Mexico, University of, Health Sciences Center
Current Performing Organization:	New Mexico, University of, Health Sciences Center
Web Approval Date:	04-27-2023

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Proposal Title:	A Novel Microvesicle-Based Therapeutic Strategy of Osteoarthritis
Log Number:	PR220470
Current PI Name:	Kin-Hing Lau
Award Number:	HT9425-23-1-0061
Current Contracting Organization:	Loma Linda Veterans Association for Research and Education (LLVARE)
Current Performing Organization:	Loma Linda Veterans Association for Research and Education (LLVARE)
Web Approval Date:	12-23-2022

This proposal has two immediate goals regarding the potential therapeutic utility of microvesicles (MV) on OA. MV is formed by budding directly from plasma membrane of living cells and is a major form of the released extracellular vesicles (EV), which are nanosized, plasma membrane-bound vesicles released by cells that transport DNA, RNA, or protein biological cargoes between distant cells as a form of intercellular communication. Our first goal is to establish that MV released by bone destruction cells (osteoclasts) can act on chondrocytes to promote the repair of the damaged articular cartilage and on cells of synovium to suppress the acute and chronic inflammation in response to osteoarthritis (OA). It also acts on bone cells to rebuild the degenerated bone structures underneath the articular cartilage (subchondral bone). We believe that these skeletal activities would allow the MV to simultaneously address three important but distinct etiologies of OA, rendering it an excellent candidate for a comprehensive therapy for OA. Thus, our second goal is to evaluate the feasibility of the MV-based therapy for OA. Accordingly, this proposal is related to the Fiscal Year 2022 Peer Reviewed Medical Research Program Portfolio of Orthopaedic Medicine, Topic Areas of Arthritis and Musculoskeletal Disorders, and Strategic Goal of development and testing of novel and improved intraarticular treatments for joint injuries.

Articular cartilage is a white, dense, specialized connective tissue covering the bony articulating ends inside the joint, and its primary function of articular cartilage is to provide cushion and lubrication to facilitate natural joint motion. Injuries to the joints, chronic or increased joint loading, or genetic predisposition can lead to lesions of the articular cartilage, which often causes acute and chronic synovial inflammation and OA. OA is the most common degenerative joint disease and afflicts ~50 million people annually in the United States alone. An estimated 12% (or ~6 million) of all OA are resulted from an acute trauma event to the joint and are referred to as PTOA. The estimated aggregate financial burden specifically of OA/PTOA (in 2011) is >3.06 billion dollars annually. While primary OA affects mostly people older than 60 years, PTOA can afflict younger and more physically active population. Due to the high physical demands of military-related training and activities or to combat-related traumatic injuries to the joints, the military population has a much greater incidence of PTOA than the general population.

There is no cure for OA/PTOA. Current therapies are limited to pain management and inflammation reduction. The highly invasive surgical alternatives are unable to restore a normal cartilaginous surface and suffer from poor integration with the surrounding host bony tissues. The frequent eventual endpoint is joint replacement with a prosthetic device or even amputation, which are not viable options for young or middle-aged patients. It is speculated that a major reason for the lack of a highly effective therapy for OA is because OA is a multifaceted disorder, involving many complicated etiologies. Thus, a comprehensive therapy for OA is one that can simultaneously address most major etiologies of the diseases, e.g., (1) persistent erosion of articular cartilage due to acute and chronic inflammation of the injured/damaged synovium; (2) inability of the damaged articular cartilage to repair itself; and (3) dysfunctional remodeling of subchondral bone structure. Unfortunately, no current approved therapy can address more than one etiology. As a result, the benefit of the current therapies is often transient, marginal, and unsatisfactory.

This proposal has advanced two novel concepts: The first concept is that osteoclastic MV has potential pro-chondrogenic, anti-inflammatory, and osteogenic activities in relevant cells within the injured joint. This concept was based on the recent preliminary findings that treatment of articular chondrocytes and bone cells with osteoclastic MV enhanced its cartilage-forming and bone-forming abilities. The concept that osteoclastic MV has anti-inflammatory activity was based on the finding that injections of osteoclastic MV into injured knee joint reduced the OA-related increase thickness of that synovium, which is a consequence of inflammation. The second concept is that osteoclastic MV can be used to treat OA and related joint disorders. The most saline characteristic of this proposed therapy is that this MV-based therapy may target three key etiologies of OA, and thus it could provide a more comprehensive treatment than any current OA therapies, all of which targets only a single etiology.

The immediate objectives of this work are two-fold: (1) It tests the first hypothesis that osteoclastic MV has pro-chondrogenic, anti-inflammatory, and bone-forming activities by establishing that treatment of articular chondrocytes, cells of synovium, and bone cells would enhance cartilage formation of chondrocytes, reduce the release of pro-inflammatory factors by cells of synovium, and increase bone formation activity of bone cells, respectively. (2) It tests the second hypothesis that injections of osteoclastic MV into injured joints prevent OA development and progression after a joint injury. We expect that the results of this work would strongly support both hypotheses.

This work has three innovations: (1) The use of MV, a physiological cell product, as the therapeutic for OA is a provocative concept, and it has not been proposed by others before. The use of a natural product would have an important theoretical benefit in that it would not expect to cause immune or inflammatory responses. Thus, it would have a good safety profile. (2) Osteoclastic MV has multiple skeletal activities relevant to OA treatment. As a result, this therapy may simultaneously target multiple distinct etiologies of OA, rendering it an ideal comprehensive therapy. (3) The use of cells of monocytes/macrophage lineage to produce the MV. It would allow the use of patient’s own blood as the cell source, which is clinically beneficial. MV is believed to be stable in circulation and in body fluids, as the membrane structure of MV would protect itself from being cleared or degraded by circulating proteases. It follows that the MV-based strategy should have a relatively long biological half-life, which would lead to prolonged potency.

Proposal Title:	micro-RNA29a as a Novel Therapeutic Treatment in Inflammatory Bowel Disease
Log Number:	PR220562
Current PI Name:	Agnieszka Czopik
Award Number:	HT9425-23-1-0094
Current Contracting Organization:	Texas, University of, Health Science Center at Houston
Current Performing Organization:	Texas, University of, Health Science Center at Houston
Web Approval Date:	02-21-2023

The burden of inflammatory bowel disease (IBD) is rising globally, and there are significant economic and quality of life costs for the IBD patients and for the society, with 1 in 200 individuals affected by the disease. In the U.S. military population, prevalence of IBD is estimated to be 348 cases out of 100,000 individuals. There are significant difficulties in finding a common treatment for sufferers of IBD as each person responds to therapy differently. Even with successful management therapies, many patients encounter a diminishing therapeutic response as their treatment continues, so it is critical to develop novel, diverse approaches that can be used sequentially and interchangeably to increase the quality of life for IBD sufferers. To achieve the goal of effective individualized and targeted treatments, it is crucial to have a clear understanding of the cells and molecules involved in the IBD disease progression.

The gut is an area of high immunological challenge where tolerating what is inside the intestine and delivering protection against intestinal pathogens must be maintained. Lymphocytes are key players in mediating both the protective and the tolerizing responses, but their aberrant activation in IBD can also lead to intestinal inflammation. Very low-oxygen conditions develop within an inflamed, damaged bowel leading to the activation of a protective hypoxic response that activates the program of repair and healing in the inflamed intestine. Hypoxic signaling controls a class of broad-acting molecules called microRNAs. These microRNAs are powerful regulators in a wide variety of diseases, and their role in IBD has just begun to be appreciated. MicroRNAs can decrease activation of lymphocytes and restrain their disease-causing potential. The small size and portability of microRNAs make them attractive candidates for therapeutic interventions in the clinic as they can be efficiently and easily delivered to patients.

We propose to study the regulation of lymphocyte function by the microRNAs that show great promise to be employed as future therapeutic agents in human IBD patients. We anticipate that microRNA therapeutics have the potential to become a major new class of drugs in the treatment of bowel inflammation. We are planning to focus on the role for miR-29, a hypoxia-regulated microRNA, in the development of colitis and in the regulation of T-lymphocyte function. The miR-29 microRNA shows promise as an injectable therapeutic agent that could have clinical applications for alleviating disease progression in IBD patients. With the help of translational medicine, we hope to take bench-top science to the bedside of patients and make new treatments available for IBD sufferers and to combat the growing incidence of IBD morbidity in military personnel and the Veteran population.

Proposal Title:	Astrocyte Aryl Hydrocarbon Receptor Signaling Is Necessary and Sufficient for Mediating Metabolic Homeostasis of Gut Microbiota-Derived Tryptophan Metabolites
Log Number:	PR220578
Current PI Name:	Frank Duca
Award Number:	HT9425-23-1-0058
Current Contracting Organization:	Arizona, University of, Tucson
Current Performing Organization:	Arizona, University of, Tucson
Web Approval Date:	12-23-2022

Obesity and diabetes rates continue to rise in both the general U.S. population and specifically in Veterans, underscoring a critical need to develop novel therapeutics to treat these metabolic diseases. Recent work has highlighted the importance of the gut bacteria in host metabolism, but the exact mechanism remains elusive. In the current proposal, we aim to determine the impact of tryptophan metabolites derived from ingested nutrient breakdown via the gut bacteria. We hypothesize that changes in these metabolites contribute to the development of obesity and diabetes via direct action in the brain on specific sites that regulate energy and glucose homeostasis. This proposal is highly innovative as it will demonstrate a direct link between the diet and gut microbiome with central nervous system function. This could lead to development of probiotics and specific dietary formulas to treat obesity and diabetes. This project is a great fit for several Peer Reviewed Medical Research Program Topic Areas. For example, this work corresponds with the “Nutrition and Metabolism” Portfolio Category, specifically in the Diabetes and Nutrient Optimization Topic Areas. Indeed, it fits multiple Strategic Goals: as a foundational study that aims to “Understand correlations between nutrition and disease susceptibility,” and as a Prevention study to “Develop evidence-based diet and exercise recommendations to decrease obesity, improve nutrition, and optimize energy balance to prevent metabolic diseases.” Indeed, this proposal will understand how diet-induced changes in tryptophan metabolism can impact metabolic disease and how treatment with these metabolites can prevent or ameliorate the development of obesity and diabetes. Furthermore, it aims to understand how the gut microbiome can impact specific areas in the brain that regulate metabolism, an extremely novel hypothesis.

Proposal Title:	Evaluation of Pirfenidone as a Novel Therapeutic Strategy Against Recurrent Acute Pancreatitis
Log Number:	PR220585
Current PI Name:	Vikas Dudeja
Award Number:	HT9425-23-1-0900
Current Contracting Organization:	Alabama, University of, at Birmingham
Current Performing Organization:	Alabama, University of, at Birmingham
Web Approval Date:	09-11-2023

The current project aims to evaluate a new therapy for recurrent acute pancreatitis in clinical trial. Pancreatitis has been identified as a topic area for Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP). Furthermore, development of novel therapies for the treatment of pancreatitis is also a Strategic Goal.

Acute pancreatitis is an inflammatory disorder of the pancreas. It is a major cause of health care cost in United States. Patients with acute pancreatitis presents with severe abdominal pain. While most patients with acute pancreatitis get better by supportive treatment and do not need any further treatment, up to 30% of patients will develop repeated episodes of pancreatitis. These patients are labeled to have recurrent acute pancreatitis. Not only these recurrent bouts of pancreatitis lead to unnecessary suffering, these patients are at high risk of death, decreased quality of life, and high risk of progression to chronic pancreatitis, a disease characterized by chronic pain, lack of digestive enzymes, malnutrition, diabetes, and increased risk of cancer.

Currently, there is no specific treatment, which can prevent future bouts of pancreatitis in patients with recurrent acute pancreatitis. Smoking, alcoholism, and drug abuse are the key risk factors for development of pancreatitis. Military personnel and Veterans are very susceptible and at a very high risk of developing pancreatitis, as alcohol intake, smoking, and drug abuse have been commonly observed in this population.

The Principal Investigator (PI) of the current grant has demonstrated in his laboratory that pirfenidone, a novel anti-inflammatory molecule is very effective against animal models of pancreatitis. These results from animal studies are very exciting for multiple reasons. (1) Pirfenidone treatment not only prevents development of pancreatitis, but even if pancreatitis was to develop, pirfenidone treatment reduces its severity. (2) Furthermore, pirfenidone treatment of animals, which have recurrent episodes of pancreatitis, reduces the progression of this disease to chronic pancreatitis with its associated sequelae. These studies thus mirrored the clinical presentation of the patients with recurrent acute pancreatitis. Furthermore, the fact that pirfenidone is already approved for clinical use in patients with idiopathic pulmonary fibrosis facilitates the execution of this clinical trial. Based on our data, we have put together a clinical trial for the evaluation of safety, tolerability, and efficacy of pirfenidone in patients with recurrent acute pancreatitis.

In the current study, we will conduct a clinical trial to test the safety, tolerability, and efficacy of pirfenidone in improving recurrent acute pancreatitis. This clinical trial will be performed at three major academic centers, namely, University of Alabama at Birmingham; Mayo Clinic, Rochester; and Brigham and Women' s Hospital. We have put together a strong study team to ensure the success of this clinical trial. The PI of the current grant, Dr. Dudeja, has extensively studied pirfenidone in laboratory and has generated all the data that forms the basis of the current clinical trial. The Co-PI, Dr. Vege, is a world-renowned clinical expert in pancreatitis and has run two successful clinical trial of drugs in acute pancreatitis. The Co-Investigators, Drs.

Julia McNabb-Baltar and Charles Mel Wilcox, are also world-renowned experts in the management of pancreatitis and have busy clinical pancreas practices. The expertise of our team members provides confidence that we will be able to execute this clinical study without difficulty.

Successful execution of the proposed studies will lead to emergence of pirfenidone as the first disease-modifying treatment for recurrent acute pancreatitis. Thus, the proposed studies are very translational and have the potential to revolutionize the treatment of pancreatitis. Development of novel treatments for pancreatitis will improve the outcome of a large U.S. patient population including Veterans and military personnel suffering from this formidable disease.

Proposal Title:	Characterization and Validation of a New Mouse Model of Spontaneous Endometriosis to Implement Translation of Basic Research to the Clinic
Log Number:	PR220589
Current PI Name:	Emanuele Pelosi
Award Number:	HT9425-23-1-0147
Current Contracting Organization:	University of Queensland
Current Performing Organization:	University of Queensland
Web Approval Date:	02-22-2023

Background: Endometriosis is a chronic, debilitating disease in which endometrial tissue, which normally lines the uterus, grows outside the uterus. Endometriosis is associated with infertility, severe pain and reduced quality of life and is estimated to affect 10% of women worldwide. Despite its prevalence, the causes of endometriosis remain poorly understood. The most accepted theory is that endometriosis is the result of a process called retrograde menstruation, where endometrial cells are sloughed off into menstrual fluid, flow backwards through the fallopian tubes and implant into tissues in the peritoneal cavity. In support of this theory, endometriosis develops in over half of women affected by uterine obstruction, which can occur during embryonic development. In these women, retrograde menstruation is greatly exacerbated because outflow of menstrual fluid is impeded. For reasons that are still unclear, although retrograde menstruation occurs in most women, only a minority will develop endometriosis. Hence, there is a pressing need for an improved understanding of the mechanisms underlying endometriosis in order to deliver better diagnostic and therapeutic options for patients, as invasive surgery is currently the only means to definitively diagnose this condition.

One reason that endometriosis is poorly understood is that there are currently no small animal models of spontaneous endometriosis. This negatively impacts our ability to study this disease, test potential treatments, and find new diagnostic strategies. Because laboratory mice do not normally develop endometriosis, most research has been conducted by artificially introducing uterine tissue into the peritoneal cavity. These experiments typically require surgical removal of the ovaries and injection of hormones to support endometriosis growth. This has made it difficult to study the most common form of endometriosis in humans, which are lesions that grow directly on the ovaries called endometriomas. While endometriomas greatly reduce fertility and increase the risk of ovarian cancer, the exact mechanisms by which this occurs are still unclear.

Recently, we have developed the first mouse model of spontaneously occurring endometriosis. We have done this by suppressing a gene called Hnf1b in the mouse uterus, which causes uterine obstruction. We have found that Hnf1b mutant mice develop endometriosis and show signs of ovarian disease also observed in humans, offering a world-first model to study the pathological mechanisms of endometriosis as they arise spontaneously without surgical or pharmacological interventions.

Here, we will extend our breakthrough by using our Hnf1b mutant mice to address two major knowledge gaps in the endometriosis field. We will:

1) Define the mechanisms of endometriosis in the Hnf1b mutant mouse model.

2) Determine the impact of endometriomas on fertility and ovarian health.

By answering these questions, we will address the Strategic Goal to Improve understanding of long-term complications and comorbidities of associated diseases and conditions within the Fiscal Year 2022 Peer Reviewed Medical Research Program Topic Area of Endometriosis (Internal Medicine portfolio). We anticipate that comprehensive analyses of our Hnf1b mutant model will reveal new mechanisms of disease progression and improve our understanding of the impact that endometriosis has on fertility and cancer risk.

Innovation: A new strategy is needed to address long-standing limitations in endometriosis research and fill critical knowledge gaps, especially in the fields of infertility and ovarian cancer. We have developed the first mouse model of spontaneous endometriosis. With this innovation, we have established the necessary resources to place our work at the forefront of endometriosis research. The implementation of our animal model will open new research avenues, including the validation of findings from previous models, the refinement of research strategies, and the development of new experimental designs.

Impact: These animal models hold strong potential to completely transform the field, allowing new opportunities for translation to the clinic. This will result in substantial breakthroughs that will uncover critical mechanisms involved in endometriosis development and progression. We contend that this research has significant potential to inform future preclinical trials as well as clinical guidelines and practices. Most importantly, this study will improve our understanding of the impact that endometriosis has on fertility and ovarian disease, enabling clinicians to better counsel affected patients.

Proposal Title:	Determining a Role for PP2A in the Development and Treatment of Endometriosis
Log Number:	PR220601
Current PI Name:	Caitlin O'Connor
Award Number:	HT9425-23-1-0046
Current Contracting Organization:	Michigan, University of
Current Performing Organization:	Michigan, University of
Web Approval Date:	12-23-2022

Endometriosis is a reproductive disorder affecting 5%-15% of women of reproductive age, caused by endometrial tissue aberrantly growing outside of the uterus. Despite the high prevalence and debilitating nature of this disease, the causes of endometriosis are still not well understood, and many women diagnosed with endometriosis have few therapeutic options. Discovering new clinical interventions and therapies is critical for both the health of those dedicated to protecting our country and to our population as a whole.

Recent studies have suggested that the invasive phenotype of endometriosis shares similar mechanisms with tumor metastasis. In support of the link between endometriosis and cancer, recent genetic studies have identified cancer mutations present in endometriosis tissues. Researchers believe that there may be shared mechanisms in the development of endometriosis and cancer. One particular tumor suppressor, Protein Phosphatase 2A (PP2A), is frequently turned off in a wide range of human cancers, and has recently been found to be turned off, or mutated, in endometriosis. Based on this, we hypothesize that PP2A is also a key suppressor of endometriosis. PP2A is built through the assembly of a scaffolding A-subunit, a catalytic C-subunit, and one of four classes of regulatory B-subunits. Our lab has classified a class of small molecules, called Small Molecule Activators of PP2A (SMAPs), which take PP2A from an off state to an on state by stabilizing the fully assembled A/B/C complex. These drugs represent one of the first known and published examples of small molecules that turn on the brakes to kill cancer cells.

An imbalance in estrogen and progesterone signaling contributes to endometriosis development. PP2A is known to regulate both signaling pathways. Here, we seek to understand if turning off PP2A, by mutation, leads to altered estrogen and progesterone signaling and ultimately endometriosis development. We will also explore the effects of turning on PP2A, by SMAPs, on estrogen and progesterone signaling and determine if SMAPs can stop the growth of endometriosis tissue, like how SMAPs can stop the growth of cancer cells.

This proposal will test the contribution of PP2A mutations on endometriosis development for the first time. Additionally, this proposal will determine if small molecule activation of PP2Ais a new therapeutic strategy for endometriosis treatment. Combined, this proposal covers the Peer Reviewed Medical Research Program (PRMRP) Topic Area of Endometriosis and two key PRMRP Strategic Goals: epidemiology, where novel mouse models will be developed, and treatment, where we will explore if SMAPs are a novel fertility-sparing and non-surgical therapeutic for the treatment of endometriosis. Together, with the successful completion of this proposal, we hope to contribute to improving the treatment options and quality of life of patients suffering from endometriosis.

Proposal Title:	Development of Clinical Candidates for the Treatment of Myotonic Dystrophy
Log Number:	PR220632
Current PI Name:	Matthew Disney
Award Number:	HT9425-23-1-0336
Current Contracting Organization:	Florida, University of
Current Performing Organization:	Florida, University of
Web Approval Date:	05-03-2023

Background: Myotonic muscular dystrophy type 1 (DM1), also known as Steinert's disease, is the most common adult-onset form of muscular dystrophy, affecting ~1:8000 individuals. Symptoms include muscle weakness and atrophy, difficulty relaxing muscles (myotonia), insulin insensitivity, cataracts, and cardiac arrhythmia. DM1 is caused when three DNA building blocks, or nucleotides, are repeated too many times in a gene, also known as triplet repeat expansion. In DM1, the major cause of toxicity and symptoms occurs when the gene is transcribed into a biomolecule known as RNA. During this transcription process, the triplet repeat expansion, CUG, adopts into a 3D structure that looks like a bobby pin, or a hairpin-like structure. This structure binds and inactivates a protein named muscleblind-like 1 (MBNL1) that is responsible for regulating other RNAs. The interaction between the DM1 hairpin and (MBNL1) is central to the pathology of DM1; when CUG sequesters (binds and activates) MBNL1, other RNAs are not processed correctly by the cell and form defective proteins. Among many others, two RNAs processed incorrectly encode the muscle-specific chloride ion channel and insulin receptor proteins, direct ties to muscle weakness and insulin insensitivity associated with DM1.

Alignment with Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Areas and Strategic Goals: DM1, as well as other diseases caused by repeat expansions including frontotemporal dementia (FTD), DM type 2, and fragile X-associated tremor ataxia syndrome (FXTAS) are all FY22 PRMRP Topic Areas and share common causes of disease pathology. Our studies to de-activate the RNA that causes DM1 are broadly applicable including to myotonic dystrophy type 2 (DM2), FTD, and FXTAS. The proposed research directly aligns with the PRMRP Strategic Goal: "Develop and evaluate novel treatments, strategies or therapeutic targets, including research to repurpose existing drugs, for associated neurological diseases and psychological conditions."

Proposed Studies: Under our previously funded work, we developed an innovative approach to use the body' s immune system to degrade selectively the CUG repeat expansion that causes DM1. Importantly, the drug only degrades its intended target in disease-affected cells and has no effect on healthy cells. This approach, which we call RiboTACs, could revolutionize how repeat expansion diseases are treated. Briefly, our RiboTACs comprise a lead drug that selectively targets the DM1 RNA and a building block that activates a cellular protein that functions in the immune system by destroying viruses. Usually, this protein is only activated when a cell encounters a virus. We will recruit this protein to seek out and destroy only the toxic DM1 RNA with a drug-like small molecule, causing its selective degradation. By selectively degrading the RNA, we can alleviate multiple modes of DM1 toxicity. Our proposed research is focused on developing these lead RiboTACs into clinical candidates for the treatment of DM1, accomplished by three aims: (i) optimize lead, drug-like compounds for inhibiting DM1 disease pathology; (ii) comprehensively evaluate the compounds in patient-derived muscle cells and study cellular selectivity across all human RNAs; and (iii) evaluate lead molecules in mouse models by measuring pharmacokinetics and tissue exposure, safety profiles, and therapeutic efficacy. Collectively, our studies will afford clinic candidates for treatment of DM1 by eliminating the disease-causing agent and broadly demonstrate that DM1 and other diseases caused by RNA are viable small molecule drug targets.

Ultimate Applicability and Impact:

Short-Term Impact: The therapeutic modality of choice for RNA targets has long been antisense oligonucleotides (ASOs). Unfortunately, ASOs suffer from therapeutic liabilities including poor cellular and tissue permeability, and clinical trials of ASOs have been halted due to dose-limiting toxicity often caused by thrombocytopenia. Therefore, alternative strategies are required for therapeutic development. Herein, we will provide late-stage preclinical candidates that are the small molecule equivalent of ASOs for the treatment of DM1.

Long-Term Impact: We will develop small molecules that eliminate highly structured, toxic RNAs that cause human disease. This highly groundbreaking approach, supported by in vivo studies, will establish a completely new paradigm for therapeutic development for toxic structured RNAs. Indeed, we will develop these compounds into late-stage preclinical and then clinical candidates. Importantly, they establish a pipeline for the development of therapeutics for other microsatellite disorders causes by RNA repeat expansions.

Clinical Application and Benefit: Any neurological or neuromuscular disease that affects military personnel affect our military readiness. Indeed, military Service is associated with an increased risk to develop both ALS and FTD. Further, myotonia in general and myotonic dystrophy in particular have been diagnosed in military personnel and affect Service fitness. Thus, understanding how to short-circuit disease processes with medicines would advance new therapeutic paradigms for these important classes of disorders and will also make our military more prepared to deal with threats.

Proposal Title:	Clinical Assessment of CX-011 for Pain Relief, Increased Function, and Potential Disease-Modifying Treatment for Knee Osteoarthritis
Log Number:	PR220638
Current PI Name:	Hassan Serhan
Award Number:	HT9425-23-1-0289
Current Contracting Organization:	CarthroniX, Inc.
Current Performing Organization:	CarthroniX, Inc.
Web Approval Date:	04-30-2023

Topic Area: Arthritis; Strategic Goal: Treatment.

Osteoarthritis (OA) is a degenerative joint disease, characterized by the progressive loss of cartilage that causes stiffness, swelling, and pain. Often resulting from a joint injury, OA affects nearly 10% of the world's population, making it the most common form of arthritis and one of the most common pathological conditions. In the United States, there are over 27 million people affected by OA, with this number projected to climb steeply due to a rapidly aging population and increasing obesity rates. Each year, over $185 billion is spent to treat OA globally, establishing this disease as a major burden on global health and economics.

Service Members develop OA earlier in life and more often than civilians, necessitating long-term care and often reducing their ability to serve. Moreover, Soldiers injured by roadside bombs or other blast-related injuries often develop OA within 2 years, or five times faster than civilians suffering injuries. OA is the most common condition that renders Soldiers unfit for duty, thereby decreasing morale and readiness. In addition, Veterans are twice as likely to suffer from OA than civilians.

There are currently no treatments to slow the loss of cartilage in patients with OA. Instead, doctors focus on modifying lifestyle, managing pain, and improving joint function, with the overall goal of delaying joint replacement surgery. For those with mild OA, initial therapies include weight loss, physical therapy, and pain management using over-the-counter non-steroidal anti-inflammatory drugs (NSAIDs). As the condition progresses to a moderate stage, injections into the joint space are used to increase joint lubrication and/or reduce inflammation while continuing physical therapy and other exercises. If the disease progresses to a severe stage, opioid-based pain control is introduced in conjunction with the therapies used in previous stages. Finally, if none of these previous tares able to lessen the pain, total joint replacement surgery is considered. There are over 1 million knee and hip replacements performed each year in the U.S, at a cost of over $50 billion. Unfortunately, mechanical joints have a limited lifetime, and conducting multiple joint replacements on the same joint is very difficult on the body and has a high failure rate; this is particularly troubling for our Wounded Warriors who may require joint replacements early in life.

Here we propose to conduct the first human studies of a potential new therapy for OA called CX-011. This medicine would be injected into the joint, where it would block a signal that drives cartilage loss and pain. By intercepting this key signal that drives the development of OA, CX-011 would be the first medicine to prevent disease progression instead of just treating the symptoms. Other possible benefits would include reducing pain and increasing mobility, as well as potentially regenerating cartilage. These improvements would be likely to occur in the weeks following the first injection, with additional injections every few months resulting in further benefits. Our results so far have shown that the effects are strongly correlated with the dose; if more of the drug is injected, experimental animals see larger improvements in joint structure and function, as well as pain. The drug has been shown to be very safe, with no side effects observed even at extremely high doses. By advancing the development of a drug to help Veterans and Service Members with OA, the medicine could greatly reduce the burden and progression of OA in Soldiers

as well as the general public, thereby improving their lives and those of their families and caregivers. Our team includes the Chief of Orthopaedic Surgery as the West Los Angeles VA Medical Center, the fourth largest VA in the country; she will discuss the trial with Veterans and refer as many as possible for evaluation and enrollment so we can directly begin to see the potential benefit of CX-011 in our Service Members. If the results from this trial show the medicine to be safe and potentially effective, we will expand the trial to more patients, both civilian and military, to fully understand how effective it can be. While the first potential application of this medicine would be OA, it could also potentially benefit Veterans and patients suffering from other diseases driven by the same signal, including heart disease, diabetes, and rheumatoid arthritis.

Proposal Title:	Dissecting Mechanisms of Emphysema and Lung Fibrosis in COPD
Log Number:	PR220648
Current PI Name:	Charles Dela Cruz
Award Number:	HT9425-23-1-0034
Current Contracting Organization:	Yale University
Current Performing Organization:	Yale University
Web Approval Date:	12-22-2022

Topic Area: Respiratory Health

The burden of medical and social resources slated for tobacco-smoke-related diseases is predicted to be US$47 trillion by 2030 and is especially relevant for the Military and Veteran population with high incidence of smokers. Chronic Obstructive Pulmonary Disease (COPD) is a composite entity that includes chronic bronchitis and emphysema. Many of these patients also develop fibrosis or lung scarring. It is the fourth leading cause of death in the U.S. with tobacco smoking to be one of the major causes. Fifteen million people in the U.S. have COPD, with another 10 million undiagnosed. Unfortunately, the prevalence of tobacco use among Veterans (39%) is staggeringly higher than the general population (19.8%). Even though it is clear that cigarette smoke (CS) is the major cause of COPD, only 10%-15% of the patients who smoke develop COPD, suggesting that CS alone is not enough. Additional events are needed to induce the disease process.

Respiratory infections also have more severe consequences in individuals who have been exposed to CS than in those who are not. The mechanisms that underlie the exaggerated virus-induced pathological lung responses in CS-exposed individuals have not been adequately addressed. Respiratory viruses cause increased levels of inflammation, tissue destruction, and fibrosis in CS-exposed mice. Influenza (Flu) infections are more severe, with more cough, phlegm production, and breathlessness in smokers. CS exposure was an important risk factor in the 1978 H1N1 influenza epidemic in healthy young military recruits.

Pulmonary fibrotic changes (where the lung tissues are scarred due to many factors) often occur with emphysema (where the alveolar sacs important for gas exchanged are destroyed and become enlarged). This has been described in COPD patients with emphysema and with parenchymal fibrosis as combined pulmonary fibrosis and emphysema (CPFE). These interstitial lung abnormalities (ILA) such as fibrosis in COPD patients are associated with a greater risk of mortality. There is a group of COPD patients who have frequent flares or exacerbation of their COPD that results in increased rate of disease progression and loss of lung function. Acute exacerbations due to viruses are more severe, last longer, and are associated with heightened responses than exacerbations due to other non-viral causes.

One of the biggest questions in COPD is how, in one patient, destructive emphysematous changes and proliferative fibrotic processes can take place in the same lung. The origin and nature of the underlying signals that dictate the destruction of the tissue or the proliferation of the tissue are not known. We propose to study how the lung epithelium, which lines the inside of the lungs, in COPD dictates these signals in close proximity to cause varying lung pathologies. It is possible that either the composition of COPD matrix or the properties of COPD lung epithelium dictate these divergent signals in response to CS with or without viral infections.

The overall goal of this project is to determine what drives the COPD lung epithelium to signal to either fibrotic or emphysema response in the same lung given the same exposure of CS and/or viral infections. We

will leverage spatial core sectioning of COPD lungs with both emphysema or fibrosis, single cell RNA sequencing, matrix protein analyses, in vivo cell-matrix and cell-cell co-culture models to address our questions.

For Aim 1, we will identify the different signaling in the lung epithelium at the fibrotic foci and emphysematous foci and in between normal lung using single cell RNA sequencing and matrix protein analyses on precisely core-cut blocks of human COPD lungs.

For Aim 2, we will determine the impact of the lung epithelium on matrix modified by composition and physical stiffness. For this aim, we will use a static model of air liquid interface using lung epithelial cells overlaying a tunable extracellular matrix.

For Aim 3, we will study the cell-cell interactions as origin of divergent epithelial cell signaling mimicking emphysematous and fibrotic signaling. We will use in vitro epithelial-fibroblast cell co-culture systems.

Through these proposed experimental strategies, our goal is to have a better and novel understanding how different pathologies seen in the COPD lung is regulated. Our proposal will help shed mechanistic insights into what drives these pathological emphysematous and fibrotic changes in these patients. Better understanding on how the lung epithelium drives pathology will lead to more effective therapeutic options such as anti-fibrotics and others for all our COPD patients.

Proposal Title:	Modulating the Stem Cell Secretome for the Treatment of Ehlers-Danlos Syndrome
Log Number:	PR220656
Current PI Name:	Ganna Bilousova
Award Number:	HT9425-23-1-0019
Current Contracting Organization:	Colorado, University of, at Denver
Current Performing Organization:	Colorado, University of, at Denver
Web Approval Date:	12-22-2022

Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Portfolio Category: Internal Medicine.

FY22 PRMRP Topic Area: Ehlers-Danlos Syndrome.

FY22 PRMRP Strategic Goal: Treatment: Develop and test novel treatments, and/or improve upon existing treatments for associated diseases and conditions.

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of genetic skin and connective tissue disorders characterized by hyperextensible skin, joint hypermobility, cutaneous fragility, delayed wound healing, and chronic pain. The disease is caused by defective proteins that participate in the assembly of extracellular matrix (ECM), a mix of molecules and minerals that form a matrix between cells. There are no treatment options for EDS patients except for symptomatic care and pain management. Because of overlapping symptoms and a poorly defined genetic basis, diagnosing EDS remains a challenge, and many patients are left undiagnosed, including military personnel.

In this application, we propose to develop a so-called protein replacement therapy for EDS by replacing defective proteins in ECM with normal variants. This can be achieved by using small membrane vesicles called exosomes that are released by adult stem cells, such as mesenchymal stem/stromal cells (MSCs). MSCs are already being tested in clinical and preclinical studies for many conditions such as stroke, limb ischemia, and diabetic wounds, and their therapeutic potency is being attributed to the release of exosomes filled with a variety of factors, including proteins and messenger RNAs (mRNAs) that encode these proteins. These exosomes can release factors into the extracellular space and/or transport mRNAs into neighboring diseased cells, providing a template to produce functional proteins by these cells.

However, using exosomes isolated straight from healthy MSCs may not be the most effective way to treat diseases since exosomes will only contain the factors that are naturally produced by MSCs. We hypothesized that the therapeutic potency and consistency of MSC-derived exosomes can be enhanced by tailoring the factors that are secreted by MSCs to a disease of interest. For EDS, tailoring the production and secretion of proteins that are affected by the disease is not a trivial task due to the large size of these molecules. For this reason, we developed a novel technology that allows us to increase the production of proteins that are commonly affected by EDS in healthy MSCs without any permanent genetic modifications. For that, we use a modified version of the gene editing protein Cas9, which, instead of modifying the genome of the cells, induces the activation of genes in a specific manner. The activation of EDS-specific genes in MSCs using our system should enrich the content of MSC-derived exosomes with target proteins and mRNAs encoding these proteins. The delivery of these modified exosomes into EDS affected tissues will promote the release of functional ECM proteins into extracellular space and/or production of functional versions of affected proteins by neighboring cells from provided mRNA templates. Using our humanized model of EDS that we generated previously, we will validate if exosomes isolated from MSCs with enhanced production of

functional copies of EDS-associated proteins can improve the EDS characteristics observed in our mouse model. If successful in our small-scale study, the strategy will need to be validated in a large-scale study outside the scope of this proposal. While the current study will only assess the effect of a local delivery of our exosomes on EDS skin phenotype, the study can potentially be expanded to systemic delivery.

EDS affects connective tissues and delays wound healing. Therefore, the exosomes that are effective in treating EDS are likely to be effective for treating other connective tissues disease, such as osteoarthritis, and for healing acute and chronic wounds, which affect both military personnel and civilians alike. Exosomes can be easily frozen and shipped to combat zones, where they can be administered as an initial treatment strategy to promote more efficient wound healing in military burn patients and prevent the development of non-healing wounds and scarring. Thus, if successful, our exosome-based therapy for EDS can potentially provide a novel cell-free off-the-shelf therapy for wounds and potentially other conditions affecting connective tissues, such as osteoarthritis.

Proposal Title:	Antibody Discovery for Non-Human Coronaviruses with the Potential for Human Emergence
Log Number:	PR220667
Current PI Name:	Ivelin Georgiev
Award Number:	HT9425-23-1-0070
Current Contracting Organization:	Vanderbilt University Medical Center
Current Performing Organization:	Vanderbilt University Medical Center
Web Approval Date:	02-21-2023

Antibodies – protein molecules that are derived from the immune system – are effectively used in diagnostics, therapy, and prevention against numerous diseases, such as infectious disease, cancer, autoimmunity, and others. A paramount feature of antibodies is the typically exquisite specificity that they possess against their targets (referred to as antigens). Over the last several decades, antibody discovery efforts have met with notable success, resulting in the approval of numerous effective antibody-based clinical products. However, to date, antibody discovery efforts have focused on identifying antibodies against pathogens that have already crossed into humans, focusing primarily on samples (such as blood) with prior exposure (through infection or vaccination) to these pathogens. In the case of SARS-CoV-2, even though effective monoclonal antibodies and vaccines were discovered and validated within months, the COVID-19 pandemic still has had a devastating effect, resulting in the loss of millions of human lives, extreme potentially long-lasting burden on the health care system, and economic turmoil. Therefore, the preemptive development of countermeasures against a diversity of pathogens, even ones that have not yet crossed into humans – and especially before they have crossed into humans – is of utmost significance. To address this challenge, here we propose to develop a program for the discovery of countermeasures against a broad diversity of coronaviruses, specifically focusing on coronaviruses that have not yet emerged in humans.

Current “pandemic preparedness” approaches focus on antibody discovery against human pathogens. The goal of such approaches is to generate a repository of antibodies as countermeasures for future significant outbreaks of pathogens that are already observed in the human population but that are currently with lower frequency of occurrence, restricted to specific geographic regions, and/or have limited public health burden. However, in the general case, these “pandemic preparedness” approaches will not be able to prepare us against pathogens that are yet to emerge in humans. Hence, in this project, we will aim to develop a platform for the high-throughput discovery of antibodies against non-human pathogens with the potential for human emergence. Our proposed efforts fall within the Viral Diseases Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Area, with a focus on the following FY22 PRMRP Strategic Goal: “Develop or optimize vaccine strategies, platforms, or compounds, to include active or passive immunoprophylaxis, especially for Dengue, Lassa, and Crimean-Congo Hemorrhagic fever viruses, beta-coronaviruses.”

As an initial focus of our efforts, we will target the development of this platform in the context of coronaviruses, a family of pathogens with clear potential for future pandemics. In particular, we will focus on the discovery and characterization of antibodies against a wide range of non-human coronaviruses, as potential therapeutic candidates and templates for broadly protective vaccines, should any of these coronaviruses emerge into humans in the future. A critical advantage of our efforts is our recently developed LIBRA-seq technology (LInking B-cell Receptor to Antigen specificity through sequencing) for antibody discovery and characterization of antigen-specific monoclonal antibodies. Unlike other antibody discovery approaches, LIBRA-seq is unique in its ability to simultaneously screen candidate antibodies against a theoretically unlimited set of targets. LIBRA-seq therefore provides a unique opportunity for screening for

antigen-specific antibodies that are capable of recognizing a wide range of coronaviruses. Overall, the discovery of antibodies against diverse coronaviruses will become a first line of defense against new outbreaks with previously unencountered coronaviruses, if (and when) these coronaviruses emerge in humans. Further, the antibody discovery platform that we will build as part of this proposal will be generalizable to other viruses that have the potential for human emergence and will therefore be of exceptionally broad potential impact.

Proposal Title:	The Role of Circadian Rhythm Disruption in Polycystic Kidney Disease Progression
Log Number:	PR220678
Current PI Name:	Reena Rao
Award Number:	HT9425-23-1-0011
Current Contracting Organization:	Kansas, University of, Medical Center Research Institute, Inc.
Current Performing Organization:	Kansas, University of, Medical Center Research Institute, Inc.
Web Approval Date:	01-11-2023

This Discovery Award grant is within the Topic Area of Polycystic Kidney Disease (PKD). It falls under the Strategic Goal to improve the understanding of long-term complications and comorbidities of associated diseases and conditions.

Background: Autosomal dominant Polycystic kidney disease (ADPKD) is a familial disease that affects the kidneys and livers of over 12.5 million people worldwide and an estimated 1 in 500 military personnel in the United States. It is usually diagnosed between the ages of 30 to 50, an age group that is trying to make a living, serve their country, raise a family, and have a social life. So how do sleepless nights and untimely eating due to working night shifts, training, or deployment, repeated time zone changes, crying babies, or nights out on the town, affect disease progression in ADPKD patients? We made a novel finding that the body's timekeeper called the circadian clock is disrupted in the cystic kidneys of ADPKD mouse models and in human ADPKD kidneys. Chronic disruption of circadian rhythm is known to stimulate disease progression in various diseases, but its role in PKD is unknown.

In the PKD kidneys, fluid filled cysts develop and grow over time, ultimately progressing to kidney failure. The objective of this grant is to determine the role of circadian rhythm disruption in ADPKD progression. Circadian rhythms are cyclical 24-hour rhythms that maintain equilibrium in the body by regulating our body functions. Each cell in our body has a clock, composed of clock proteins, which help to maintain these circadian rhythms. It is well known that disruptions to these circadian clock rhythms aggravate progression of some diseases such as diabetes, hypertension, various cancers, and Alzheimer's disease. It is unclear if disruption of the circadian clock rhythms contributes to disease progression in ADPKD.

Although no systematic study has been done to examine if individuals with ADPKD have circadian disruption, disturbed sleep, night-time urination, and non-dipping hypertension are known to occur in ADPKD patients, which indirectly suggests possibly circadian disruption. We have now obtained novel evidence that the circadian clock is disrupted in ADPKD kidneys. We found that an important clock protein called BMAL1 is abnormally expressed in the ADPKD kidneys. We hypothesize that the disrupted circadian rhythms due to abnormal expression of BMAL1 promotes disease progression in ADPKD kidneys. The study design involves two aims, which are based on strong preliminary data supporting our hypothesis and feasibility of the proposed studies. Aim 1 will inactivate BMAL1 in the early or later stages of disease progression in an ADPKD mouse model, and Aim 2 will determine the mechanism by which BMAL1 regulates ADPKD progression.

Impact and Innovation: This would be the first study to determine the role of circadian disruption on ADPKD progression. This study is important because circadian disruption could be a risk factor that accelerates disease progression in ADPKD. Disease progression in individuals with ADPKD is not uniform, with rapid cyst growth starting anywhere between infancy to 80 years of age. If our hypothesis is correct, based on our preliminary data, we expect disruption of the circadian rhythm to accelerate ADPKD progression. We also expect to define the molecular mechanisms that accelerate ADPKD progression in

mice with circadian disruption and the role of BMAL1. We expect to identify novel targets for treatment that could be beneficial for ADPKD patients. Innovation includes a novel hypothesis that the circadian rhythm is disrupted and is pathogenic in ADPKD kidneys and that reducing BMAL1 levels can slow or stop ADPKD progression. Our novel approach includes the use of an adult ADPKD mouse model, tissue-specific inactivation of BMAL1 gene and use of unbiased approaches to determine how BMAL1 regulates disease progression. This research will generate data that will form the foundation for future research that tests if disruption of the circadian rhythm due to environmental factors such as altered sleep patterns and mistimes feeding accelerate cyst growth in PKD and determine methods to synchronize the circadian clock and slow cyst growth in ADPKD patients.

Proposal Title:	Oligonucleotide-Based Therapy for Frontotemporal Degeneration
Log Number:	PR220679
Current PI Name:	Mohammad Moshahid Khan
Award Number:	HT9425-23-1-0043
Current Contracting Organization:	Tennessee, University of, Health Science Center
Current Performing Organization:	Tennessee, University of, Health Science Center
Web Approval Date:	12-15-2022

Military personnel face a unique set of risk factors such as the use of improvised explosive devices and other explosives in recent military conflicts. In the recent wars in Iraq and Afghanistan, traumatic brain injury (TBI) has been one of the most common types of injury sustained by Soldiers and military personnel. Persistent and multiple blast-related TBI can lead to behavioral, neurodegeneration, and cognitive effects. One common form of this deleterious outcome is frontotemporal degeneration (FTD), which is a progressive neurodegenerative process marked by atrophy of the frontal and temporal lobes, a common form of dementia affecting behavior, cognition, and language. It is the most common cause of dementia for people under age 60, affecting more than 50,000 Americans. At present, there are no therapeutic interventions that prevent frontotemporal dementia or slow its progression. The available symptomatic treatments have limited efficacy and are often associated with significant side effects. Thus, there is a critical need to identify potential disease-modifying treatments that provide a higher quality of life for individuals affected with FTD. Frontotemporal dementia with parkinsonism-17 (FTDP-17), is one of the major degenerative dementias caused by mutations in the tau gene, which encodes a microtubule-binding protein. Tau promotes the stabilization of microtubules and provides structural support to the neurons. In pathological conditions, the mutation in the tau gene disrupts the normal structure and function of tau and facilitates tau aggregation to form intraneuronal neurofibrillary tangles within neurons and other brain cells. Given that accumulation of pathological tau directly correlates with cognitive impairment, tau-based therapy that suppresses pathological tau formation and aggregation is a potentially impactful approach to counter FTD. DNAzyme (DNZ) is a relatively novel type of gene therapy and offers a potentially superior method to reduce protein expression because it specifically cleaves targeted RNA, it is easier to control its dosing, and it does not show off-target effects. DNZs are an excellent choice to target FTD because they can be designed to selectively eliminate the mutant tau mRNA without any adverse effect, and they cross the blood-brain barrier, thus avoiding the need for direct central nervous system injection. We have designed a novel, brain penetrant, and specific anti-human tau DNAZyme (TDNZ) that selectively targets mutant forms of tau.

The goal of this application is to determine the therapeutic potential of anti-Tau DNZ in reducing pathological tau burden, neurodegeneration, and behavioral deficits (cognitive and motor) in rTg4510 mice, a well-characterized mouse model linked with FTD. The rationale of this study is that a medical intervention that selectively reduces the amount of RNA (the messenger molecule used during gene expression) coming from the mutant pathologic allele is predicted to reduce the severity of symptoms. The objective of Aim 1 of this study is to determine the effective dose and length of DNAzyme treatment to obtain therapeutic benefits. The objective of Aim 2 is to investigate the therapeutic benefits of DNAzyme for the amelioration of cognitive function, and neurodegeneration in a mouse model of FTD. The proposed experiments will utilize genetically engineered mice that harbor mutations known to cause FTD in humans. Any observed benefits of TDNZ therapy in a mouse model of FTD will form the basis for translational testing in human cases.

Proposal Title:	Epithelial Tissue Mechanics: A New Key to Relapse in Chronic Inflammatory Bowel Disease
Log Number:	PR220695
Current PI Name:	Alpha Yap
Award Number:	HT9425-23-1-0088
Current Contracting Organization:	University of Queensland
Current Performing Organization:	University of Queensland
Web Approval Date:	01-20-2023

Peer Reviewed Medical Research Program (PRMRP) Portfolio: Autoimmune Disorders and Immunology; Fiscal Year 2022 (FY22) PRMRP Topic Area: Inflammatory Bowel Disease; FY22 PRMRP Strategic Goal: Foundational Studies – Identify factors, to include environmental exposures, lifestyle triggers, and past medical history, impacting the onset and progression of associated immune- mediated diseases

Rationale: Crohn's disease and ulcerative colitis are inflammatory bowel diseases (IBD) that are often diagnosed in otherwise healthy people in their twenties and thirties; carry symptoms that are painful, embarrassing, and debilitating; and characteristically have a chronic course, distinguished by periods of relapse and remission. IBD is a medical bar to joining the military, and its unpredictable symptoms often prevent patients from serving active roles. IBD is a priority area for the military, since several factors associated with military service can increase the chances of developing this condition, including the stress of intensive training, gut infections, and psychological stress. IBD also affects Veterans and the dependents of Service people, creating a burden for the Department of Veterans Affairs (VA) health system.

A major goal of therapy is to prevent IBD from relapsing and thus prolong disease-free remission. At the present time, such therapies are based on the idea that low-grade inflammation occurs in the gut of IBD patients, even when they appear to be in remission. This low-grade inflammation then triggers the development of full-blown disease. Therefore, measures to prevent relapse include the use of agents directed against that low-grade, subclinical inflammation. However, these approaches are not ideal. Specific biological agents directed against the inflammatory process often wear off after time; and less-specific anti-inflammatory drugs (such as steroids) carry significant side effects. There is a clear need for new approaches that would help prevent relapse and prolong the disease-free periods for IBD patients.

Hypothesis and Aims: This proposal tests a new concept for how subclinical inflammation may provoke overt disease. Based on our pilot findings, we propose that the inflammatory factors produced during subclinical disease (i.e., even in remission) alter the mechanical properties of the lining of the gut (the epithelium). This mechanical change prevents the gut lining from eliminating dying cells. Importantly, cell death is a common, normal feature in the gut, which usually does not cause any problems, because dying cells are readily eliminated. However, if they cannot be eliminated, dying cells can themselves provoke inflammation; we propose that these retained dying cells are critical for provoking IBD relapse.

These findings carry the implication that correcting tissue mechanics may reduce the risk of the disease breaking out, even though low-grade inflammation may persist. In this Discovery Award, we seek to test this idea using animal models of IBD. We will test how the mechanical properties of the gut are altered as inflammation develops, even before disease breaks out, and how drugs that correct abnormal mechanics may delay or prevent the onset of full-blown disease.

Impact: If successful, this project will establish a new way for us to understand what causes IBD relapse, new knowledge that can lead to new types of treatments to prevent relapse. The long-term aim of this work

is the potential to use new drugs that correct gut tissue mechanics to help prevent or delay the relapse of IBD. These drugs act on the skeleton of cells and therefore are independent of conventional immune-based treatments designed to reduce low-grade inflammation. Thus, these drugs could be used as alternatives or in combination with current immune therapies, to prolong disease-free life of patients with IBD.

Proposal Title:	Comprehensive Approach to Upregulate and Stabilize Frataxin mRNA Using Antisense Oligonucleotides
Log Number:	PR220715
Current PI Name:	Marek Napierala
Award Number:	HT9425-23-1-0337
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	04-26-2023

Friedreich's ataxia (FRDA), a severe progressive neurodegenerative disorder, is caused by an increasing number of specific DNA sequences, termed GAA repeats, that are present in the Friedreich's ataxia gene (FXN). This error in DNA causes a block in the flow of the information from DNA to RNA, and ultimately leads to a deficiency of the final FXN product, a protein called frataxin. Frataxin is a small protein that is critical for proper function of other proteins in the body, especially those involved in production of the energy needed for our organs to properly work. Consequently, shortage of frataxin results in progressive destruction of organs and systems that require a large amount of energy, such us neurons of the brain and spinal cord, or the heart. That, in turn, leads to development of debilitating movement discoordination termed ataxia along with frequent and life-threatening heart disease, diabetes, loss of vision and hearing, and ultimately premature death. This relentless disease frequently manifests initially in children, adolescents, and young adults. Our preliminary studies showed that: (i) due to the presence of the GAA repeats and block of the information flow, FRDA patients produce a large amount of an incorrect RNA (message) that cannot be made into the correct frataxin protein and (ii) all FRDA patients produce a small but detectable amount of correct frataxin that functions properly, yet the amount is not enough to maintain healthy cells.

In the proposed project, we will take a new approach aimed first to increase production of the correct frataxin RNA message and subsequently to increase the amount of frataxin protein in patient cells. We will use molecules called antisense oligonucleotides, or ASOs, which are small and specific DNA fragments that can spontaneously enter diseased cells, locate frataxin RNA, block synthesis of the incorrect RNA, and stabilize the correct frataxin RNA message to increase its "molecular lifespan" in patient cells. This strategy targets the process of frataxin production on two stages: increases production of the correct frataxin message RNA and subsequently allows existing frataxin RNA to be available longer to make frataxin protein. We predict that the result of oligonucleotide treatment will be an increased amount of frataxin protein in FRDA patient cells. In summary, this work contributes to the development of a new strategy to treat frataxin deficiency in Friedreich's ataxia. Importantly, while no oligonucleotide-based therapy exists for Friedreich's ataxia, therapies using similar technology have been recently approved by the United States Food and Drug Administration for treatment of Spinal Muscular Atrophy (SMA) or Duchenne Muscular Dystrophy (DMD), paving the path for broader use of oligonucleotides for other neurodegenerative diseases, including FRDA.

Proposal Title:	Novel Calcitropes as Targets for Cardiomyopathy Treatment
Log Number:	PR220724
Current PI Name:	Jonathan Satin
Award Number:	HT9425-23-1-0655
Current Contracting Organization:	Kentucky, University of
Current Performing Organization:	Kentucky, University of
Web Approval Date:	10-03-2023

This application addresses the Topic Area of Cardiomyopathy. Cardiovascular diseases are the leading cause of death in the United States and the incidence of cardiomyopathies are rising. As many as one in 500 adults may have cardiomyopathy; it is a leading cause of heart failure, with cost of care in the Veterans Affairs system exceeding $3.5 billion. Cardiomyopathies can be broadly classified into two groups, acquired and inherited. This reflects the varied initiating events for cardiomyopathy. Acquired cardiomyopathies refer to primary effects outside of the heart itself; a common cause is chronic high blood pressure (hypertension). Inherited cardiomyopathies are typically initiated by a mutated gene encoding a protein that functions within the heart. Regardless of the origin of the cardiomyopathy, it results in damage to the muscle tone of the heart and reduces the heart's ability to pump blood to the rest of the body. A major goal of this proposal targets a novel mechanism that safely increases the ability of the heart to circulate blood. Thus, a major long-term benefit of successful completion of the proposed studies is the establishment of a new, effective broad-spectrum treatment for the diseased heart.

Our laboratory discovered that a key molecule called Rad dynamically controls the activity of a class of proteins called calcium channels. Calcium channels are present in a wide variety of tissues. The core function of calcium channels is to open, in response to an electrical stimulus, to allow calcium ions to pass into the interior of a cell. In the heart, calcium channels play key roles in contributing to the electrical function of the heart (visible as the signal on an electrocardiogram) and in contributing to the force of contraction of the heart. The stronger the heart contracts, the more blood can be distributed into the lungs and oxygenated blood away from the heart to the rest of the body. During exercise, in battle, and in any stressful situation, our hearts adapt to an increased need for more activity by pumping more blood. The key transmitter for this response is commonly referred to as adrenaline, and the physiological response is colloquially called the "fight or flight" response. To increase cardiac output, calcium channels become more active. We discovered that the protein Rad interacts within the calcium channel to limit activity. When we genetically eliminate Rad from the heart, there is a profound increase of calcium current, heart force production, and cardiac output. These findings suggest that a major physiological function of Rad is to mediate the "fight or flight" response.

A key research tool that we developed that motivates this proposal is a genetically modified animal that allows us to delete the Rad gene in only the muscle tissue of the heart (myocardium). Within 7 days of deleting myocardial Rad, we discovered that heart function dramatically increased, and in pilot studies we discovered that Rad-deleted animals show enhanced heart function into old age. When we experimentally imposed an equivalent of chronic high blood pressure to these animals, we discovered that Rad deletion prevented the usual onset of pathological heart failure. We also discovered that deletion of Rad protects the heart against a human-genetic model of cardiomyopathy. These exciting findings motivated the present proposal. We will now use a human ex vivo heart preparation to perform a head-to-head test of effectiveness in comparison to pharmacological approaches, the latter of which are in early-stage clinical trials. We complement human ex vivo experiments with in vivo animal studies to gain finer knowledge of mechanisms.

The work is divided into two complementary aims. The first aim will compare human to animal impact of Rad reduction. The second aim will explore how Rad reduction confers beneficial heart remodeling in the setting of cardiomyopathy. We envision that at the completion of these studies we can move from laboratory

experiments to the development and application of new drugs to treat Veterans and the general population from the degradation in quality of life and premature death caused by cardiomyopathy.

Proposal Title:	Nutritional Modulation of the Gut Microbiome to Mitigate Hypoxia-Induced Cognitive Impairment
Log Number:	PR220729
Current PI Name:	James Karl
Award Number:	CDMRP-22-PR220729
Current Contracting Organization:	U.S. Army Research Institute of Environmental Medicine (USARIEM)
Current Performing Organization:	U.S. Army Research Institute of Environmental Medicine (USARIEM)
Web Approval Date:	04-02-2023

Most individuals, including military personnel, who rapidly ascend to mountainous, high altitude regions experience impairments in physical and mental function due to low oxygen availability. The severity of impairment experienced varies across individuals and ranges from minor nuisance to debilitating. Why this variability exists has not been determined. Additionally, existing strategies for reducing altitude-induced impairments in physical and mental performance are often impractical or have side effects that prevent their use. Therefore, research to identify factors contributing to the unexplained variability and new strategies for reducing performance impairments are needed.

Emerging science indicates that the community of microbes living in the human gastrointestinal tract, known as the gut microbiome, may be one important factor influencing the severity of performance impairments individuals experience at high altitude. Importantly, the gut microbiome is strongly influenced by diet. Therefore, diet interventions that target the gut microbiome could help reduce altitude-induced performance decrements. This research will test that hypothesis and aims to demonstrate that: (1) the gut microbiome contributes to impairments in mental performance at high altitude and (2) nutrition interventions targeting the gut microbiome can reduce those impairments.

Research objectives will be met by using controlled animal experiments that incorporate human samples and data being collected as part of two ongoing human studies. First, human fecal samples collected during a carefully controlled nutrition and high-altitude study will be transplanted into mice that do not have a gut microbiome. These experiments will determine whether altitude-induced changes in the human gut microbiome impair mental function and whether changing the human gut microbiome using a nutrition intervention reduces those impairments. Second, fecal samples and data collected from an observational human study will be used to identify bacteria associated with resilience against mental impairments at high altitude. These bacteria will then be fed to mice to determine whether the bacteria can reduce mental impairments at high altitude. Bacteria that do will be considered candidate markers that may help identify people who may be more resilient to high altitude and will also be considered candidate probiotics for reducing mental impairments at high altitude. Collectively, this research will directly address the Fiscal Year 2022 Peer Reviewed Medical Research Program Portfolio Nutrition and Metabolism Nutrition Optimization Topic Area Strategic Goal of foundational studies to develop and test novel nutrition-based approaches to enhance performance in operational environments and extreme climates. The research is also applicable to the Neuroscience Program Area Strategic Goal of optimizing cognitive functioning in occupational environments.

This research will be the first to directly implicate the human gut microbiome as a factor contributing to impairments in mental performance experienced by most individuals who rapidly ascend to high altitudes. In the short term, the work will provide foundational knowledge that will contribute to the development of new gut microbiome-targeted nutrition interventions for reducing performance impairments at high altitude.

Ultimately, this knowledge will be incorporated into documents that define military nutrition and ration standards and provide guidance for conducting high altitude operations and will be used to develop nutritional products for optimizing military performance at high altitude.

Proposal Title:	Nutritional Modulation of the Gut Microbiome to Mitigate Hypoxia-Induced Cognitive Impairment
Log Number:	PR220729P1
Current PI Name:	Elaine Hsiao
Award Number:	HT9425-23-2-0007
Current Contracting Organization:	California, University of, Los Angeles
Current Performing Organization:	California, University of, Los Angeles
Web Approval Date:	04-02-2023

Most individuals, including military personnel, who rapidly ascend to mountainous, high altitude regions experience impairments in physical and mental function due to low oxygen availability. The severity of impairment experienced varies across individuals and ranges from minor nuisance to debilitating. Why this variability exists has not been determined. Additionally, existing strategies for reducing altitude-induced impairments in physical and mental performance are often impractical or have side effects that prevent their use. Therefore, research to identify factors contributing to the unexplained variability and new strategies for reducing performance impairments are needed.

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Ultimately, this knowledge will be incorporated into documents that define military nutrition and ration standards and provide guidance for conducting high altitude operations and will be used to develop nutritional products for optimizing military performance at high altitude.

Proposal Title:	The Role of Medial Amygdala Circuits in Mediating the Effects of Diet on Post-Traumatic Behavioral and Metabolic Complications
Log Number:	PR220736
Current PI Name:	Sarah Stanley
Award Number:	HT9425-23-1-0244
Current Contracting Organization:	Icahn School of Medicine at Mount Sinai
Current Performing Organization:	Icahn School of Medicine at Mount Sinai
Web Approval Date:	04-26-2023

Most U.S. adults (68%-89%) have experienced a severe traumatic event, and this proportion is even higher in military and Veteran populations (99%) due to exposure to combat and related situations. Trauma exposure increases the risk of a range of mental health disorders, from heightened fear and increased arousal characteristic of post-traumatic stress disorder (PTSD), to anxiety, eating disorders, and sleep disturbances. Recent studies report that the prevalence of PTSD in U.S. military Veterans is as high as 31%, while other mental health disorders affect over a third of military personnel. In parallel, trauma increases the risk of metabolic diseases such as obesity, diabetes, and cardiovascular disease. In the U.S., one in nine people have diabetes and 84 million people are pre-diabetic, but incidence is even higher in at-risk groups, such as Veterans. One in four veterans has diabetes, and there is a strong association between PTSD and metabolic diseases such as diabetes. Mental health disorders and metabolic diseases place enormous burdens on the affected individual, their family, and the larger community.

Mental health disorders and metabolic diseases after trauma are difficult to treat. Existing treatments for PTSD and additional mental health disorders after trauma have limited efficacy. There are few methods to prevent the psychological complications of trauma. Similarly, preventing and treating metabolic disease is difficult. A healthy diet and exercise regimen that reduces weight decreases the risk of diabetes, but these changes are difficult to maintain over time. So, a broader range of and better therapies to lower risks and to treat mental health and metabolic disorders after trauma are clearly needed. The studies proposed here will form a foundation for new therapies to prevent and treat the complications of psychological trauma.

Despite the known associations between intense stress, mental health disorders, and metabolic disease, systematic studies examining the mechanisms that link these factors are missing. The goal of our studies is to understand the interactions between traumatic stressors and nutrition in the development of psychological disorders and metabolic disease. To do so, we will dissect the key roles of a crucial brain region called the medial amygdala in integrating information about trauma and high-fat diet on behavior and metabolic health. Our pilot studies already show that intense stress combined with over-nutrition result in anxiety-like behavior, abnormal feeding, and increased blood glucose. Using new and improved imaging techniques, we can see that medial amygdala neurons are switched on by intense stress in real time, as well as by over-eating. Additionally, using improved technologies, we can switch on or switch off the neurons in the medial amygdala and so rapidly change activity, feeding, and blood glucose, showing this region plays key roles in regulating behavior and glucose control. Finally, our novel approach allows us to identify the crucial cell types, their location, and the genetic mechanisms that are critical for interactions between trauma and nutrition. In this proposal, we will be using our novel combination of innovative techniques to understand how trauma and nutrition interact to modulate neural activity, function, and gene expression in the development of mental health disorders and metabolic disease.

These studies will provide the foundation for understanding how trauma and nutritional environment interact to increase vulnerability to mental health disorders and metabolic diseases. By identifying the mechanisms contributing to behavioral and metabolic abnormalities after trauma and how they are disrupted by poor diet,

we will be able to identify at-risk individuals and target interventions such as behavioral modifications, diet, or drugs to prevent or treat the devastating consequences of trauma. The information from this project is a critical first step towards the development of effective treatments to improve mental health and metabolic outcomes after trauma.

Proposal Title:	The Role of Medial Amygdala Circuits in Mediating the Effects of Diet on Post-Traumatic Behavioral and Metabolic Complications
Log Number:	PR220736P1
Current PI Name:	Abha Rajbhandari
Award Number:	HT9425-23-1-0245
Current Contracting Organization:	Icahn School of Medicine at Mount Sinai
Current Performing Organization:	Icahn School of Medicine at Mount Sinai
Web Approval Date:	04-26-2023

Most U.S. adults (68%-89%) have experienced a severe traumatic event, and this proportion is even higher in military and Veteran populations (99%) due to exposure to combat and related situations. Trauma exposure increases the risk of a range of mental health disorders, from heightened fear and increased arousal characteristic of post-traumatic stress disorder (PTSD), to anxiety, eating disorders, and sleep disturbances. Recent studies report that the prevalence of PTSD in U.S. military Veterans is as high as 31%, while other mental health disorders affect over a third of military personnel. In parallel, trauma increases the risk of metabolic diseases such as obesity, diabetes, and cardiovascular disease. In the U.S., one in nine people have diabetes and 84 million people are pre-diabetic, but incidence is even higher in at-risk groups, such as Veterans. One in four veterans has diabetes, and there is a strong association between PTSD and metabolic diseases such as diabetes. Mental health disorders and metabolic diseases place enormous burdens on the affected individual, their family, and the larger community.

Mental health disorders and metabolic diseases after trauma are difficult to treat. Existing treatments for PTSD and additional mental health disorders after trauma have limited efficacy. There are few methods to prevent the psychological complications of trauma. Similarly, preventing and treating metabolic disease is difficult. A healthy diet and exercise regimen that reduces weight decreases the risk of diabetes, but these changes are difficult to maintain over time. So, a broader range of and better therapies to lower risks and to treat mental health and metabolic disorders after trauma are clearly needed. The studies proposed here will form a foundation for new therapies to prevent and treat the complications of psychological trauma.

Despite the known associations between intense stress, mental health disorders, and metabolic disease, systematic studies examining the mechanisms that link these factors are missing. The goal of our studies is to understand the interactions between traumatic stressors and nutrition in the development of psychological disorders and metabolic disease. To do so, we will dissect the key roles of a crucial brain region called the medial amygdala in integrating information about trauma and high-fat diet on behavior and metabolic health. Our pilot studies already show that intense stress combined with over-nutrition result in anxiety-like behavior, abnormal feeding, and increased blood glucose. Using new and improved imaging techniques, we can see that medial amygdala neurons are switched on by intense stress in real time, as well as by over-eating. Additionally, using improved technologies, we can switch on or switch off the neurons in the medial amygdala and so rapidly change activity, feeding, and blood glucose, showing this region plays key roles in regulating behavior and glucose control. Finally, our novel approach allows us to identify the crucial cell types, their location, and the genetic mechanisms that are critical for interactions between trauma and nutrition. In this proposal, we will be using our novel combination of innovative techniques to understand how trauma and nutrition interact to modulate neural activity, function, and gene expression in the development of mental health disorders and metabolic disease.

These studies will provide the foundation for understanding how trauma and nutritional environment interact to increase vulnerability to mental health disorders and metabolic diseases. By identifying the mechanisms contributing to behavioral and metabolic abnormalities after trauma and how they are disrupted by poor diet,

we will be able to identify at-risk individuals and target interventions such as behavioral modifications, diet, or drugs to prevent or treat the devastating consequences of trauma. The information from this project is a critical first step towards the development of effective treatments to improve mental health and metabolic outcomes after trauma.

Proposal Title:	Systems Pharmacology Model of Heart Failure with Preserved Ejection Fraction
Log Number:	PR220740
Current PI Name:	Sang Ging Ong
Award Number:	HT9425-23-1-0104
Current Contracting Organization:	Illinois, University of, at Chicago
Current Performing Organization:	Illinois, University of, at Chicago
Web Approval Date:	01-20-2023

Heart failure with preserved ejection fraction (HFpEF) affects more than 3 million people in the United States (~1% of the population) and carries a 75% 5-year mortality. In HFpEF patients, while the heart is able to contract normally, it is too stiff to fill properly. There is currently a lack of evidence-based therapies for HFpEF; this is in part due to its complex heterogenous nature, which makes identification of drugs/therapies challenging. There is therefore an urgent need to develop novel therapeutic drugs to reverse HFpEF. Such a drug would provide unprecedented hope to both civilian and military patients who have limited options in their battle against HFpEF.

Traditionally, efforts to combat cardiac diseases have focused on a single causative gene, which we believe is not the ideal approach when it comes to a multifactorial disease such as HFpEF. Hence, in this proposal, we will employ a systems approach that considers the more extensive network of signaling interactions and U.S. Food and Drug Administration (FDA)-approved drugs that are viable candidates for drug repurposing. Instead of targeting a single gene, we will generate a genome-wide transcriptional signature of genes that are perturbed in HFpEF and apply a systems pharmacology model to predict drugs that cause a broad reversal of genes that are dysregulated in HFpEF. Our early experiments using a single model of HFpEF have shown promising results by identifying novel compounds that can reverse impaired relaxation of cardiac cells. We will expand our pipeline to include more models of HFpEF including mice, rats, and human transcriptomic data, and test predicted compounds for their potency in alleviating HFpEF both in vitro and in vivo. Overall, these studies will establish a systems pharmacology model, new computational insights into how drugs modulate cardiac diastolic dysfunction, and a wealth of new experimental data that will validate these predictions.

Proposal Title:	Investigating if cccDNA Is the First Viral DNA Species to Be Cleared During HBV Clearance
Log Number:	PR220743
Current PI Name:	Yong-Yuan Zhang
Award Number:	HT9425-23-1-0121
Current Contracting Organization:	HBVtech
Current Performing Organization:	HBVtech
Web Approval Date:	02-14-2023

Hepatitis B is one of important diseases in Infectious Diseases Portfolio of Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Area. This proposal aims to validate a new concept that hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) is the first viral DNA species lost from HBV-infected cells, and the evidence generated from this proposal is expected to prompt the HBV field to think a new HBV cure strategy that shall focus on blocking cccDNA replenishment, not the existing cccDNA molecules. Thus, this project meets one of FY22 PRMRP Strategic Goals, i.e., improving treatment of severe/chronic diseases.

HBV chronically infects 292 million people worldwide. Nearly one million people die of HBV-related liver diseases each year. Current HBV cure strategies aim to directly eliminate existing HBV cccDNA in the nuclei of infected cells, which serve as HBV transcriptional template. cccDNA molecules have been conventionally considered long-lived in the infected cells. A lack of therapeutics that directly eliminate cccDNA is the main hurdle to conducting HBV curative treatment.

The Principal Investigator (PI) started questioning the view of cccDNA longevity because average cccDNA pool per cell in HBV-infected human livers is very small, ranging between 1 to a few copies while rcDNA (relaxed circular DNA), another viral DNA species is 100-fold higher than cccDNA. The low copies of cccDNA imply that cccDNA likely is the first viral DNA species to get lost from infected cells. Subsequently, the PI obtained preliminary data suggesting that cccDNA is spontaneously lost in a fraction of infected cells.

The PI hypothesizes that cccDNA is the first viral DNA species lost during persistent HBV infection, but the lost cccDNA is frequently replenished through new rounds of infection.

In this proposal, the PI aims to establish direct evidence at a single cell level that cccDNA is the first viral DNA species lost from infected cells. In addition, this project will also generate the results that the size of infected cells cleared of cccDNA is significantly expanded in HBV-infected animals treated with anti-HBs antibody than untreated animals.

If this hypothesis is validated through this project, the persistent presence of cccDNA in infected cells does not mean the longevity of cccDNA, rather a result of spontaneous cccDNA loss and subsequent replenishment. This discovery will likely direct a new HBV cure strategy that focuses on blocking cccDNA replenishment, a shifting from eliminating the existing cccDNA pool. Since blocking cccDNA replenishment can easily get done with anti-HBs antibody, curing chronic HBV infection would soon become a reality.

Innovations of this proposal include: (1) A novel concept that cccDNA is the first viral DNA species lost from infected cells. (2) A new HBV cure strategy that includes anti-HBs antibody as an indispensable part of

new HBV curative treatment, for interrupting cccDNA replenishment. (3) An improvement in HBV cccDNA and rcDNA detection that allows specifically and simultaneously to detect both cccDNA and rcDNA in the same infected individual cells.

Proposal Title:	AMPK-Activator Metformin to Promote Podocyte Survival in FSGS: A Pilot Trial
Log Number:	PR220744
Current PI Name:	Madhav Menon
Award Number:	HT9425-23-1-0454
Current Contracting Organization:	Yale University
Current Performing Organization:	Yale University
Web Approval Date:	10-03-2023

Nearly 600,000 Americans suffer from end-stage kidney disease (ESKD), a failure of kidney function that necessitates either dialysis or a kidney transplant. Medicare expenditures for ESKD was $37 billion (B) in 2019 (increased from $27B in 2009). Similarly, the Veterans Health Administration (VHA) spent $19B on chronic kidney disease (CKD) and ESKD care in 2014 (up 26% from 2012). The majority of ESKD in the U. S. is due to glomerular disease - a process in which the filtering component of the kidney is damaged, sometimes resulting in massive leakage of protein in the urine known as nephrotic syndrome (NS), a Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Area.

Focal Segmental Glomerulosclerosis (FSGS) (FY22 PRMRP Topic Area), a form of NS, is the most common glomerular disease in the U.S. that leads to ESKD. FSGS is typified by injury and loss of podocytes - specialized cells that are located in the filtering component of the kidney which critically cannot be renewed once lost. On the other hand, another NS called Minimal Change Disease (MCD) shows podocyte survival despite similar injury with very rare progression to ESKD. Indeed, MCD can look identical to early FSGS, and some MCD cases will "switch" to FSGS, and sustain higher risk of disease progression. Hence, understanding mechanisms in MCD could reveal targets that also help keep FSGS podocytes alive. Using human biopsies, we demonstrated that signaling by the enzyme AMP-activated protein kinase (AMPK) is increased in MCD, and markedly reduced during FSGS. Using numerous animal models of podocyte injury, we then reported that activating AMPK in FSGS using drugs including Metformin (MF) promoted podocyte survival and reduced CKD - a potential switch mechanism in injured podocytes between survival and loss. Fortuitously, MF, an antidiabetic drug, is the fourth most prescribed drug in the U.S. and has co-incidental observable benefit for diabetic kidney diseases. A clinical trial testing MF for podocyte injury in FSGS cases has not been done before.

In a pilot randomized placebo-controlled trial, we will test the hypothesis that AMPK-activation by MF for 6 months (in addition to usual care) mitigates podocyte loss in FSGS.

In Aim 1, in adults with a new diagnosis FSGS on kidney biopsy, we will serially study mechanism-related biomarkers that also correlate with improved outcomes using (i) urine, (ii) blood, and (iii) biopsy samples. Between baseline and follow up data, we will apply: (1) conventional approaches including serial serum /urine markers for podocyte loss; and (2) new innovative approaches including artificial intelligence-based biopsy-reads, gene- and protein-expression profiles within kidney podocytes to test our hypothesis.

In Aim 2, we will test whether MF therapy (with usual care) also improves clinical outcomes including remission rates, proteinuria, and renal function decline (versus controls) in FSGS, as well as test safety of MF in this context. By repurposing a widely prescribed and safe agent, and by including novel and specific mechanistic signals with efficacy/safety data, this study is a crucial proof-of-concept first step to develop a larger phase-III clinical trial using MF in clinical FSGS. Successful completion of this proposal will lead to an inexpensive and efficacious potential agent in the fight against the progression of FSGS to ESKD in the U. S. and have application to resource-poor settings across the world.

Proposal Title:	Web-Based Provider Training for Brief Cognitive Behavior Therapy (BCBT) for Suicide Prevention
Log Number:	PR220750
Current PI Name:	Justin Baker
Award Number:	HT9425-23-1-0299
Current Contracting Organization:	Ohio State University, The
Current Performing Organization:	Ohio State University, The
Web Approval Date:	04-10-2023

This proposal addresses the Fiscal Year 2022 Peer Reviewed Medical Research Program Portfolio category of Neuroscience, Topic Area of Suicide Prevention, and Strategic Goal of "Develop and test treatment strategies to manage symptoms and improve quality of life for those affected by associated neurological and psychological conditions." We plan to do this by developing and disseminating a web-based platform that will enable providers to access expert training in Brief Cognitive Behavioral Therapy (BCBT) and Crisis Response Planning (CRP), two evidence-based suicide prevention treatments.

Critical Problem to Be Addressed: Suicide rates in the United States remain elevated with over 12 million civilians in 2020 reporting suicidal ideation, 3 million making a suicide plan, and 1 million attempting suicide. Suicide rates for military and Veterans are alarmingly high, with recent data suggesting military and Veteran suicide rates are more than double that of the general U.S. population. Therefore, suicide remains a pressing public health concern, particularly among military personnel and Veterans, prompting researchers to develop and test treatments for vulnerable individuals. Two evidence-based suicide prevention treatments, Brief Cognitive Behavioral Therapy (BCBT) and Crisis Response Plan (CRP), have been demonstrated to be effective in reducing suicide related behaviors; however, few mental health providers within the Defense Health Agency (DHA) are trained to deliver these interventions.

In response to the lack of trained providers in BCBT and CRP within the DHA, the overall objective of this proposed project seeks to reduce suicide risk in at-risk Service Members through the development, implementation, and dissemination of BCBTweb. BCBTweb will be an efficient and user-friendly web-based training platform that will enable military behavioral health providers to access online trainings in BCBT and CRP from anywhere with internet access.

The proposed project will develop and evaluate a web-based training program for BCBT and CRP building upon our experience developing other, successful web-based trainings for evidence-based treatments. The web-based training, BCBTweb, will be fully sustainable and accessible with minimal costs and time constraints for providers. BCBTweb will be built and refined over five strategic phases. The inception phase will bring together leaders in the field of military suicide prevention to provide input on the BCBT manual and scripts for the training videos. In the iteration phase, agency leaders, decision-maker consultants, and 50 providers will complete BCBTweb testing and provide feedback, after which revisions will be made. In the release phase, 100 providers will complete additional testing and will provide feedback, after which more revisions will be made. In the production phase, 200 providers will complete either BCBTweb or a live workshop. Provider reactions/satisfaction, learning will be compared as well as provider fidelity as assessed through simulated treatment sessions with a standardized patient.

The immediate impact of this proposed project will be the dissemination of BCBT and CRP training via BCBTweb to current military, Department of Veterans Affairs (VA), and civilian providers. In the DHA alone there are approximately 3,000 behavioral health providers who would be given the opportunity to access this training and deliver the therapy to over 270,000 Service Members struggling with suicide

ideation and/or related behaviors. Previous web-based trainings developed by our team have trained over 6,500 providers within the first 2 years of launching the website. Providers reported previous web-based trainings as highly positive, noting advantages over traditional in-person trainings like on-demand access to the training materials that can be reviewed as needed. The use of internet technology to increase reach and efficiency of offering an interactive and expert-led online training is a strength of the proposal.

The long-term goal of the proposed project will be to have a fully trained and competent behavioral health workforce able to deliver evidence-based suicide prevention treatments with high fidelity. Suicide rates are continuing to rise making the development and dissemination of scalable training opportunities in evidence-based suicide prevention treatment necessary.

Proposal Title:	Inhibiting Mitochondrial Permeability Transition Pore Opening to Treat Mitochondrial Myopathy
Log Number:	PR220761
Current PI Name:	Erin Seifert
Award Number:	HT9425-23-1-0798
Current Contracting Organization:	Thomas Jefferson University
Current Performing Organization:	Thomas Jefferson University
Web Approval Date:	04-04-2023

Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Area: Mitochondrial disease.

Strategic Goal: “Treatment: Develop and test novel treatment strategies for mitochondrial diseases, especially those ready to progress to the clinic, including repurposing existing drugs or non-prescription treatment option.”

Critical problem to be addressed: Mitochondria provide the bulk of energy for the cell and perform numerous other functions for the cell. However, mitochondria can become dysfunctional and cause disease. Primary mitochondrial disease is caused by mutations in nuclear- or mitochondrial-encoded DNA, or from drugs or toxins. Energy deficit, per se, is not the major driver of pathology in mitochondrial disease. Rather, mitochondrial dysfunction initiates adaptive and maladaptive changes in metabolism and cell signaling that are linked to pathology. However, what initiates, sustains, and modifies these changes in vivo, and how they lead to myopathy and worsening of myopathy, are unknown. This knowledge could reveal novel therapeutic strategies.

Skeletal muscle is among the most severely affected organs; “mitochondrial myopathy” is characterized by a poor capacity for physical activity, muscle wasting and weakness. Furthermore, when a muscle biopsy can be obtained allowing for the muscle to be imaged at very high resolution, swollen mitochondria with little internal structure (cristae) are evident and suggest that a process called opening of the mitochondrial permeability transition pore (mPTP) is occurring in the muscle. Opening of the mPTP prevents mitochondria from producing energy and also might cause cell death. Evidence for opening of the mPTP is also evident in the muscle of mouse models of mitochondrial myopathy. The uptake of Ca2+ into mitochondria can be a potent trigger for mPTP opening, especially in conjunction other aspects of mitochondrial function that are frequently present when mitochondria are severely dysfunctional as occurs in mitochondrial disease. We have made the novel observation that the protein complex that allows mitochondrial Ca2+ uptake – called the “uniporter” – is greatly increased in abundance in mouse models of mitochondrial disease; this was detected from our own data from two different mouse models of mitochondrial myopathy, and in published data from six other mouse models of mitochondrial myopathy. We nominate the uniporter and the mPTP as drivers of mitochondrial myopathy, and thus as potential therapeutic targets. Indeed, small molecules that target the mPTP have been developed and continue to be in development, as are small molecules that target the uniporter.

Basic experimental approach: Basic approach: Opening of the mPTP will be inhibited by (1) genetic knockdown of the Ca2+ uniporter or (2) pharmacological (NIM811) or direct genetic targeting of mPTP. These strategies will be performed on two different mouse models of mitochondrial myopathy (loss of mitochondrial phosphate carrier, loss of Frataxin). Outcomes: (1) Signs of mPTP opening will be evaluated by imaging muscle using electron microscopy. (2) Exercise capacity will be tested by treadmill running using defined protocols. (3) Whole-body O2 consumption during treadmill running will be measured to

evaluate mitochondrial energy production during exercise. (4) Blood lactate will be measured at rest and after treadmill running, to estimate the reliance on non-mitochondrial energy production. (5) Extent of “Integrated Stress Response” (ISR) activation will be analyzed in muscle by measuring well-accepted outcomes of ISR activation. (6) ISR activation can lead to lower muscle mass; muscle mass will be measured and muscle cross-sectional area evaluated by histology.

Innovation: There are three innovative aspects of this proposal. First, the idea to target the mPTP in the context of mitochondrial myopathy has, to our knowledge, never been proposed and thus is innovative. The second innovative aspect is the insight on which this proposal is based, namely that the expression of the uniporter is increased in multiple models of mitochondrial disease that represent different perturbations in mitochondrial function. The third innovative aspect of this project is the basic concept that mitochondrial Ca2+ uptake and mPTP opening might drive the progression of mitochondrial disease. Thus, beyond identifying two potential therapeutic targets, the proposed project is expected to increase our basic understanding of mitochondrial myopathy pathogenesis.

Ultimate applicability and impact of the research: Small molecule inhibitors of the mtCU are currently under development, and small molecule inhibitors of the mPTP already exist (e.g., NIM811) and interest in their further development continues because the mPTP is a target of interest to treat other pathologies. Thus, this project has the potential to reveal two new therapeutic targets to treat mitochondrial myopathy, for which small molecules are under development or already exist.

Proposal Title:	The Role of GPR87 in Idiopathic Pulmonary Fibrosis
Log Number:	PR220762
Current PI Name:	Johad Khoury
Award Number:	HT9425-23-1-0126
Current Contracting Organization:	Yale University
Current Performing Organization:	Yale University
Web Approval Date:	02-08-2023

Fiscal Year 2022 Peer Reviewed Medical Research Program Portfolio Category: Respiratory Health; Topic Area: Pulmonary Fibrosis; Strategic Goal: Develop and test novel treatments, including precision medicine approaches, to slow progression or reverse lung injury/disease.

Pulmonary Fibrosis (PF) describes a condition in which lung tissue becomes scarred over time in response to unknown injuries, leading to shortness of breath and dry cough, that gradually progress and ultimately lead to death within 3-5 years. This condition can be idiopathic, as in idiopathic pulmonary fibrosis (IPF), or secondary to genetic disorders, lung involvement in autoimmune disorders, or to exposure to environmental toxins, chemical warfare, drugs, foreign antigens, or radiation. The most common and lethal forms, idiopathic pulmonary fibrosis (IPF), fibrotic hypersensitivity pneumonia (FHP), and autoimmune pulmonary fibrosis (CTD-PF) are also more common among military personnel and Veterans. While U.S. Food and Drug Administration (FDA)-approved anti-fibrotic treatments like pirfenidone or nintedanib can slow disease progression, lung transplantation remains the only cure. Thus, novel therapeutic approaches are of supreme interest.

Recently, using single cell RNA sequencing, a technology that enables to analyze the gene expression based on the RNA of each cell, we identified a specific cell population that exists only in fibrotic lungs, to which we refer as aberrant basaloid cells. These cells are located in the dense fibrotic regions of the lungs and express a rare combination of genes, including genes typically seen in basal cells, epithelial–mesenchymal transition, senescence cell markers, and most importantly, markers and regulators of fibrosis. Thus, it is thought that these cells have a major role in lung fibrosis. Indeed, since our report in 2020, these cells have been in the spotlight of multiple research projects worldwide. One of the cell membrane receptors expressed in aberrant basaloid cells, and can potentially serve as a therapeutic target, is G-protein coupled receptor 87 (GPR87). This gene is expressed exclusively in different types of cancer cells, and as we discovered recently, in fibrotic lungs, mainly in aberrant basaloid cells, and for lesser extent in basal cells, club cells, and goblet cells. We found that the expression of this gene correlates inversely with lung function and volumes. Moreover, this is a receptor of lysophosphatidic acid (LPA), a molecule that induces downstream signaling in the cell, leading eventually to fibrosis. Previously, it has been showed that blocking GPR87 led to withdrawal in cancer, in animal models.

We aim to discuss the role of GPR87 in pulmonary fibrosis and the therapeutic role of GPR87 blocking or silencing, as a novel therapeutic approach for pulmonary fibrosis.

First, we will isolate aberrant basaloid cells from fibrotic lungs, based on their specific markers. But because the number of these cells can be insufficient, we have succeeded previously to generate similar cells from basal cells, using aberration induction model. Second, we will co-culture these cells with different cell types: alveolar epithelial (AT) type 1 cells, AT2 and basal cells. We will discuss the effect of aberrant basaloid cells on the different cell types, based on the genetic and proteomic expression. Then we will block the expression of GPR87 and examine the effect in these cells.

Second, we will use human precision cut lung slices (very thin lung slices) from healthy lung donors. Previously, using a well-documented protocol for fibrosis induction, we revealed a higher expression of GPR87. We will examine GPR87 expression blocked as a protective against fibrosis induction.

Third, using bleomycin-induced lung fibrosis in a mouse model, we identified higher levels of GPR87 7 and 14 days after bleomycin injection. We will block GPR87 using different approaches, like silencing GPR87 expression, blocking the receptor with an antibody, using knocked out mice that cannot express GPR87, and discuss the effect in lung fibrosis development after bleomycin injection.

Our proposal will help understanding the role of aberrant basaloid cells, and specifically the role of GPR87 in the development of pulmonary fibrosis, and the therapeutic role of blocking of GPR87 as a novel approach that can lead to stabilizing and reversing pulmonary fibrosis.

Proposal Title:	Plasma Membrane Repair in the Lung
Log Number:	PR220774
Current PI Name:	William Chirico
Award Number:	HT9425-23-1-0140
Current Contracting Organization:	New York, State University of, Downstate Medical Center
Current Performing Organization:	New York, State University of, Downstate Medical Center
Web Approval Date:	03-30-2023

The research described in this proposal will fill a fundamental gap in understanding how an understudied gene family – the extended synaptotagmins (ESYTs) – helps repair the surface (termed the plasma membrane) of lung cells. This gap in knowledge must be addressed to develop therapeutic approaches for respiratory health based on this gene family. The long-term goal is to develop new therapies to accelerate plasma membrane repair and bolster membrane integrity. The plasma membrane, also called the cell membrane, is essential for life because it covers and protects all cells. It mediates the transport of compounds and is the cell's platform for communication with the extracellular environment. ESYTs link the plasma membrane to important intracellular portions of the cell, such as the cortical endoplasmic reticulum (cER), a long membrane structure located just behind the plasma membrane. The cER can protect the plasma membrane from damage and contribute to its repair. The plasma membrane can be damaged by toxins, chemicals, smoke, and pollutants. Under battlefield conditions, the lungs of active-duty Service Members can sustain damage from such airborne contaminants. In addition, military deployment is associated with the initiation of cigarette smoking and repeated usage. Prolonged exposure to airborne pollutants can lead to emphysema, chronic obstructive pulmonary disease (COPD), and lung cancer. Although cells can repair moderate damage to the plasma membrane rapidly, our knowledge of the repair pathway and ways to accelerate repair remain rudimentary. The central hypothesis is that the ESYT gene family is essential for membrane repair in the lung. This hypothesis is based on preliminary data generated in the applicant's laboratory, demonstrating for the first time that loss of ESYT-like genes in yeast profoundly impairs plasma membrane repair, and that the amount of certain ESYTs is reduced in human bronchial epithelial cells from COPD patients. The two specific aims of this proposal are to quantify the contribution of the ESYT proteins to plasma membrane repair in (1) a human lung cell line and (2) in the lungs of mice. In Aim 1, stable human lung cell lines that lack one or more of the ESYT genes will be established. These cell lines and a control cell line will be exposed to membrane-damaging compounds, such as smoke and hydrogen peroxide, and then plasma membrane repair efficiency will be measured. In Aim 2, a mouse colony lacking all Esyt genes will be established. These mice and control mice will be exposed acutely and chronically to smoke, lung physiology will be comprehensively evaluated, and plasma membrane repair efficiency of freshly isolated and cultured primary lung cells will be measured. The research described in this proposal is innovative because it focuses on a gene family – the ESYTs – newly implicated in plasma membrane repair. The proposal is novel because it translates the discovery of a membrane repair role of ESYT-like proteins in yeast – a simple model system – to humans. The research is significant because it may lead to developing therapies that will limit lung damage and accelerate lung repair.

Proposal Title:	Discovery of Novel, Early Response Biomarkers for Trials in ADPKD
Log Number:	PR220784
Current PI Name:	Alan Yu
Award Number:	HT9425-23-1-0025
Current Contracting Organization:	Kansas, University of, Medical Center Research Institute, Inc.
Current Performing Organization:	Kansas, University of, Medical Center Research Institute, Inc.
Web Approval Date:	01-10-2023

Autosomal dominant polycystic kidney disease (ADPKD) is a devastating disease with limited treatment options. Few drugs have been tested in clinical trials, and only one has been approved. The major barrier to ADPKD drug development is the lack of an early response biomarker, meaning an indicator of clinical response to an intervention that can be used to assess the efficacy of a drug expeditiously (commonly known as clinical "proof of concept"). This step is critical in determining whether to move forward with development of a drug and invest substantial money and time in the much larger and longer clinical trial required to obtain regulatory approval.

The overall goal of this proposal is to identify candidate response biomarkers using a broad-based, discovery approach. This addresses a Strategic Goal of the Polycystic Kidney Disease Topic Area, namely, to develop technologies for tracking progression of associated diseases and conditions. Because a project of this scale is not feasible in patients, we will use mouse models of ADPKD, treat them with drugs or genetic manipulation, and screen the entire genome for RNA biomarkers in their kidneys. The goal is to identify one or a small number of candidates that can subsequently be validated in future studies using biospecimens from human clinical trials.

If we can find informative response biomarkers that could be used in clinical proof-of-concept studies, this will have a huge impact in the field because it will enable the de-risking and acceleration of ADPKD drug development and thereby expedite the approval of effective therapies for patients with this disorder.

Proposal Title:	Virus-Induced Loss of Tolerance Against IgG as a Driving Force in Rheumatoid Arthritis
Log Number:	PR220829
Current PI Name:	Miriam Shelef
Award Number:	HT9425-23-1-0124
Current Contracting Organization:	Wisconsin, University of, Madison
Current Performing Organization:	Wisconsin, University of, Madison
Web Approval Date:	03-02-2023

Rheumatoid arthritis (RA) carries a lifetime risk of ~3% and causes pain, disability, early mortality, and billions of dollars annually in the U.S. alone. There are no cures or preventative treatments for RA because we do not understand exactly, how, when, or why immune tolerance for self-molecules is lost and thus cannot develop preventative strategies or accurately identify who will imminently develop RA. Two main classes of antibodies develop prior to the swollen joints of RA: anti-citrullinated protein antibodies (ACPAs) that bind to many different citrullinated proteins and rheumatoid factors (RFs), antibodies that bind to parts of other antibodies that also form in other conditions like infections. However, the original targets and triggering stimuli for these antibodies are unknown. We recently discovered that both ACPAs and RFs can bind antibodies, making antibodies themselves a shared and potentially original target of both autoantibody types in RA. However, the stimuli for the loss of immune tolerance for antibodies in RA remains a mystery. Viral infection can lead to increased RFs and has long been believed to be a RA trigger, but determining if this is true has been prevented by a lack of unique biomarkers for immune activation by viruses and uniform groups of patients who recovered from viral infection to study over time. We recently found that about half of COVID-19 patients generate RFs that bind unique part of antibodies, different parts than those uniquely bound in RA. Thus, the COVID-19 pandemic provides both a unique opportunity to study virus-induced loss of tolerance, as well as, given the >500 million people infected with SARS-CoV-2, an urgent need to address the critical problem of determining if viruses could trigger RA and how. Indeed, some post-COVID-19 patients may already be close to RA, in particular, long COVID patients with joint pain but not swelling.

The question to be addressed by this project is “Could loss of immune tolerance for antibodies caused by viral infection be a key part of RA development?” thereby addressing the Fiscal Year (FY22) Peer Reviewed Medical Research Program (PRMRP) Strategic Goal to identify factors impacting the onset and progression of associated immune-mediated diseases in the Rheumatoid Arthritis Topic Area and Autoimmune Disorders and Immunology Portfolio. To answer this question, we break down previous barriers by taking the innovative approach of utilizing the unique RFs that we recently discovered in RA and COVID-19 as biomarkers as well as people with a SARS-CoV-2 infection at a known time as a uniform cohort of post-viral patients. More specifically, we will evaluate conventionally detected RFs, viral RFs, RA RFs, and multiple ACPAs in (1) individuals who recovered from COVID-19 and provided blood multiple times over 4 years after infection, (2) individuals who developed RA who provided blood at multiple times over the course of ~20 years prior to the development of RA, and (3) Veterans with long COVID and new joint pain. We expect to detect viral RFs at key points after infection (known infection in the case of COVID-19 and previously undetected in the case of pre-RA) that disappear over time followed by the presence of RA RFs and ACPAs, suggesting that (1) viral infection could be a trigger for the autoimmune antibody repertoire and loss of tolerance that drives RA and (2) that the subset of long COVID characterized by joint pain is a precursor of RA.

The successful completion of this project will demonstrate that loss of tolerance for antibodies triggered by viral infection could drive RA. In the short term, our findings will provide new insights into the field as well as the theoretical groundwork and essential preliminary data to serve as the foundation for future research

projects that will evaluate virus-induced loss of tolerance in larger patient groups and after different infections. Further, demonstrating that at least some long COVID patients with joint pain have preclinical RA will address a second Strategic Goal (Improve understanding of long-term complications of infections [e. g., long COVID] in the Viral Diseases Topic Area in the Infectious Diseases Portfolio) as well as open new horizons in long COVID treatment that incorporate anti-RA drugs. In the long term, the viral RFs and RA RFs that we will detect at key points prior to clinical RA can be leveraged to design clinical biomarkers in pre-RA (addressing another Strategic Goal to identify biomarkers to predict onset and/or progression of associated immune-mediated diseases in the RA Topic Area) as well as inform the intelligent design of interventions to prevent RA with its pain, increased mortality, and financial burdens, thereby improving the health of Service Members, Veterans, military beneficiaries, and the American public.

Proposal Title:	S1P-Mediated Podocyte Injury in Focal Segmental Glomerulosclerosis
Log Number:	PR220830
Current PI Name:	Sandra Merscher
Award Number:	HT9425-23-1-0054
Current Contracting Organization:	Miami, University of, Coral Gables
Current Performing Organization:	Miami, University of, Coral Gables
Web Approval Date:	01-12-2023

In the United States, about 37 million adults (15% of the adult population) are estimated to have chronic kidney disease (CKD), making kidney disease a leading cause of death. Approximately 90,000 Americans die from kidney disease each year, more than from breast and prostate cancers combined.

One of the Topic Areas of the Peer Reviewed Medical Research Program is Focal and Segmental Glomerulosclerosis (FSGS). FSGS is a protein-spilling disorder that affects the kidney filtration apparatus (glomerulus). About half of the patients diagnosed with FSGS require dialysis or kidney transplantation within 5 years from the time the diagnosis is made. Interestingly, people of West African descent, including African Americans, have a four-fold greater risk than the general population to have FSGS. Researchers believe that this increased risk is due to the presence of a variation (mutation) in a gene called Apolipoprotein (APOL1) in the genome of some African Americans. In addition to APOL1, many other genes were found to be associated with the development of FSGS or with the inability to respond to the initial treatment, which is usually a cortisone-based therapy. Among these mutations, the most common ones in patients that are affected by familiar FSGS and do not respond to cortisone-based therapy are mutations in a gene that makes collagen IV (COL4), an important constituent of the filtration apparatus. Among these FSGS causing mutations, a recently described one causes the accumulation of a form of lipid called sphingosine-1 phosphate (S1P) in the kidney filtration apparatus. While more and more genes and pathways that contribute to FSGS are being investigated, there is an unmet need to understand how these different genetic variations and pathways of injury can amplify each other and are associated with a worse disease prognosis. It is ultimately only through a better understanding of the mechanisms linking known genes variations, pathways of injury and clinical presentation that a definitive cure can be identified.

The overall scope of this proposal is to investigate a new mechanism causing protein spillage in FSGS, to understand if this mechanism is responsible for the higher susceptibility to FSGS we see in African Americans and to propose a new therapeutic strategy for affected individuals. We have utilized a unique bedside to bench approach where the research being proposed stem from the clinical observation that in the kidney filtration apparatus of patients with FSGS there is the deficiency of a newly identified protein called Apolipoprotein M, APOM.

We generated strong clinical and experimental preliminary data suggesting that reduced APOM expression in patients with FSGS is associated with loss of renal function. We confirmed these findings in an experimental model of FSGS, where administration of APOM was sufficient to reduce protein spillage in the urine and was able to block injury induced by accumulation of S1P in the kidney, confirming that the APOM /S1P pathways are related and suggesting the therapeutic potential of targeting these pathways. Finally, we analyzed cells that were established from the urine of African American patients with FSGS with and without the APOL1 gene variations to demonstrate that patients with the APOL1 risk variations are more susceptible to S1P-dependent injury.

Based on these observations, we hypothesize that decreased APOM expression renders kidney filtration cells susceptible to S1P-mediated injury and that the presence of APOL1 gene variations amplifies this pathway

of injury. The proposed study is highly feasible, as we have developed all the tools needed to address this hypothesis. The risks associated with our approach to find a cure for FSGS is minimal, as our idea stems from and is supported by strong clinical observations. The proposed study is significant, as experiments proposed herein are likely be sufficient to lead to the clinical development of a new therapeutic approach to FSGS. Finally, the proposal is innovative as it utilizes unique clinically relevant tools investigating a pathway that has never been studied before.

The long-term impact of this application resides in the opportunity to identify a new pathway that contributes to disease progression in FSGS with the goal to identify new therapeutic targets that will ultimately lead to the development of personalized therapeutic strategies for patients with FSGS carrying different APOL1 gene variants.

Because APOM expression was found to be low also in larger cohorts of patients with CKD, the knowledge derived from this application may also benefit the entire population of patients affected by CKD.

Proposal Title:	Targeting of Photodynamic Therapy-Resistant Port Wine Birthmarks
Log Number:	PR220832
Current PI Name:	Wenbin Tan
Award Number:	HT9425-23-1-0008
Current Contracting Organization:	South Carolina, University of
Current Performing Organization:	South Carolina, University of
Web Approval Date:	11-30-2022

Vascular malformations are congenital vascular anomalies. They present at birth, grow proportionally with age, and do not regress naturally. They are considered as a result from differentiation impairments of vasculatures including veins, arteries, capillaries, and lymphatic vessels during embryonic development. Vascular malformations cause a variety of severe symptoms, depending on the locations of the lesions, the types of blood vessels involved, and the stage of disease, which makes clinical management very challenging. Development of novel treatments for vascular malformations is among the Strategic Goals in the Fiscal Year 2022 Peer Reviewed Medical Research Program Discovery Award.

Port Wine Birthmark (PWB) is one of the most common types of vascular malformations. PWB mainly occurs on the face with initial appearance as flat red macules in childhood; lesions tend to darken progressively to purple and by middle age they often become raised as a result of the development of vascular nodules, which are susceptible to spontaneous bleeding or hemorrhage. The pulsed dye laser (PDL) or photodynamic therapy (PDT) is the common treatment of choice for PWB; unfortunately, complete removal occurs in less than 10% patients treated. These inadequate clinical outcomes mainly result from: (1) incomplete ablation of PWB blood vessels located in the deep dermis where light from the PDL cannot reach effectively, (2) the regrowth of PWB blood vessels post-PDL, and (3) laser-resistant PWB. In order to tackle these challenging clinical barriers, this proposal aims to develop a novel modality referred as a synergistic PDT plus stem cell signaling modulator (SPSM), to achieve an enhanced therapeutic outcome for PWB treatment, particularly laser-resistant PWB lesions.

In this proposal, we will take advantages of utilizing our current advancements in the generation of PWB-derived inducible pluripotent stem cells (IPSCs) and their differentiated lineages such as endothelial cells (ECs) as the cell models to design and validate a novel approach of SPSM. These cells present a patient-matching phenotype such as laser resistance and are particularly valuable in the field of vascular malformations for circumventing the lack of animal models. This novel strategy comprises two central components: (1) PDT, to treat PWB blood vessels locating deeply in dermis, and (2) a compound to regulate stem cell laser-resistant phenotypes, thus leading to an enhanced efficacy. Specifically, the following research objectives will be pursued: (1) What is the molecular mechanism underlying SPSM-induced photosensitizing enhancement? (2) What are the optimal parameters to define a SPSM protocol including near infrared (NIR) photosensitizer, stem cell signaling modulator, and laser fluence?

This proposed study is highly innovative because it lays the groundwork for a novel approach to the successful treatment of PWB patients with laser-resistant lesions. The formulation of SPSM is novel in stem cell biology and light therapy since no such design has been ever reported. The development of SPSM is very significant because it is designed to directly address the clinical limitations of current treatment of PWB: (1) the NIR wavelength can penetrate deeper into human skin than PDL, thus targeting those blood vessels in the reticular layer of the dermis; (2) a stem cell signaling modulator can manipulate the laser-resistant phenotype, thus leading to an enhanced efficacy in destruction of PWB blood vessels; and (3) the data generated from PWB patient-derived IPSCs and ECs are clinically translational. The SPSM is also of significance as a method for broad new research applications for other types of vascular malformations with serious complications and limited treatment options, such as cerebral arteriovenous malformations, etc.

Proposal Title:	Potential-Independent Mitochondrial Targeting
Log Number:	PR220839
Current PI Name:	Lin Zhu
Award Number:	HT9425-23-1-0119
Current Contracting Organization:	Texas A&M University System Health Science Center, College Station
Current Performing Organization:	Texas A&M University System Health Science Center, College Station
Web Approval Date:	02-21-2023

Mitochondria are a vital organelle supporting a wide range of biological processes. Mitochondrial defects or dysfunctions result in many human diseases and disorders. Mitochondria are one of the most important therapeutic targets, and selective delivery of therapeutics or imaging probes to mitochondria is highly desirable for studying mitochondrial functions and treating mitochondrial diseases.

Unfortunately, mitochondria are difficult to access for many therapeutics due to their unique structures and functions. The current mitochondrial targeting strategies mainly rely on the binding affinity between the “negatively charged” mitochondria and positively charged targeting ligands. However, it is known that the mitochondrial membrane potential (charge), a key indicator of mitochondrial health, is not always constant in the cell life cycle, particularly under drug treatments, which may greatly influence the effectiveness of these potential-dependent strategies. Furthermore, the positive charge of current mitochondrial targeting ligands is not always favorable to biomedical applications and may cause undesirable side effects.

In this application, the novel charge-neutral polymers are proposed as nanocarriers for potential-independent mitochondrial targeting. Unlike the potential-driven targeting strategies, the proposed strategy will not influence the mitochondrial potential or be influenced by the mitochondrial potential. Therefore, it will be more reliable for various biomedical applications. If successful, the proposal will (i) provide the first potential-independent tool for reliable mitochondrial imaging and targeting, (ii) promote the clinical translation of mitochondria-acting drugs, and (iii) be a new strategy for repurposing other types of drugs for treating mitochondrial diseases. The proposed strategy will advance the diagnosis and treatment of mitochondrial diseases and will ultimately benefit the military Service Members, Veterans, and their beneficiaries as well as other people who are suffering from mitochondrial diseases (one of the Fiscal Year 2022 Peer Reviewed Medical Research Program Topic Areas and Strategic Goals).

Proposal Title:	DNA-Based Hydrogels for Peripheral Nerve Repair
Log Number:	PR220856
Current PI Name:	Remi Veneziano
Award Number:	HT9425-23-1-0037
Current Contracting Organization:	George Mason University
Current Performing Organization:	George Mason University
Web Approval Date:	12-15-2022

Every year, thousands of returning wounded Soldiers and Veterans undergo treatment or surgery for temporary or permanent disabilities caused by peripheral nerve injuries (PNIs). Peripheral nerves are responsible for transmitting information from the brain to the organs and regulate vital functions like breathing and heartbeat. Therefore, damage to these nerves can have severe consequences for the health of wounded Service Members that extend beyond the injury itself. PNIs generally result from combat-sustained traumatic injuries, such as blast injuries, but are also common consequences of toxin exposure, burn pit exposure, or infections by pathogens that might occur on the battlefield. PNIs affect patients across all ages and health conditions and currently represent one of the most challenging problems faced by the military health system. The steady improvement in Warfighters' protective gear has drastically reduced combat fatalities, with the vital organs being most efficiently protected; however, simultaneously, more Soldiers and Veterans are returning from battle with greater incidence of PNIs, mainly in the lower and upper limbs that are less protected than the head and the torso.

Because severe PNIs do not spontaneously heal efficiently, the need for nerve transplants and/or other surgical reconnection of the severed nerves is required. However, despite being of one of the most common injuries treated in the military health system, there is currently no treatment or procedure that enables fast and complete recovery of patients after PNIs. Often patients have to undergo multiple long and complex surgical procedures that have a very limited success rate. Because of this lack of efficient therapeutic strategies, many of these patients will experience lifelong symptoms such as chronic pain, weakness, and limb paralysis, which drastically affect their quality of life. There is an unmet need for tissue engineering solutions that enable fast regeneration of peripheral nerves in desired locations only.

To solve this unmet need, here we propose to design and assess the efficacy of a novel class of hydrogel-based biomaterials that could be used to provide the physical and biological support to neurons that is necessary to promote their growth and facilitate peripheral nerve repair. Although engineered biomaterials are not new in the treatment of PNIs, our strategy could change the way these hydrogels are currently designed and manufactured. The innovation of our solution derives from the use of DNA nanotechnology tools that allow us to use DNA as a building block for these hydrogels. The extraordinary flexibility and strength of DNA as a biomaterial will allow us to tune the mechanical and electrical properties to match those of the native nerve tissue with unprecedented precision, providing the most realistic environment possible for the nerve cells that is ideally conducive to PN repair. In addition, the pre-assembled DNA skeleton will help guide the conjugation of molecules that promote tissue repair. The DNA skeleton will be further reinforced with conductive polymers to provide conductivity to the hydrogel, which is a critical features of peripheral nerve tissues.

Pending demonstration of success in this simple in vitro proof-of-principle study, we will pursue additional efforts to improve the formulation and the stability of our DNA-based hydrogel to support animal models of PNIs. We will particularly focus our efforts on incorporating this hydrogel formulation in hollow nerve guidance conduit that could be used to reconnect the two nerve stumps of a dissected peripheral nerve. The results obtained in this project will be foundational to our long-term efforts to acquire U.S. Food and Drug Administration approval for a novel tissue engineering candidate to treat peripheral nerve injuries. Moreover, if our strategy is successful, it will serve as proof of principle for the application of this novel and

versatile scaffold for other tissue engineering and regenerative medicine applications, such as wound healing and treatment of burns, which represent other common injuries for Warfighters.

Proposal Title:	The Skeletal Stem Cell Basis for Skeletal Fragility
Log Number:	PR220870
Current PI Name:	Shawon Debnath
Award Number:	HT9425-23-1-0002
Current Contracting Organization:	Joan and Sanford I Weill Medical College of Cornell University
Current Performing Organization:	Joan and Sanford I Weill Medical College of Cornell University
Web Approval Date:	12-15-2022

This project will address Musculoskeletal Disorders, which is a Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Area. Here, we will conduct a mechanistic study elucidating the cellular basis of “bone to fat” tissue remodeling observed in skeletal fragility disorders of low bone mass such as osteoporosis. This study is in alignment with FY22 PRMRP Strategic Goals under Foundational Studies, which focuses on understanding mechanisms underlying the pathobiology of associated musculoskeletal disorders.

Critical Problem: Osteoporosis is a skeletal disorder resulting in bone fragility and increased fracture risk. A signature feature of osteoporosis and many other skeletal disorders (such as aging-associated bone loss, glucocorticoid-induced bone loss, irradiation, and anorexia nervosa) is bone loss with a gain in marrow fat content. The collective impact of these disorders is tremendous. Although osteoporosis is more prevalent in women (8 million, 1 in 5 U.S. women), it also affects over 2 million American men. Among these patients, half of all women and a quarter of men aged 50 years or more, will encounter lifetime fracture. These fractures cause deadly complications such as pneumonia, blood clots, and postoperative complications that ultimately lead to death. In the United States, Veterans are at a higher risk of developing osteoporosis compared to civilian populations due to their lifestyle and health issues related to military service, such as excessive alcohol use, spinal cord injury, lack of weight-bearing exercise, obesity, and prolonged corticosteroid use.

Current treatment options for osteoporosis and other disorders of low bone mass have strong limitations due in part to both the few anabolic agents available and the restrictions on the use of these agents, necessitating new approaches to understand the pathogenesis of and therapeutic targets for these disorders. New insights into basic bone biology for osteoporosis will lead to therapeutic advances for this disorder.

Innovation: In this project, we will scientifically perform mechanistic studies to understand the pathobiology of “bone to fat tissue” remodeling occurring in osteoporosis or other disorders of low bone mass. Here, we will provide first direct determination of the cellular basis for the decrease in the bone-forming cells, osteoblasts, and the increase in fat containing cells in the marrow, adipocytes, that lies in the heart of various disorders of low bone mass. A core innovation underlying this project is providing a key insight into the identity of a candidate adipocyte/osteoblast bipotent cell by combining all the markers that define current skeletal stem cells (SSCs) and adipocyte progenitors. Our work provides key evidence that heterogeneity exists within the current SSC definition and will further divide it into true stem and non-stem fractions. This work will also harness technical innovations to accomplish project goals, including utilization of 18+ color FACS (Fluorescence Assorted Cell Sorting) for deep phenotypic segregation of SSCs and a combination of novel in vivo transplantation used together with high-resolution 3D tissue imaging to allow for unambiguous resolution of the in vivo adipogenic capacity of specific cell populations.

Impact: In summary, this project will provide the first identification of this key cell where the decision to form bone-forming osteoblasts versus fat-forming adipocytes occurs, and the first resolution of the cellular pathogenesis of osteoporosis and related disorders down to key specific cellular populations. This study will be a paradigm-establishing discovery, as it will enable the first direct determination of the cellular basis for the decrease in bone-forming osteoblasts and increase in fat-forming adipocytes that lies at the heart of

osteoporosis and other related disorders of low bone mass. This mechanistic insight into the cellular basis of skeletal tissue remodeling associated with skeletal fragility disorders will lead to development of druggable candidates and their screening by directly targeting the appropriate cell where the lineage decision occurs.

Proposal Title:	Determine Drug-Gene and Drug-Drug Interaction Using a Zebrafish Polycystic Kidney Disease Model
Log Number:	PR220879
Current PI Name:	Zhaoxia Sun
Award Number:	HT9425-23-1-0020
Current Contracting Organization:	Yale University
Current Performing Organization:	Yale University
Web Approval Date:	01-12-2023

Topic Area: Polycystic Kidney Disease

Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disease affecting more than 600,000 Americans and an estimated 12.5 million people worldwide. Genetic studies revealed that mutations in either PKD1 or PKD2 are responsible for ADPKD. The two genes encode two proteins named Polycystin 1 and Polycystin 2, respectively. Past research suggests that the two proteins function together. It is also known that a cell surface finger-like structure called the cilium plays an important role in ADPKD. In addition to kidney cyst formation, ADPKD patients have increased risk in multiple conditions outside of the kidney, including heart disease and brain aneurysm, highlighting the need of a more holistic approach.

Since ADPKD is a slow-progressing disease, it is likely that patients are exposed to a variety of U.S. Food and Drug Administration (FDA)-approved drugs. In addition, some patients are treated with tolvaptan. In this project, we propose to take advantage of the zebrafish model system to systematically evaluate drug-gene and drug-drug interaction in intact animals holistically and across multiple organs. Specifically, we will identify both adverse and beneficial effects of a library of FDA-approved drugs on zebrafish with defective Pkd2. We will also test whether these drugs affect tolvaptan treatment. Results from this project will be informative for selecting proper medicine for routine care of patients with ADPKD and will generate leads for repurposing existing drugs for ADPKD treatment. This project is directly relevant to the Topic Area of Polycystic Kidney Disease. Since the proposal addresses medication toxicity, it is pertinent to the Strategic Goal of Epidemiology.

Proposal Title:	A Novel Immunotherapy for Treatment of Traumatic Brain Injury
Log Number:	PR220880
Current PI Name:	Manish Bhomia
Award Number:	HT9425-23-2-0002
Current Contracting Organization:	Henry M. Jackson Foundation
Current Performing Organization:	Uniformed Services University of the Health Sciences (USUHS)
Web Approval Date:	01-26-2023

Traumatic brain injury (TBI) is a leading cause of death and disability worldwide. The long-term neurological sequelae after experiencing single or multiple events of TBI are too often associated with a neurodegenerative cascade in the brain. Currently, there are no effective therapeutics that can reduce the immediate and long-term morbidity and mortality associated with it. Hence, a novel therapeutic is urgently needed to treat TBI. SARM1 (Sterile Alpha and TIR Motif Containing 1) is a protein that is found in human brain and has been shown to be a key mediator of long-term neurodegeneration after a TBI, which is responsible for neurobehavioral deficits in individual with history of single or multiple episodes of TBI. Recent studies have shown that targeting this protein improves the TBI outcomes in animal models; hence, it is an attractive target to develop novel TBI therapeutics. However, the major challenge in targeting this protein is to deliver drugs effectively to the brain. Most of the drugs cannot pass through a barrier that exist between blood and brain due to their large size. Additionally, toxicity of the drugs is another major challenge to address. To address these key issues, we propose to target the SARM1 protein with a novel approach of Nanobodies (Nb), which are a smaller version of antibodies and are found in camels. Their small size and low toxicity make them an attractive novel therapeutic drug. Nbs have been shown to accumulate in brain when administered through intravenous injections and causes little to no toxicity. In this proposed study, we plan to use the human SARM1 protein and develop Nbs in camels. These Nbs will be purified and will be tested foe their ability to target SARM1 protein in human and mouse cells. The Nbs that show promise in inhibiting this protein in cell culture studies will be tested in an experimental model of TBI in mice. Completion of the proposed studies will provide a potential novel therapeutic approach to minimize the neurodegenerative morbidity and mortality associated with severe TBI.

Proposal Title:	Novel DNA Repair-Independent Roles of DNA-PKcs in Hypercholesterolemia-Induced Atherosclerosis
Log Number:	PR220886
Current PI Name:	Hamid Boulares
Award Number:	HT9425-23-1-0229
Current Contracting Organization:	Louisiana State University Health Sciences Center
Current Performing Organization:	Louisiana State University Health Sciences Center
Web Approval Date:	03-15-2023

A high level of cholesterol in the blood stream predisposes individuals to severe cardiovascular diseases. The World Health Organization reported that 17.9 million people die every year due to cardiovascular diseases. Several risk factors have been identified for the development of such pathological conditions: physical inactivity, excessive use of tobacco and alcohol, and unhealthy diet. Despite a heathy lifestyle and the substantial improvement in the treatment of hypercholesterolemia, essentially due to the introduction of statins in the standard therapy, many patients still fail to lower the levels of lipids recommended by guidelines, resulting in a loss of clinical benefits. Defining the molecular and cellular processes underlying the complexity of the disease is essential. One of the major consequences of high levels of cholesterol in the blood is the formation of plaques on the surface of the arteries, making them very narrow. This condition is called atherosclerosis. Atherosclerosis is a silent disease that can stay without symptoms for years. Most of the time, patient realize that they have the disease only when they experience some consequent major event such as stroke or heart attack. We identified a new potential target for the treatment of such condition. We demonstrated that a protein, primarily known for its role on DNA repair (DNA-PKcs), could also play a major role in cardiovascular inflammation and the development of atherosclerosis. Initially, naively, we speculated that the relevant function of this protein was strictly linked to DNA damage. However, with more knowledge, it became evident that the role of this enzyme might be independent of its traditional role in DNA repair. This is due to two issues: (1) the enzyme is highly abundant in cells and (2) the DNA repair process requires minute levels of the enzymes (e.g., less than 1% of DNA-PK). Our laboratory previously found that genetic inhibition of DNA-PKcs is sufficient to block asthma, another inflammatory disease. Such effects correlated with a marked reduction in production of several specific inflammatory molecules. We hypothesize that the role of DNA-PKcs may be a master regulator of inflammation during the development of atherosclerotic plaques. The role of DNA-PKcs in such inflammation does not require the assistance of other factors that are requisite for DNA repair. We propose DNA-PKcs to be considered as a unique enzyme that is completely distinct from its trimer DNA-PK responsible for DNA repair processes. We will use sophisticated approaches that include an animal model of hypercholesterolemia-induced atherosclerosis and a platform of cell culture system to unravel the mechanism by which DNA-PKcs functions during inflammation. We plan also to test Nedisertib, a drug already approved for clinical trial, in our animal model. We are confident that the completion of the proposed studies will allow us to establish a completely novel and unprecedented DNA-repair independent function for DNA-PKcs in atherosclerosis and determine the mechanism(s) by which this new enzyme plays a critical role in inflammation of arteries. This proposal puts forth a very novel and paradigm-shifting concept that challenges the current understanding on the role of DNA-PKcs in cellular and tissue processes. Unraveling the mechanism by which the kinase function provides a platform on which new drugs can be developed in such a way that DNA repair processes are not interfered with. Even if our proposed study is highly relevant for the improvement of the health of military uniformed Service Members currently serving or Veterans, because of their higher risk to develop cardiovascular disease, we would like to emphasize that the general public could also benefit from our research. More importantly, the benefit of our study can also be extended to other inflammatory diseases.

Proposal Title:	PACAP as a Potential Therapeutic Intervention for Mild TBI-Induced Mood Dysregulation
Log Number:	PR220889
Current PI Name:	Fereshteh Nugent
Award Number:	HT9425-23-2-0003
Current Contracting Organization:	Henry M. Jackson Foundation
Current Performing Organization:	Uniformed Services University of the Health Sciences (USUHS)
Web Approval Date:	02-28-2023

The proposed research relates to the Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Area of Neuroscience/Trauma with Strategic Goals as Foundational Studies to provide preclinical validation for targeting a specific hypothalamic hormone, the pituitary adenylate cyclase-activating polypeptide (PACAP), as a potential neuroprotective mechanism against mood disorders as the long-term consequences of mild traumatic brain injury (mTBI) in military as well as civilian populations. U. S military Service Members as well as non-deployed military personnel are at increased risk for mTBI-induced psychiatric disorders than civilians, both from explosive devices and from an increased risk of impact injury from vehicle accidents. Enhancing PACAP in the brain is shown to exert neuroprotective and neurotrophic effects in pathological conditions associated with PACAP deficiency such as aging, neurodegenerative disorders as well as traumatic brain injury, but whether PACAP can also provide neuroprotection on the negative effects of mTBI in mood-related brain circuits and associated affective and emotional dysregulation is unknown. In this proposal, we aim to test the preventive efficacy of a novel PACAP agonist with enhanced stability and blood-brain barrier penetration on long-term negative effects of mTBI on social and motivated behaviors through regulation of lateral habenula (LHb) activity (a critical brain region involved in pathophysiology of psychiatric illnesses including depression and anxiety) in mice. Our innovative and collaborative approach of drug discovery, behavioral pharmacology and mechanistic circuit-based study designed and executed by a strong team of investigators will provide the translational validity for potential therapeutic effects of PACAP analogues in prevention of prolonged negative consequences on mood, motivation and emotional regulation by a relevant and established preclinical repetitive closed head mouse model of mTBI. The results of this proposal will have translational applications by providing potential validation for novel pharmacotherapy focused on PACAP signaling for mood disorders as the long-term consequences of mTBI in military as well as civilian populations. Therefore, our preclinical studies focused on prevention interventions in mTBI have the potential for translation to clinical applications with a significant impact in the health of military as well as clinical population.

Proposal Title:	Optimizing Small Molecule Therapeutics to Treat Focal Segmental Glomerulosclerosis
Log Number:	PR220893
Current PI Name:	Neil Hukriede
Award Number:	HT9425-23-1-0480
Current Contracting Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Current Performing Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Web Approval Date:	10-03-2023

Focal segmental glomerulosclerosis (FSGS) is a debilitating kidney disease characterized by the abnormal leak of blood proteins into the urine (proteinuria). This disease causes fluid accumulation and swelling, high blood pressure, and high cholesterol, and patients have a high risk of developing complete kidney failure, which requires kidney dialysis or transplantation for survival. FSGS is much more common in African Americans, a population that is highly represented among military personnel and Veterans, than Caucasians. African Americans also have more severe disease and a four-fold higher risk of developing complete kidney failure due to FSGS. There are very few treatment options for FSGS, and those that do exist often result in severe side effects and exhibit limited improvement. Even kidney transplantation is associated with a 30% -40% recurrence of FSGS in the transplanted organ. Thus, there is an urgent need to develop better treatments for FSGS.

Our preliminary research showed that modulating the activity of the protein HDAC8 with certain small molecules shows very promising results in an animal model of FSGS. Importantly, these molecules are effective after the onset of disease, a critical factor in treating human FSGS which occurs without warning. However, the currently available compounds have significant liabilities that preclude translation to a human therapeutic. Based on compelling data, we hypothesize that by careful medicinal chemistry design, we can generate small molecules that will be efficacious in models of FSGS, and be appropriate, after key drug development studies, for advancement to clinical trials. Therefore, the objective is to develop novel therapeutics that will prevent the progression of FSGS in military patients. This application is in response to the Peer Reviewed Medical Research Program Portfolio, Internal Medicine, with the Topic Area FSGS, and the Strategic Goal for Treatment to "Develop and test novel treatments, and/or improve upon existing treatments for associated diseases and conditions." These models were selected and designed to reflect the same types of kidney injury that would affect combat and retired military personnel. Therefore, these studies will identify candidate drugs that can be quickly translated into human clinical trials.

Proposal Title:	A Tacrolimus-Releasing Nanofiber Nerve Wrap to Enhance Motor and Sensory Recovery in Injured Peripheral Nerves
Log Number:	PR220909
Current PI Name:	Gregory Borschel
Award Number:	HT9425-23-1-0081
Current Contracting Organization:	Indiana University
Current Performing Organization:	Indiana University
Web Approval Date:	01-20-2023

Background and Objectives: Nerve injuries from penetrating or blunt trauma often result in lifelong disability with loss of sensation and paralysis. Recovery is usually poor. A key barrier to recovery is the naturally slow axonal regeneration rate which consequently deprives the target muscle and skin of nerve supply and results in permanent loss of function. Drug-based therapies that accelerate axonal regeneration may decrease the denervation time and thereby improve functional recovery. However, no such treatments to augment the results of surgery are currently available. Local delivery of the transplant antirejection drug, also known as tacrolimus, via biodegradable gels to the nerve repair site results in enhanced nerve regeneration. A more convenient, surgeon-friendly delivery system is needed for clinical use in the operating room. We aim to validate a reliable, effective, and easy-to-use, off-the-shelf product for local drug delivery following nerve surgery and demonstrate its effectiveness. We have therefore designed a biodegradable wrap for the delivery of tacrolimus that surgeons can place around the site of injured nerves during surgery.

Methods: We will fabricate nerve wraps made out of polymers sheets loaded with tacrolimus. Based on our preliminary studies on gel-based application of tacrolimus, these devices will provide sustained tacrolimus release to the nerve repair site for 4 weeks or longer. We will assess effectiveness in a rat model by wrapping the devices around nerve repair sites. Adult rats will undergo hindlimb nerve transection and immediate repair. Intraoperatively, rats will receive either the nerve wrap loaded with tacrolimus (local treatment), an empty nerve wrap without tacrolimus, daily subcutaneous tacrolimus injections, or undergo nerve repair only as a control group. After 21 days, we will assess the number of sensory and nerve cells that regenerate to the skin and muscle using tracer dyes. To determine the effect of local tacrolimus delivery on recovery of movement rats will undergo unilateral median (forearm) nerve cut and repair and will be randomly allocated to tacrolimus nerve wrap treatment or vehicle alone. Time to return of active finger flexion is monitored by daily grasping tests.

Anticipated Results: We anticipate that the devices will increase the number of nerve regeneration to the muscle and skin, resulting in improved and accelerated functional recovery. The results will form the basis for human device trials to enhance outcomes following nerve trauma.

Proposal Title:	IgA-Seq Analysis to Advance the Study of Host Immunity-Microbiome Interactions in Osteoarthritis
Log Number:	PR220910
Current PI Name:	Matlock Jeffries
Award Number:	HT9425-23-1-0101
Current Contracting Organization:	Oklahoma Medical Research Foundation
Current Performing Organization:	Oklahoma Medical Research Foundation
Web Approval Date:	01-20-2023

Post-traumatic osteoarthritis (PTOA) is a chronic, debilitating musculoskeletal disease that disproportionately affects military personnel, resulting in pain, mobility loss, increased morbidity, and early mortality. PTOA is the most common form of arthritis among active-duty military personnel and the most common condition resulting in permanent disability in the military, and roughly 95% of PTOA cases have a history of prior combat injury. In fact, even with the best currently available treatment, the risk of PTOA following significant joint injury exceeds 50%. Soldiers are more than five times more likely to develop this form of arthritis than the general population, and these rates are accelerating over time; for example, the rate of PTOA doubled in the decade from 2005 to 2014.

Despite the significant burden of PTOA in the military, we have no treatments to prevent or slow the development of PTOA. In fact, our therapeutic arsenal is limited to pain relief and eventual joint replacement. Previous studies have shown that PTOA is a disease of local and systemic inflammation, although the ways in which the immune system interacts with environmental factors to produce PTOA are unclear. One potential mechanism of environmental-immune interaction are microbiome effects. In this study, we will evaluate both the pathogenic and potential protective effects of changes in body microbiomes along with their interactions with the immune system in PTOA, using cutting-edge microbiome and immune cell subtyping analyses. We will use a well- established PTOA animal model, the disruption of medial meniscus (DMM) mouse model. We will compare the microbiome and immune cell responses to induction of osteoarthritis (OA) by DMM in both wild-type mice and mice protected from PTOA (the MRL/MpJ mouse) and evaluate how the immune system interacts with the microbiome in each of these conditions.

Success in our study would lead not only to a new understanding of the ways in which environmental factors affect susceptibility to PTOA, but also to potential paradigm-shifting treatments for PTOA, which would be both accessible and readily deployable in the battlefield.

Proposal Title:	Accelerated Treatment for Co-Occurring Insomnia, Nightmares, and PTSD
Log Number:	PR220924
Current PI Name:	Carmen McLean
Award Number:	HT9425-23-2-0014
Current Contracting Organization:	Palo Alto Veterans Institute for Research
Current Performing Organization:	Veterans Health Administration - Palo Alto, CA
Web Approval Date:	10-03-2023

Rationale: An estimated 20% of active-duty Service Members suffer from clinically significant insomnia, 20% of Service Members suffer from clinically significant nightmares, and 15% suffer from posttraumatic stress disorder (PTSD). These disorders are highly interrelated, with an estimated 50% of U.S. Army with insomnia having comorbid PTSD, and 60% of National Guard with nightmares alone or with insomnia having PTSD. Conversely, approximately 80% of patients seeking treatment for PTSD report clinically significant insomnia and approximately 75% report clinically significant nightmares. Untreated insomnia, nightmares, and PTSD negatively impact military readiness and are linked with other mental and physical health problems such as suicide risk and substance use, difficulties in daily functioning, increased risk for accidents, and impairments in occupational performance. There is an urgent need to provide effective and efficient treatment for military personnel impacted by these three co-occurring problems.

Evidence-based behavioral treatments are the first line recommended interventions for each of these disorders and have been validated in the military by our group. However, the sleep treatments do not directly target PTSD and PTSD treatments do not target sleep problems. Additionally, each of these treatments are typically delivered in weekly sessions over multiple months. If provided sequentially, it would take approximately 4 months for a Service Member to get a full dose of each treatment. This treatment length is associated with greater dropout and might not be feasible for military personnel who are very mobile and who are also managing busy occupational demands with other responsibilities at home.

Evidence-based treatments for insomnia, nightmares, and PTSD have demonstrated acceptability, feasibility, and clinical benefit when delivered separately in brief or accelerated formats. Delivering an integrated treatment for insomnia, nightmares, and PTSD in an accelerated treatment could increase feasibility for treating these disorders in the military, increasing access to care and the likelihood that patients receive a full dose of effective treatment and optimal outcomes. This efficient treatment format would also fast-track recovery from insomnia, nightmares, and PTSD to achieve better functioning and military readiness.

Objectives: The proposed study aims to test an integrated, accelerated treatment for clinically significant symptoms of three common and interrelated problems among military personnel: insomnia, nightmares, and PTSD. The goals of the study are to determine the efficacy of cognitive behavioral treatment for insomnia and nightmares in reducing sleep problems when integrated with PTSD treatment and delivered in an accelerated treatment format (e.g., over 5 consecutive days with a preparatory session before and a booster session after). We expect that treating insomnia and nightmares related to PTSD in an accelerated treatment format will lead to significant improvements in sleep outcomes. We will also explore the impact of sleep treatment on PTSD symptoms.

Topic Area and Area of Encouragement: The proposed project addresses the Neuroscience Topic Area "Sleep Disorders and Restriction" and Strategic Goal within Treatment to "Develop and test treatment strategies to manage symptoms and improve quality of life for those affected by associated neurological and psychological conditions." We propose to test a brief evidence-based treatment for insomnia, nightmares,

and PTSD delivered in an accelerated and integrated format (daily sessions) on key indices of psychological and neuropsychological functioning and quality of life.

Ultimate Applicability and Impact: Providing treatment for insomnia, nightmares, and PTSD in an integrated, accelerated treatment format will make it easier for the many military personnel impacted by these problems to seek and complete treatment. We expect that the proposed study will positively impact the lives of the participating military personnel who might otherwise not have an opportunity to benefit from evidence-based psychotherapy for these common and interrelated disorders. If the accelerated treatment format is effective, we also expect that this model could be used throughout the Department of Defense to help many military personnel receive effective treatment and recover from insomnia, nightmares, and PTSD.

Proposal Title:	Machine Learning Strategies for Predicting the Risk of Suicide Using Clinical Note Text
Log Number:	PR220927
Current PI Name:	Jiang Gui
Award Number:	HT9425-23-1-0267
Current Contracting Organization:	Dartmouth College
Current Performing Organization:	Dartmouth College
Web Approval Date:	05-03-2023

Preventing death by suicide is a national imperative. According to the Center for Disease Control and Prevention, suicide was the tenth-leading cause of death in the United States in 2018, claiming over 45,000 American lives. Veterans make up a substantial proportion of all suicide deaths in the U.S., accounting for approximately 18% of suicide deaths. The risk for suicide is 22% higher in the Veteran population compared to the general U.S. population. Early prediction, however, can make a difference. The development of effective tools that identify risk for suicide is vital to ensure that individuals in need receive prompt life-saving support.

Given these high rates of Veteran death by suicide, the U.S. Department of Veterans Affairs (VA) has prioritized improving suicide prediction. As a means to establish more effective suicide risk prediction, the VA recently developed a prediction method that evaluates VA users' electronic medical record (EMR) for suicide risk. Even with this innovation, however, detecting individuals who are at risk for suicide remains a major challenge. The VA's current model automatically evaluates all users based on established risk variables. One of the problems with this method is that there are many relevant risk variables that are not uniformly measured or even assessed and therefore not included within risk evaluation. Previous research indicates that analysis of mental health providers' clinical notes provides useful information that is not always present in numeric variables.

Whereas the VA's current suicide prediction method evaluates risk variables related to users' demographics and health care usage, the proposed study evaluates a comprehensive network of nuanced psychological variables associated with suicide. We are able to develop these variables by utilizing Natural Language Processing (NLP), a machine learning technique that analyzes semantic patterns in written text. As VA providers write notes summarizing each patient encounter, each user's notes spans their treatment history. Our proposed study uses NLP to analyze providers' notes, scanning them for variables associated with suicide. This work builds on our previous research that demonstrated the feasibility and added accuracy of this innovative approach when compared to the VA's currently used method.

Within our proposed study, we will develop a dataset of VA user's that received mental health services between 2015-2018 and died by suicide (over 25,000) and then match these individuals with a larger dataset of VA users (over 150,000) that received mental health services but did not die by suicide. These two groups will be matched on the VA's currently used numeric suicide risk variables. We will then extract the groups' mental health provider notes and analyze the two groups' semantic differences. As we controlled for variables used by the VA's current risk prediction method, any increase in predictive accuracy will be over and above current standards. Harnessing the most recent advances in machine learning, we will select the most predictive semantic variables and develop a reproducible model that can be used broadly within the VA. Moving forward, this improved model could be integrated within the VA's suicide risk alert system, directing providers to target those with increased need.

As death by suicide is a very rare event, it takes a large treatment population to accurately study suicide risk. Although we have demonstrated the feasibility and efficacy of our proposed model with a smaller VA population (250 deaths by suicide compared to 2,000 non-deaths by suicide), including a larger sample will be allow us to develop a model that is representative of VA users nationwide. The proposed study facilitates a leap forward in suicide research, leveraging advances in machine learning to extract clinically relevant information from provider's written records. This research will improve suicide prediction, allowing the utilization of providers' written notes within systematic risk evaluation. It will both increase knowledge about suicide risk factors and advance an innovative technique that can be widely implemented in the VA and among active-duty military populations. By the conclusion of this project, outcome results will be disseminated in the Department of Defense, VA, Veterans service organizations, and scholarly publications. Results will be submitted to the Medical Simulation and Information Sciences research program within the Health Information Technology and Informatics portfolio. We will also work with closely with colleagues that developed the VA's current suicide risk prediction metric to integrate our findings within their alert system. By improving risk prediction, providers can better reach those most in need and save lives.

Proposal Title:	Development of Neurophysiological Biomarkers in Rett Syndrome
Log Number:	PR220939
Current PI Name:	Jeffrey Neul
Award Number:	HT9425-23-1-0004
Current Contracting Organization:	Vanderbilt University Medical Center
Current Performing Organization:	Vanderbilt University Medical Center
Web Approval Date:	01-26-2023

Rett syndrome (RTT) is a devastating neurological disorder that emerges in early childhood. RTT is made particularly tragic by the fact that children destined to be diagnosed with this disorder appear to be developing normally and gaining skills early in life. But then, between 18 and 30 months of age children experience loss of the ability to speak and use their hands and have problems walking or cannot walk at all. Unfortunately, these skills are not regained in any meaningful way, causing affected individuals to be severely impaired for the rest, leading to them requiring lifelong assistance in performing basic activities of daily living like feeding themselves and using the toilet. In addition to the loss of skills, people with RTT have many other problems, such as seizures, severe constipation, problems chewing and swallowing, poor growth, movement problems, changes in breathing patterns, as well as a number of problematic issues. Many of these problems are seen in other brain disorders that affect both children and adults. There are no approved therapies for RTT, and the approach to treatment is entirely based on giving medicines and therapies to treat the symptoms (like seizures), and the ability of these symptomatic treatments to make meaningful change is very limited.

Nearly all people with RTT have a mutation in a gene called Methyl CpG binding protein 2 (MECP2), which “reads” signatures embedded in everyone’s DNA code that is important for how this code is used to control the activities of cells in the body. The MECP2 gene is on the X chromosome; for this reason, the most affected individuals are girls and women. Animal models of RTT have been created, and they show many of the same problems as those observed in people with RTT. Excitingly, the problems seen in these RTT animal models can be reversed, even after the problems are present, by turning the gene back on. This gives great hope that treatments can be developed for people that will change the course of the disease, or even reverse the problems seen in people with RTT.

While work is underway to create new therapies for RTT, there are challenges in the ways available to do clinical trials of these therapies in a rare disease such as RTT as there are only a limited number of people who can participate in the trials. A missing element is that lack of “biomarkers” in RTT. A biomarker is something that can be measured and can tell or predict if someone has a disease, how well the body is functioning, or if there is a response to a treatment. For a rare disease like RTT, having a biomarker that shows response to treatment before clinical improvement could help make clinical trials faster and need less participants. An additional benefit would be a biomarker that can be seen in both people with RTT and in animal models of RTT, as this could be used in studies that evaluate new treatments in animal models, and then help guide human clinical trials.

We found changes in brainwave activity and response to stimulus (measured with non-invasive EEG [electroencephalogram]) in people with RTT that are also seen in animal models. These EEG changes correlate with severity in people and animals with RTT, which makes them promising as biomarkers of response to treatment. To see if that is the case, in this proposal we will see if these EEG features improve when we turn the MECP2 gene on in a mouse model of RTT, and if they improve when we treat RTT mice with ketamine, which has been shown to improve the problems seen in these mice. Successful completion of this project will show if EEG can be used as a treatment responsive biomarker in RTT. Because these EEG features can be measured in animal models and people, this would help improve how therapies are testing in animal models and then in human clinical trials.

This proposal addresses the Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Area of Rett Syndrome (within the Neuroscience Portfolio), with the specific FY22 PRMRP Strategic Goal of Treatment. We are directly studying mouse models of RTT and developing treatment responsive biomarkers in these mice. The development of these biomarkers will help enable the discovery and evaluation of novel treatments for RTT by helping improve and speed preclinical studies of these novel therapies in animal models of RTT. These biomarkers, which are also present in people with RTT, can then be used in clinical trials of these novel therapies to accelerate and improve such trials. Additionally, such biomarkers may also be useful in other neurological disorders that share clinical features with RTT. Ultimately, using these biomarkers to help find and test new therapies that meaningful improve the lives of people with RTT and their caregivers, including military families who are caregivers to people with RTT.

Proposal Title:	Use of Droplette Micromist Technology Device (DMTD) for Deep Tissue Treatment of Pressure Ulcers
Log Number:	PR220947
Current PI Name:	Lakshmidevi Pulakat
Award Number:	HT9425-23-1-0524
Current Contracting Organization:	Tufts Medical Center
Current Performing Organization:	Tufts Medical Center
Web Approval Date:	10-03-2023

This project addresses the Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Portfolio "Internal Medicine" and the Topic Area "Pressure Ulcers." The FY22 PRMRP Strategic Goal addressed here is "Develop and test novel treatments, and/or improve upon existing treatments for associated diseases and conditions." The overarching goal of this project is to develop a new deep tissue treatment for non-healing Stage 3 and Stage 4 pressure ulcers (PUs) that often lead to limb amputations and cause extreme pain. PUs affect 2.5 million people per year and costs $2.6 billion. In Veterans Affairs (VA) hospitals, the incidence rate of PU is almost double that of national average. The fact that 53% of active-duty military casualties in Operation Iraqi Freedom had pressure injuries and there is a 22% incidence rate of PU across most military medicinal facilities, underscores the increased risk for PUs in active-duty military personnel. PU treatment is one of the most challenging clinical problems in hospitals across the world, especially among patients with spinal cord injury, elderly patients, immune compromised patients, and in patients with metabolic diseases such as diabetes. Clinical studies show that recurrence of PUs occurs at a high rate (11% to 29% in cases with postoperative complications and 6% to 61% in cases without postoperative complications). This is due to the incomplete healing of deep tissues that are damaged at the site of pressure ulcer due to lack of availability of molecules that stimulate deep tissue repair. Currently, there are no effective treatments to completely heal damaged deep tissues and prevent recurrence of pressure ulcers.

PUs and pressure injuries are caused by localized damage to the skin and/or underlying tissue from direct pressure, or pressure in combination with shear. PUs usually develop over a bony prominence, but can also develop in skin areas under constant pressure load from medical devices or environmental conditions that cause deformation of skin and deeper tissues near the contact region. Such constant external pressure induces deep tissue ischemia, cell death, inflammation and edema, and tissue necrosis resulting in PUs. Proper debridement and offloading are essential, but not sufficient to heal chronic PUs such as those in pathology Stages 3 and 4. Current PU treatments use skin substitutes (natural or artificial) placed on top of the wounds. Skin substitutes can carry stem cells that can differentiate into epithelial cells, and collagens and growth factors to promote wound closure from the top of the wound. However, as pointed out in the 2020 Technical Brief (Project ID: WNDT0818) of the U.S. Department of Health and Human Services, none of the clinical trials with skin substitutes show that they prevent recurrence of PUs. Thus, there is a desperate medical need for new treatments to completely heal damaged deep tissues, particularly in Stage 3 and Stage 4 PUs and prevent PU recurrence.

The lack of efficient methods of delivery for growth factors, cytokines, and reparative gene or protein therapy to the deep wound beds of PUs impedes development of effective treatments to reduce the high rates of PU recurrence. To overcome this issue, we propose to use our custom made Droplette Micromist Technology Device (DMTD), a hand-held, needle-free, transdermal delivery device that generates a micromist that can package and deliver high molecular weight biologics through intact skin. DMTD is a patented product of Droplette Inc., Boston, Massachusetts, custom-made for the proposed studies. Our published studies show that DMTD-delivered high molecular weight antibiotics on the skin could successfully attenuate E. coli infection as deep as ~6mm in the skin of a rat model. Our recent studies show

that DMTD-delivered plasmid DNA expressed new proteins in skin cells located at a depth of ~5mm from skin surface in pig and rat models. Inflammatory immune cells such as macrophages increase inflammation at PU sites that impedes healing. We found that DMTD-delivery of DNA could change gene expression in macrophages present in a human 3D skin model. Based on these unprecedented observations, we have developed a conceptually innovative hypothesis that states DMTD-delivered gene and protein therapeutics will reach damaged deep tissues of PUs within seconds and change gene expression in the wound bed to induce deep tissue repair and complete healing that prevents PU recurrence.

To test our hypothesis, we propose two specific aims. In Aim 1, we will determine the efficacy, translational feasibility, and safety of DMTD-delivered plasmid DNA that increases gene expression of a wound-healing molecule, FGF2, and an siRNA that can inhibit overexpression of a molecule named TNF alpha that is responsible for increasing chronic inflammation in non-healing PUs. In Aim 2, we will take advantage of a new protein therapeutic, our custom-made human mesenchymal stem cell conditioned medium (MSC-CM) and test if DMTD-delivered MSC-CM will effectively and safely heal skin, muscle, and bone tissue and restore blood circulation in Stage 3 and Stage 4 PUs.

We will use an in vitro/ex vivo/in vivo testing platform comprised of young and old, male and female, rat, pig, and human preclinical models, which includes in vitro human 3D skin-like tissues that mimic the skin of young and aged men and women, and inflamed human skin tissues from amputated limbs of PU patients at Tufts Wound Clinic, to evaluate the efficacy, safety, and translational feasibility of these DMTD-delivered therapeutics in PU healing and preventing recurrence.

Efficacy of this treatment in achieving complete healing of PU that prevent recurrence must meet the following criteria: Absence of redness at the site of PU, loss of scab, restoration of normal skin color, histological evidence of tissue restoration, lack of leukocyte infiltration at PU site, reduction in inflammatory cytokines and proteins as determined by the cytokine and proteome profiles, and the lack of PU recurrence during the waiting period after wound closure.

Positive data from this study will pave the way for a needle-free deep skin tissue therapy for PU that uses state-of-the-art gene and protein therapeutics to ensure complete healing of PUs safely that will prevent PU recurrence and improve patient outcomes. The next step will be to evaluate the efficacy of this new DMTD-based deep skin tissue therapy for PU in a phase 1 clinical trial at the Tufts Wound Clinic. A Funding Level 2 proposal will be prepared using data from this proposal as proof of concept. Our multidisciplinary Physician-Researcher investigative team is committed to validate the efficacy and safety of our new PU treatment and eager to move this life-saving treatment to clinic to fully heal chronic PUs and improve patient outcomes.

Proposal Title:	Multi-Site Automated Segmentation and Multi-Parametric MRI Quantification to Assess the Effect of Treatment of Venous Malformations
Log Number:	PR220948
Current PI Name:	Craig Jones
Award Number:	HT9425-23-1-0032
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	12-19-2022

Topic Area: Vascular Malformations

The human body is composed of a complex network of blood vessels that are crucial for circulating oxygen and nutrients to organs. Vascular malformations are a type of abnormally formed blood vessels that usually develop before birth and persist throughout life. The most common subtype is venous malformations (VMs), which specifically involve veins and lymph vessels, and affect about 1 in 100 people. Most venous malformations happen sporadically and without any underlying cause. They can involve any body part and affect patients of any age as they grow throughout life. As a result, patients can suffer from various symptoms, including debilitating pain, functional loss (e.g., limping, limited movement), cosmetic deformity (and social stigma), swelling, bleeding, or weakness. VMs are currently treated with a local image-guided treatment called sclerotherapy, which includes injection of certain agents into the lesion (abnormal region) that causes local inflammation and eventual reduction of the volume of the lesion.

In recent years, there have been advances in both image-guided and pharmaceutical treatments for VMs with the introduction of new systemic therapies. However, the effects of these therapies, either standard or new, are incompletely understood because there are no objective ways to assess treatment outcomes. Most clinical assessment is highly subjective, with physicians interpreting patients' descriptions of ongoing symptoms or changes in those symptoms after treatment. Recently, the first clinical outcome measurement tool has been published for the assessment of treatment outcomes in patients with VMs. Using this tool, patients report the changes in their quality of life without the influence or interpretation of a clinician.

VMs differ widely in location, size, extent, and imaging characteristics across patients. Therefore, an objective assessment of treatment outcome is considered challenging especially if a lesion was treated in multiple sessions and/or with different agents across time. We believe that magnetic resonance imaging (MRI) will provide a robust and objective outcome measure for VMs. However, MRI images consist of many different adjustable components, and it is not established which changes in these components may be best used to assess VMs. Further complicating this, the variability, both within and between VMs, makes it more challenging to decide which properties are most relevant to the disease burden and what changes represent a beneficial response to treatment. Therefore, an understanding of how VMs change in a way that is detectable under MRI will allow physicians to follow lesions over multiple sessions of therapy without irradiating the patient (who are often children). Furthermore, the use of automatic analysis tools, developed through artificial intelligence, should allow us to rapidly and uniformly assess complex VMs.

We hypothesize that an automated imaging method of analyzing magnetic resonance images can be used to objectively assess the treatment outcomes for VMs. Therefore, our experimental aims are as follows:

Aim 1: Create and validate a neural network (NN) to segment VMs on MRI with varying acquisition parameters, based on a combined GAN synthesis NN for image signal normalization and a U-Net NN for segmentation.

Aim 2: To quantify MRI radiomics in the segmented regions of the VMs over time, across treatments, and at two independent sites on T2-weighted and apparent diffusion coefficient (ADC) images.

Aim 3: To quantitatively correlate the MRI findings and patient-reported clinical outcomes.

This proposal is innovative in (1) its goal to develop the first-ever fully automated assessment tool for VMs; (2) providing a quantitative assessment of MRI changes occurring in VMs over serial treatments; and (3) determining the correlation between MRI findings and clinical outcomes.

As there has been no standardized method for assessment of treatment outcomes in VMs, the results of this project will provide physicians and researchers with a quantitative tool to assess treatment outcomes in patients with VMs and correlate objective MRI findings of treatment outcomes with clinical outcomes (short-term impact). The results of this study will also provide a novel technical structure and clinical understanding for researchers to run large-scale diagnostic and therapeutic trials to improve the standard of care provided for patients with VMs (long-term impact).

Proposal Title:	Novel Leptin-Islet Signaling Pathway Regulates Beta Cell Function and Proliferation
Log Number:	PR220954
Current PI Name:	Richard Cox
Award Number:	HT9425-23-1-0113
Current Contracting Organization:	Baylor College of Medicine
Current Performing Organization:	Baylor College of Medicine
Web Approval Date:	02-06-2023

This proposal addresses the Fiscal Year 2022 Peer Reviewed Medical Research Program Topic Area of Diabetes. Type 2 diabetes (T2D) has become a global health pandemic that greatly shortens lifespan and carries a significant economic burden. U.S. Veterans are particularly susceptible, developing T2D three times more often than the general population. There is a significant need for new treatments in T2D, especially for the U.S. Veteran community. T2D develops when the insulin-secreting beta cells of the pancreas fail to meet the body's insulin requirements. Drugs that can increase the number of functioning beta cells represent strong candidates to treat T2D; however, no such drugs are currently available, creating a critical unmet need in the medical community. The satiety hormone leptin can suppress the ability of beta cells to function and grow. In contrast, blocking leptin effects on beta cells can dramatically increase the number of beta cells. The mechanism is yet to be defined, but we hypothesize that leptin acts on nearby delta cells that secrete an inhibitory hormone, somatostatin, to suppress beta cells. This is an innovative concept that will describe for the first time the effects of leptin on human delta cells and define the mechanism regulating beta cell function and growth. Furthermore, this proposal represents a conceptual shift in how leptin activity outside of the brain can significantly impact metabolism and pancreatic beta cells. This proposal will address the strategic goal to understand correlations between nutrition and metabolic disease susceptibility. Ultimately, these studies will reveal new drug targets to increase the number of functioning beta cells to overcome T2D.

Proposal Title:	New Artificial Oxygen Carrier for Resuscitative Care
Log Number:	PR220987
Current PI Name:	Christi Parham
Award Number:	HT9425-23-1-0053
Current Contracting Organization:	Bondwell Technologies LP
Current Performing Organization:	Bondwell Technologies LP
Web Approval Date:	02-09-2023

Peer Reviewed Medical Research Program (PRMRP) Topic Area: Pathogen-Inactivated Blood Products

PRMRP Strategic Goal: Develop and test novel or engineered blood products that add physiological, logistical, and cost advantages over current products.

Critical Problem to Be Addressed: Severe blood loss is the number one preventable cause of death on the battlefield as well as being a primary cause of civilian mortality in trauma cases. Stopping blood loss, resuscitating, and stabilizing casualties is of prime importance in casualty care. If a casualty has lost too much blood, then resuscitation is needed to prevent succumbing to shock and death. The best resuscitation fluid is low-titer (meaning low in antibodies that could cause complications) whole O-type blood that has been stored in the cold. Whole blood is best because it replaces the lost fluid, carries oxygen (with red blood cells acting as oxygen carriers), and is capable of clotting. Derivative products such as red blood cells, plasma, platelets, or mixtures can also be used but are less effective. It should be noted that resuscitation with products containing red blood cells are preferred because of their oxygen-carrying capacity, enabling oxygen to be delivered to the body to sustain life.

Unfortunately, supplies of whole blood and related products are very limited because of storage and shipping requirements. This is only expected to become worse in future conflicts against peer or near-peer adversaries where evacuation is not possible or supply chains are slowed or cut off. Cold chain storage and supply is particularly problematic because of the energy-intensive resources needed to sustain the products that need it, otherwise they expire or never reach their destination, which is particularly problematic for something as critical as blood. Given this information, there is a clear capability gap that needs to be addressed to ensure a sustained supply of life-saving blood in severe environments. New technology is needed to develop blood or blood products that have improved stability to reduce the need for cold chain storage and mitigate problems with replenishment since the shelf life of blood is untenably short.

Innovation: We are proposing to develop a novel shelf-stable oxygen carrier for fluid resuscitation of trauma casualties. Critically, our material is based on a newly discovered matrix-forming protein that is capable of stabilizing protein structure, increasing storage temperature and extending the shelf life of derivative products. We propose to apply this stabilization matrix technology to hemoglobin to create a shelf-stable, engineered blood product that can be used in fluid resuscitation.

Our goals for the first phase of research are to match as close as possible the oxygen-carrying capabilities of hemoglobin, demonstrate chemical and temperature stability, and demonstrate that our material will not cause adverse biological reactions.

Impacts: By developing a resuscitation fluid based on our materials as a highly stable oxygen-carrying component, medics in military, wilderness, and/or rural settings will be able to preserve life, limb, and organs in prolonged, austere environments where blood or related products simply would not be available due to expiration or inadequate storage. This product can help reduce logistic burdens associated with cold storage and the need for new materials as old ones expire. This innovation directly addresses inherent challenges to providing adequate medical care on the battlefield, particularly as resource limitations are

expected to worsen in the future battlespace. Therefore, the technology proposed herein has the potential to set a new paradigm in casualty care or civilian trauma by greatly reducing the need for cold-stored blood or derivative products.

Further research and development could see additional use-case scenarios for products derived from this proposed effort. This includes the use of our oxygen-carrying platform as a medium in whole organ storage, preservation, and transport. Other potential developments include furthering the proposed technology as a platform technology for the development of other stable bio-derived materials that would otherwise be inaccessible due to degradation problems.

Proposal Title:	Ingestible Microneedles-Based Electronics for Programmable Drug Delivery
Log Number:	PR220995
Current PI Name:	Yong Lin Kong
Award Number:	HT9425-23-1-0041
Current Contracting Organization:	Utah, University of
Current Performing Organization:	Utah, University of
Web Approval Date:	01-12-2023

The proposed is the first step to realizing ingestible wireless electronics that can reside in the stomach for weeks to wirelessly deliver medication via the stomach with microneedles. This eliminates the need for painful or repeated needles injection (e.g., insulin for diabetic patients) and can resolve medication non-adherence challenges for a broad range of diseases, especially for members impeded by physical and mental conditions. The device can be swallowed without medical procedure and will not require surgery for implantation, does not require wearing, attachment, or injection of the device into the body. The electronics can be wirelessly monitored and controlled via personal devices that can also be interconnected with a broad range of electronics and medical services. The Topic Area of this proposal is “Sustained Release Drug Delivery,” and the proposal is aligned with multiple Portfolio categories including but not limited to “Neuroscience” and “Nutrition and Metabolism.”

Critical Problem to Be Addressed: The proposed research addresses the critical problem of medication non-adherence: only 50 percent of adherence is achieved even in developed countries and is especially challenging for Service Members impeded by physical or mental conditions (e.g., post-traumatic stress disorder [PTSD], addiction, and depression) where non-adherence can lead to further degradation of symptoms and deaths. Non-adherence can also be exacerbated by the lack of clinical access such as in the Service Members in remote warfare, or due to a shortage of resources (e.g., clinical staff) prior to definitive care. Non-adherence to drug therapy can also contribute to long-term military health challenges such as the development of drug-resistant strains and unnecessary loss of organs due to infections or organ rejection in transplantation.

Existing Solutions: Despite decades of development in implantable and wearable drug-delivery electronics (e. g., Microchips™ Implants was first introduced in 1999, or a commercially available insulin pump), significant challenges remain for current devices that impeded their ability to be adopted. For example, the invasive surgical procedure causes pain and discomfort and is susceptible to surgical complications, rejection, and infection, which is particularly undesirable for remote military sites. Recent advances in wearable and epidermal electronics, though promising, are fundamentally limited by the access from the skin and require penetration of the epidermis with needles-based delivery. These systems remain challenging to achieve weeklong attachment without eliciting irritation, infection, and discomfort. For a Warfighter, such attachments may not be compatible with body armor and clothing and are susceptible to interference by the motion of the body during Service activities. Further, injured Soldiers with injuries on the skin, such as burn wounds, can have difficulty leveraging skin-based electronics.

Innovations: Oral delivery leverages the naturally evolved delivery mechanism and has been the preferred route for drug delivery for decades. Critically, oral delivery of electronics can leverage the significant space and immune-tolerant environment available within the gastrointestinal tract, circumventing the need for more invasive device placement or complications associated with wearable or epidermal devices. For instance, by swallowing the electronics device once, a weeklong dosage of insulin can be delivered autonomously in a diabetic patient without causing any sensation or requiring additional actions for glycemic

management. The delivery can also be integrated with sensors and a broad range of wireless devices to ultimately enable programmable, closed-loop feedback modulated, sustained release of medications that are much more effective, safe, and convenient for Service Members and Veterans. Further, in contrast to other electronics and devices that rely on mass production of identical devices to lower the cost, the 3D printing approach can be readily personalized without significant added cost as the economy of scale with conventional manufacturing is no longer possible when personalization is needed.

Applicability and Research Impacts: This proposal is the first step in enabling digitally modulated drug delivery using ingestible electronics that can reside in the stomach for a prolonged (multi-week) period. The proposed integration of microneedles enables the delivery of medications that would otherwise require frequent painful injections. Here, we primarily aim at demonstrating the feasibility of the critical function within 2 years in a preclinical setting using large animal models. Upon completion, 3 to 4 years of optimization, and preclinical safety studies will be performed. We envision that we can resolve long-standing challenges in medical electronics implantation, ultimately enabling automated and on-demand wireless drug delivery of therapeutic agents into the body that can target a broad range of diseases.

Proposal Title:	The Role of Exosomes on the Alveolar Epithelium in Idiopathic Pulmonary Fibrosis
Log Number:	PR220998
Current PI Name:	Karim Bahmed
Award Number:	HT9425-23-1-0368
Current Contracting Organization:	Temple University-Of The Commonwealth System of Higher Education
Current Performing Organization:	Temple University-Of The Commonwealth System of Higher Education
Web Approval Date:	10-03-2023

Our application aligns with the goal of the Fiscal Year 2022 Peer Reviewed Medical Research Program and addresses the Pulmonary Fibrosis Topic Area. Also, we focus on a Strategic Goal: Develop and test novel treatments, including precision medicine approaches, to slow the progression or reverse lung injury/disease.

Studies have demonstrated that chronic lung diseases are more common among deployed veterans than non-deployed Veterans. Veterans were exposed to a very harsh and specific environment during their deployment. Inhalation of harmful chemicals and particles can induce respiratory problems and lung injury leading to several pulmonary diseases.

Idiopathic pulmonary fibrosis (IPF) is the most common and progressive fibrotic lung disease, with a 5-year mortality rate of 50%-70%. The pathophysiology of this disease is not well known. The thickened and stiff tissue makes it difficult for the patient lungs to work properly. Although there is an urgent need to develop an effective treatment for IPF, successful therapy is currently not available due to the lack of novel approaches. Lung transplantation remains the primary intervention to improve survival; however, it is linked to increased morbidity and mortality risks. A better understanding, novel targets, and effective therapies are needed.

The particles inhaled by Veterans were deposited in the lungs and not eliminated completely. Their persistence induces chronic respiratory diseases. Injured lung cells secrete exosomes, small membrane vesicles, to their microenvironment. Their content can dramatically change under pathological conditions. Therefore, most exosomes secreted from diseased cells induce inflammation, spread damage, and alter the phenotype of various neighboring cells. Exosomes can deliver harmful contents to different organs, including the lung, leading to their failure.

We will isolate exosomes from lung transplants of IPF patients in the current project. The harmful impact of exosomes will be determined on alveolar cells obtained from control organ donors whose lungs were not suitable for transplantation and were donated for medical research. Alveolar cells have stem cell potential in the adult lung. We will study fatty acid synthesis enzymes, which are critical for alveolar cell function, and whose impairment can lead to susceptibility and worseness of IPF.

Targeting harmful exosome loads can be used as a novel approach to developing novel methods to diagnose IPF accurately. It can also lead to therapeutic strategies to minimize IPF and inhibit exosomes' spreading and disease progression.

There is no commercial pharmaceutical drug to target harmful exosomes; therefore, we will also focus on the therapeutic function of RNA factors. They have proven their effectiveness in fighting several diseases, including pulmonary abnormalities, especially when conventional therapy fails. We will block the exosomes with harmful content as a novel potential therapeutic strategy. This approach can lead to novel treatments to prevent IPF development.

There is an urgent need to develop novel treatments for IPF that interrupt this destructive cycle and restore the normal lung milieu to improve the outcomes and achieve the ultimate goal of curing patients with this disease. Our study has clinical implications and can identify novel therapeutic targets.

Our short-term goal is to identify the harmful effect of exosomes obtained from IPF patients on control alveolar cells obtained from organ donors. Identifying dysregulated proteins by exosomes can lead to discovering novel IPF biomarkers and therapeutic targets.

Our long-term goal is to develop a new class of medications that block harmful exosome load, which can stimulate lung regeneration after injury.

Proposal Title:	A Non-Human Primate Platform to Assess Novel Plasmodium falciparum Interventions
Log Number:	PR221005
Current PI Name:	Hugo Valdivia
Award Number:	CDMRP-22-PR221005
Current Contracting Organization:	U.S. Naval Medical Research Unit No. 6 (NAMRU-6) Lima, Peru
Current Performing Organization:	U.S. Naval Medical Research Unit No. 6 (NAMRU-6) Lima, Peru
Web Approval Date:	04-25-2023

The disease malaria is caused by infection with a parasite belonging to the Plasmodium family. Several species of Plasmodium parasites infect humans to cause disease, with Plasmodium falciparum responsible for the majority of the >400 million cases and >600,000 deaths from malaria each year. Vaccine development for any malaria species has been difficult due to the complexity of the parasite (as compared to viral or bacterial pathogens). This includes the complex life cycle, which begins when a mosquito injects the parasite into the skin, and the parasite then migrates to the liver and finally out into the blood where it causes all disease and death. At this stage, a new mosquito can take a blood meal and with it the parasites that will complete the life cycle and allow onward transmission. In addition, there is a lack of robust animal models that can replicate this full life cycle and reliably predict the clinical success of new vaccines or interventions. This is because the human malaria parasites do not infect rodents or common laboratory monkeys, and the parasite species that do infect these laboratory animals are often very distant relatives of Plasmodium falciparum. We are overcoming these hurdles by developing better models of malaria infection and immunity in Aotus owl monkeys, which can be infected with the human malaria parasites Plasmodium falciparum and Plasmodium vivax. However, the parasite strains used in these animals only partially complete the full mosquito-to-mammal-mosquito life cycle, and it is our goal to recapitulate this entire life cycle in order to be able to test novel interventions against the natural parasite infection process.

Typically, this has involved taking a single parasite strain or variant and slowly adapting it to a new monkey species. This results in a high rate of failure over long and complicated experiments. We have recently shown that co-infecting discrete parasite strains together in a single animal, as often happens in nature, can accelerate this process. This natural selection of the most fit parasites allows us to identify parasites naturally suited to infection in our monkeys. It also provides the opportunity for the parasites to mate and create hybrid offspring that may contain new genetic variants more apt to complete the life cycle in a non-natural host.

However, we have only performed this for the less common malaria species Plasmodium vivax. Replicating this early success for P. falciparum carries a number of risks due to a more difficult life cycle and greater history of failure in this parasite-monkey combination.

Here, we will attempt to mitigate this risk of failure by using our novel multi-strain approach and by using a combination of new and promising P. falciparum strains from a variety of sources. Each parasite has been selected on one or more criteria, which makes them suitable for our model. In this manner, we hope to create the first preclinical animal model for the complete P. falciparum life cycle. This will greatly advance our ability to develop new malaria interventions (e.g., drugs, vaccines, and monoclonal antibodies) as well as open a completely new avenue for studying the biology of malaria transmission.

Proposal Title:	Systems Approach to Understanding the Metabolic Regulation of T Cell Phenotype in Autoimmunity
Log Number:	PR221011
Current PI Name:	Vinee Purohit
Award Number:	HT9425-23-1-0120
Current Contracting Organization:	Brigham and Women's Hospital, Inc.
Current Performing Organization:	Brigham and Women's Hospital, Inc.
Web Approval Date:	02-06-2023

Multiple sclerosis (MS) is a debilitating neurological ailment with no available therapies to reverse the disease. With an increasing understanding of immune cell metabolism, there is a renewed clinical interest in implementing dietary interventions to manage autoimmune and other immunological diseases. This warrants a systems-based understanding of the effect of dietary nutrients on gene expression and immune cell function. A group of such nutrients is “methyl donors,” which affect gene expression and immune cell function. While the effect of dietary methionine restriction has been studied, the role of other metabolites within the methionine/folate cycles and their interplay in directing T cell pathogenicity remain poorly understood. Herein, by combining my expertise in cellular metabolism with my current training in systems immunology, I aim to answer a fundamental question: How does a specific dietary nutrient affect the entire metabolic network of an immune cell to regulate its epigenetics and modulate its function in a particular tissue?

The analysis of T cell metabolism in tissue is challenging due to limitations of available cell numbers and current metabolic techniques. Our lab has recently developed a novel computational algorithm called Compass that can predict metabolic changes in immune cells using single cell RNA sequencing data. In the proposed study, we will use a similar approach to integrate transcriptomics (gene expression) and metabolomics of T cells within tissues to generate a holistic map of tissue-specific metabolic alterations resulting from a dietary intervention. This data, in tandem with in vivo perturbation studies on MS disease course, will provide clues to eventually devise dietary interventions for inflammatory and autoimmune diseases. Our proposal aims to address more than one Peer Reviewed Medical Research Program Strategic Goal. While our principal focus is to develop a deeper understanding of nutrition and susceptibility to autoimmune disease, the results of the proposed study will also benefit patients with immunological, neurological, and mitochondrial disorders.

Service Members and Veterans have a higher risk of developing immune disorders such as MS. This is exacerbated by limited availability of optimal dietary solutions upon installation and during deployment. With dietary interventions becoming the mainstay of disease management in MS, the outcome of my proposed studies will help develop more than one dietary/therapeutic intervention providing options to Service Members and the general population to manage/prevent immunological diseases.

Proposal Title:	Cerebrospinal Fluid Profiling in Preterm Infants with Hydrocephalus: Defining a Novel Pathway to Disability
Log Number:	PR221066
Current PI Name:	Maria Garcia Bonilla
Award Number:	HT9425-23-1-0075
Current Contracting Organization:	Washington University in St Louis
Current Performing Organization:	Washington University in St Louis
Web Approval Date:	02-14-2023

Military women face unique physical, social, and environmental challenges and are at risk for spontaneous preterm delivery, particularly within 6 months of deployment. Their preterm infants are at risk for developing hydrocephalus or “water on the brain.” This disease is a debilitating neurological condition, characterized by the buildup of excess cerebrospinal fluid (CSF) in the brain and enlarged chambers within the brain that house CSF (brain ventricles). In North America, hydrocephalus produced by the presence of blood in the brain ventricles represents the most common cause of pediatric hydrocephalus (post-hemorrhagic hydrocephalus – PHH). Additionally, it is associated with the worst neurological consequences in newborn medicine and enduring, complex neurosurgical care.

Inflammation has been suggested to play a role in the severity of hydrocephalus, which can cause damage to different parts of the brain, especially the areas surrounding the brain ventricles. Notably, the damaged cells or parts of them (i.e., small capsules from the cells called exosomes) can fall into the CSF. From there, these exosomes can travel throughout the brain and cause more inflammation.

To date, no one has identified the complete profile of cells and cellular components present in the CSF in PHH. Because these profiles could reveal promising drug targets that would improve the treatment of hydrocephalus, the proposed studies are new and urgently needed. Therefore, our goals are to: (1) define the cell composition of the CSF from infants with PHH; (2) examine the roles that exosomes from the CSF play in producing brain inflammation in hydrocephalus and altering important mechanisms that affect brain development; and (3) analyze the role of one specific inflammatory protein (S100A9) in PHH as a potential therapeutic target.

This project is innovative in that (1) no studies have analyzed the cellular composition of the CSF in human infants with PHH, and (2) our analysis of a specific target for a drug (S100A9) could help to improve the treatment of these most fragile infants. The successful completion of our studies will increase knowledge of the damage hydrocephalus causes in the brain and set the stage for future clinical trials on drug interventions for the treatment of hydrocephalus. Ultimately, drug treatments could be incorporated into the immediate management of hydrocephalus in the clinic, and improved treatments would benefit all patients who suffer the long-term consequences of hydrocephalus.

Proposal Title:	Identifying Protein Motifs in Cas9 Essential for Bacterial Virulence
Log Number:	PR221067
Current PI Name:	Rakhi Rajan
Award Number:	HT9425-23-1-0256
Current Contracting Organization:	Oklahoma, University of, Norman
Current Performing Organization:	Oklahoma, University of, Norman
Web Approval Date:	04-04-2023

Critical Problem: Bacterial infections are a serious threat to human health. The advances in developing new antibiotics have slowed down recently. In addition, several bacteria are developing antibiotic resistance at alarming rates. Thus, there is a critical need for developing novel approaches and targets for antibacterial development. Characterizing a novel protein target for each specific bacterial infection is a time-consuming process. An attractive strategy is to identify a new antimicrobial target that has been implicated in pathogenicity of several harmful bacteria. Towards this, we propose Cas9 as a novel target for antibacterial development since several studies in multiple human pathogenic bacteria have demonstrated the need of Cas9 in virulence and host cell invasion mechanisms. Additionally, since Cas9 is predominantly present in pathogenic bacteria, and not in commensal and environmental bacteria, a drug targeting Cas9 provides an avenue to remove harmful effects following an antibiotic treatment regime. The proposed work closely relates to the Peer Reviewed Medical Research Program Topic Area of “Respiratory Health and Pulmonary Fibrosis” and the Strategic Goal of “develop and test novel treatments to slow progression or reverse lung injury/disease” since streptococci, the causative agent of respiratory and pulmonary diseases, possess Cas9. The idea can also be related to Topic Area of “Guillain-Barré Syndrome, and Inflammatory Bowel Diseases,” since its causative agent, Campylobacter, uses Cas9 to infect the host.

Innovation: The most innovative aspect of the proposed study is using Cas9 as a universal protein to tackle harmful bacterial infections, with minimal impact on beneficial bacteria due to the unique protein sequence patterns of Cas9 between these groups of bacteria. Our preliminary data analyzed sequence patterns of Cas9 using computational approaches, including the use of artificial intelligence, and have identified sequence patterns (termed motifs) that are unique to pathogenic and environmental bacteria with great statistical significance. In the proposed work, we will use computational methods for reliable identification of unique motifs from Cas9 belonging to three unique habitats (pathogenic, commensal, and environmental). Unique motifs selected for pathogenic bacteria will be tested experimentally using Streptococcus pyogenes as the model organism since it is involved in respiratory health and pulmonary fibrosis. The combinatorial use of computational and experimental approaches is advantageous than the cumbersome, unguided experimental approaches.

Applicability: CRISPR-Cas systems were discovered as bacterial immune systems where intruding viruses are cleaved by sequence-specifically by protein-RNA complexes belonging to this system. Interestingly, emerging studies show that Cas9, a protein component of the CRISPR system, can regulate other bacterial processes, such as virulence. Currently, fundamental mechanisms by which Cas9 regulates bacterial virulence and regions of Cas9 that are specific to virulence, but not contributing to viral protection, are unknown. Cas9 being a large, multi-domain protein, identifying unique pockets related to virulence will enable targeted drug development. The proposed study will address this by large-scale bioinformatics and machine learning studies across several bacteria belonging to different habitats to identify Cas9 motifs that are unique among them. The effect of the identified motifs in virulence will be tested experimentally using streptococcus. If established, the pathogenic motifs can be targeted for novel antimicrobial strategies against several harmful bacteria, without impacting beneficial bacteria.

Impact: The main impact will be developing Cas9 as a universal molecule to treat infections that are caused by divergent bacteria. Several of the devastating diseases affecting military and civilian communities as well

as issues related to antimicrobial resistance can be theoretically rectified by Cas9 inhibitors. The proposed study will establish unique motifs of Cas9 in pathogenic bacteria as a determinant for a positive infection. This will enable future studies where these unique pathogenic Cas9 motifs will be targeted by drugs, which will prevent harmful effects to commensal bacteria possessing Cas9. Cas9 is currently popular due to its use in gene editing technology and its use as a gene therapy agent (Cas9 won a Nobel Prize in 2020 due to these medical impacts). Adding one more health benefit to Cas9, its use as a selective target to treat harmful bacterial infections, will significantly enhance the value of this protein in human health. Due to the vast mechanistic knowledge available for Cas9, proceeding the proposed study and other long-term goals will be easier and guided by these mechanisms, providing more success opportunities. Another impact from the proposed study is the ability to develop long-term research plans, not only in the Principal Investigator's laboratory, but also across CRISPR and microbiology fields, based on results acquired from the Discovery Award. It will also enable student and postdoc training in several important techniques (bioinformatics, machine learning, molecular biology, biochemistry, and microbiology) that can be easily translated to other scientific fields as the trainees move on in their scientific career.

Proposal Title:	Structure-Guided Development of Potent PfPKG Inhibitors for Malaria Chemoprotection
Log Number:	PR221072
Current PI Name:	Purnima Bhanot
Award Number:	HT9425-23-2-0023
Current Contracting Organization:	Rutgers, New Jersey, State University of
Current Performing Organization:	Rutgers, New Jersey, State University of
Web Approval Date:	10-03-2023

Malaria is a deadly infectious disease that infects more than 200 million people and kills upwards of 400,000 each year. Child survivors of malaria suffer serious long-term health and cognitive impairment. Thus, the disease imposes a huge burden in terms of both lives lost and socioeconomic development in malaria-endemic countries. The situation is likely to worsen due to the emergence of drug-resistant parasites and insecticide-resistant mosquitoes. Malaria is the top infectious-disease threat facing the U.S. military since it diminishes operational readiness of troops in several overseas areas of operations in Asia, Africa, and the Caribbean. Other Americans traveling to endemic countries for personal, business or government reasons, such as tourists, State Department personnel and Peace Corps volunteers, also need protection from malaria.

Eradicating malaria requires new tools for preventing infection. An adult malaria vaccine is still not available (Mosquirix™ is recommended only in children in Africa) and the effectiveness of insecticides is diminished by the emergence of insecticide-resistant mosquitoes. Malaria prevention relies greatly on a few drugs, but these have several drawbacks. Some do not attack the latent forms of the disease or have serious side-effects, e.g., anemia, in large numbers of people. Others are too expensive for widespread use by poor at-risk populations or are challenged by drug resistance in parasites. To make progress against malaria, it is essential that we stay one step ahead of these problems by investing in research into new drugs.

Malaria is caused by a parasite, Plasmodium, that is passed to humans by mosquitoes. Within the human host, the parasite begins a multi-stage developmental program in which the liver is first organ to be infected. This stage is the "weakest link" in the parasite's life cycle because Plasmodium must multiply exponentially within the liver. Success at this stage enables the parasite to increase its numbers and gain a toehold in the body from where it spreads into the bloodstream and causes symptoms. We believe in the adage "prevention is better than cure." Accordingly, our goal is to find drugs that block the parasite’s infection of liver cells and thereby, prevent malaria.

Our previous work identified a parasite protein that is required both for parasite entry and exit from liver cells. Blocking the action of this protein significantly decreases the parasite’s growth in the liver and its ability to enter the bloodstream. We will make compounds that specifically target this protein and test their ability to block parasite infection of liver cells. Our work is a collaboration with Department of Defense laboratories at the Walter Reed Army Institute of Research and could lead to the development of new drugs to protect against malaria.

Proposal Title:	Structure-Guided Development of Potent PfPKG Inhibitors for Malaria Chemoprotection
Log Number:	PR221072P2
Current PI Name:	David Rotella
Award Number:	HT9425-23-1-0473
Current Contracting Organization:	Montclair State University
Current Performing Organization:	Montclair State University
Web Approval Date:	10-03-2023

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Proposal Title:	Development of Immunotherapeutics for Heartland Virus
Log Number:	PR221075
Current PI Name:	Corey Balinsky
Award Number:	HT9425-23-1-0202
Current Contracting Organization:	Henry M. Jackson Foundation
Current Performing Organization:	Naval Medical Research Center
Web Approval Date:	03-07-2023

Heartland virus is an emerging tick-born disease and is of significant concern to public health due to the severity of disease, as well as the geographic expansion of its insect vector. Infection with Heartland can result in a potentially fatal disease and frequently requires hospitalization. To date, little is known about the biology or ecology of Heartland virus, and there are no available treatments or vaccines. In addition, there are no commercially available diagnostic tests, making the study of this virus difficult.

Antibody-based therapies have been shown to be beneficial for treatment of a wide variety of viral infections including COVID-19, MERS, Ebola, HIV, Influenza, and Zika. However, to date, none have been developed for Heartland virus. Here we aim to develop therapeutic antibodies to treat Heartland virus infection. These antibodies will also be useful for diagnostic testing and surveillance, as well as furthering our knowledge of this poorly understood virus.

Proposal Title:	Blocking Neuroimmune Communication as a Treatment for Endometriosis-Associated Pain
Log Number:	PR221085
Current PI Name:	Victor Fattori
Award Number:	HT9425-23-1-0040
Current Contracting Organization:	Children's Hospital, Boston
Current Performing Organization:	Children's Hospital, Boston
Web Approval Date:	12-15-2022

Endometriosis is an inflammatory disease that affects up to 10% of women in reproductive age with annual health care costs approaching $70 billion in the U.S. alone. Debilitating pain causes affected women to lose, on average, 11 hours of work weekly, primarily as a result of reduced effectiveness during working time, while for the U.S. Army, chronic pain leads to roughly 20,000 days of lost duty time per year. This is also extended to U.S. Army dependents, as more than 75% of U.S. Army dependents who had undergone laparoscopy or laparotomy because of pelvic pain were diagnosed with endometriosis. Moreover, this debilitating chronic pelvic pain also contributes to depressive symptoms and anxiety symptoms that, ultimately, might lead to decreased work, social engagement, and relationships with colleagues and family in endometriosis patients. Current treatments for pain in women with endometriosis are limited to the use of non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, hormonal agents such as birth control pills, and surgical removal of the lesions. However, patients often do not respond or experience limited benefit from hormonal therapies, NSAIDs present several side effects and should be used with cautious by patients with other comorbidities, and even after surgery, disease and pain recurrence are very common. Therefore, new medical therapies and targets that provide long-term benefits, including pain relief, are still urgently needed.

The perception of pain is transmitted by specialized nerve cells called “nociceptors.” Recent studies demonstrate that nociceptors not only can sense pain but also release “neuropeptides” such as calcitonin gene-related peptide (CGRP) to orchestrate inflammation and the activity of immune cells. This process is called “neuroimmune communication.” To produce its effect, CGRP acts on the receptor activity modifying protein 1 (RAMP1). Macrophage is a type of immune cell that is responsible for the engulfment and destruction of target cells and microorganisms. They are the main cells in the peritoneal cavity (abdominal space), and during endometriosis, macrophages change their activity to promote the growth and support of endometriosis lesions. We did preliminary experiments and found that mice without a specific type of nociceptor (TRPV1+ nociceptors) show less pain and grow smaller endometriotic lesions. In corroboration, we also did preliminary experiments with a U.S. Food and Drug Administration (FDA)-approved drug that blocks RAMP1 called rimegepant. Rimegepant-treated animals showed less pain, fewer lesions, and the remaining lesions were smaller when compared to the non-treated animals. This indicates that blocking CGRP/RAMP1 signaling might not only relieve pain but also actively eliminate lesions and reduce the growth of remaining endometriotic lesions. Ultimately, this indicates that CGRP or RAMP1 targeting drugs can provide long-term benefit for patients. In this project, to confirm the efficacy of this type of drug, we will test three additional FDA-approved drugs that target either CGRP or RAMP1, namely ubrogepant (small molecule RAMP1 antagonist, but structurally distinct from rimegepant), fremanezumab (monoclonal antibody against CGRP), and erenumab (monoclonal antibody against RAMP1). We hypothesize that neuropeptide CGRP release by nociceptors is the main driver of endometriosis-associated pain and lesion growth/establishment. Therefore, silencing the communication of nociceptors to macrophages by blocking CGRP/RAMP1 signaling can treat endometriosis pain.

Most work on endometriosis therapeutics targets either endocrine hormones and/or their receptors. This multidisciplinary project is conceptually innovative because it combines approaches from three disciplines (neuroscience, immunology, and pharmacology) to validate CGRP/RAMP1 as a non-opioid and non-hormonal therapy for endometriosis-associated pain. Combining different techniques (e.g., chemogenetic activation of lesion-restricted nociceptors, cell culture) with the use of FDA-approved drugs that block CGRP /RAMP1 signaling, we aim to address two biologically significant and clinically relevant questions about endometriosis:

a) Does the communication between nociceptor neurons (through the release of CGRP) to macrophages contribute to lesion growth and implantation in vivo?

b) Can CGRP/RAMP1 blocking strategies be used to treat endometriosis?

By answering question “a,” we will demonstrate that CGRP release activates RAMP1 receptor in macrophages and changes their phenotype to promote lesion growth and implantation. We will also leverage state-of-the-art techniques, such as single cell RNA sequencing, to identify macrophage populations that are responsible for endometriosis lesion growth vs. endometriosis lesion resolution upon nociceptor neuron activation and inhibition, respectively. By answering question “b,” we will demonstrate that blocking nociceptor to macrophage communication through CGRP/RAMP1 signaling with rimegepant, ubrogepant, fremanezumab, and erenumab could be a significant and innovative non-hormonal and non-opioid approach for endometriosis treatment.

This proposal responds to the Fiscal Year 2022 Peer Reviewed Medical Research Program Endometriosis Topic Area. Specifically, we address the following areas of encouragement in endometriosis research designated by the Department of Defense: (1) improve understanding of long-term complications and comorbidities of associated diseases and conditions, and (2) develop and test novel treatments for associated diseases and conditions. Overall, this project aims to reduce the negative effects of chronic pain among a substantial proportion of active-duty military members, Veterans, and their families who are burdened by the consequences of endometriosis.

Proposal Title:	A Plant-Made Vaccine for the Prevention of Herpes Simplex Virus (HSV)
Log Number:	PR221119
Current PI Name:	Mary Pardhe
Award Number:	HT9425-23-1-0708
Current Contracting Organization:	VaxSyna, INC
Current Performing Organization:	VaxSyna, INC
Web Approval Date:	10-03-2023

Herpes simplex virus (HSV) is a mucosal and sexually transmitted disease that infects over four billion people. HSV can cause numerous diseases including cold sores, genital herpes, meningitis, and other skin and brain-related illnesses. It is the leading cause of infectious blindness and causes a life-threatening disease called herpes simplex encephalitis (HSE). HSE has a 70% mortality rate without treatment and between 20% -30% with antiviral treatments. Repeated reactivation of HSV is also linked to the development of Alzheimer's disease.

The limited number of treatments for these diseases are not effective, and there is no approved vaccine against HSV. As HSV is one of most common sexually transmitted disease among active-duty Service Members, it is important to create a low-cost, effective vaccine that can be deployed to active military personnel, their families, and at-risk populations.

VaxSyna, Inc., proposes to further develop a plant-made HSV vaccine under the Portfolio Category of "Infectious Diseases," Topic Area "Plant-Based Vaccines," and Strategic Goal "Develop or optimize vaccine strategies, platforms, or compounds…." VaxSyna's HSV candidate vaccine uses their two-part vaccine technology that has previously proven successful in mice against multiple other viral diseases. Our vaccine technology has multiple advantages including: (1) high effectiveness, (2) low cost to make (estimated at less than $0.5/dose), (3) quick to produce without risk of contamination, and (4) thermally stable.

For this project, we propose to further test our HSV vaccine in four aims. Aim 1: Vaccine efficacy to protect against HSE in mice. Aim 2: Vaccine efficacy to prevent HSV-caused sores in guinea pigs. Aim 3: Optimal route of vaccine delivery. Aim 4: Safety profiles. Completion of these aims will indicate that our vaccine is effective at preventing HSV diseases in animal models and allow VaxSyna to take the next steps to make an HSV vaccine for the military and at-risk populations.

Proposal Title:	Drug Development for Dystonia
Log Number:	PR221120
Current PI Name:	Nicole Calakos
Award Number:	HT9425-23-1-0386
Current Contracting Organization:	Duke University
Current Performing Organization:	Duke University
Web Approval Date:	05-03-2023

This proposal addresses a major unmet need for an unremitting and disabling movement disorder, known as dystonia. This work addresses Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Area of dystonia research within the neuroscience category and the two strategic goals of (1) developing novel therapeutics and (2) identifying biomarkers. Dystonia is characterized by sustained involuntary postures and/or slow twisting movements. Dystonia is an unremitting and poorly treatable movement disorder that causes pain and movement disability. Dystonia can arise in many contexts – limb trauma, traumatic brain injury, stroke, neurodegenerative diseases, antipsychotic medication use, inherited genetic causes, and "sporadically." Military personnel are at increased risk to develop dystonia because of exposure to risk factors such as limb trauma, traumatic brain injury, repetitive use tasks, and certain medications. Unfortunately, both the number of treatment options for dystonia and their efficacy are severely limited. Current oral medications are limited by side effects and poor efficacy. Because of limited oral medication benefits, many people seek more invasive options like botulinum toxin injections or deep brain electrode implantation to get relief. However, these procedures are only suitable for specific subsets of dystonia. Lastly, no disease-modifying treatments for dystonia exist; all current treatments are symptomatic.

This Expansion Award seeks (1) to develop effective and orally available medications for dystonia with disease-modifying potential and (2) to identify human biomarkers that are needed for successful clinical trial design and outcome interpretation. Our proposal is a direct continuation of successful efforts made during the prior PRMRP Investigator-Initiated Research Award (FY19-FY22). To discover new drug treatments for dystonia, we developed a robust, cell-based, high-throughput assay to monitor dystonia-related cell pathology and screened >40,000 compounds. The top 10 were further studied and modified in order to gain properties that are necessary for a compound to be suitable as a drug for use in people. This effort included medicinal chemistry, testing for drug stability and toxicity, and testing the compound's effects in advanced disease-relevant models using human cells and mouse models. We now have two leading compounds with promising and distinct opportunities to develop into new oral medications for people with dystonia (one that is a U.S. Food and Drug Administration [FDA]-approved drug but cannot be simply repurposed because of limited central nervous system access in currently approved dosing/formulation and one with novel chemistry and higher potency).

With the Expansion Award, we aim to (1) perform medicinal chemistry to achieve the necessary brain penetrance and drug metabolism properties for the novel lead compound series that has already met high potency and efficacy in dystonia assays and low cytotoxicity milestones, (2) identify the protein that the drugs directly interact with to cause their "anti-dystonia" effects, and (3) discover biomarkers in human plasma and cerebrospinal fluid that will allow us to identify those subsets of people with dystonia that are most likely to benefit from the drugs' mechanisms and to monitor target engagement (i.e., that a future drug intervention is causing the intended effect in the brain during clinical trials).

The outcome of this effort will be to generate first-in-class dystonia drugs with a comprehensive surrounding preclinical data package in order to attract commercial partners. The advanced novel compound(s) will be ready to address next phases of safety in non-rodent large animals, drug product formulation, and investigational new drug application for human studies. Based on the core biological process that the lead compounds target, we anticipate that both people with rare inherited forms of dystonia (e.g., DYT1, 6, 16)

and sporadic disease (e.g., cervical dystonia) may benefit. A biomarker panel (Aim 3) will further enable testing of individuals with dystonia due to diverse causes, including traumatic brain injury and limb injury for which military personnel are at increased risk. This will help get the right drug to the right people. The ultimate goal of this work is to develop a highly effective oral drug, that may also have disease-modifying properties, for people with dystonia. Such a treatment opportunity would be a major advance because as yet, oral medications have limited benefit and no dystonia therapy is disease-modifying.

Proposal Title:	Percutaneous Cryoneurolysis: A Single-Administration, Non-Opioid, Non-Addictive, Multiple-Month Analgesic for Thoracic Trauma Free of Systemic Side Effects
Log Number:	PR221132
Current PI Name:	Brian Ilfeld
Award Number:	HT9425-23-2-0013
Current Contracting Organization:	California, University of, San Diego
Current Performing Organization:	California, University of, San Diego
Web Approval Date:	10-03-2023

Chest injuries frequently involve rib fractures, which can be very painful for 2-3 months. Unfortunately, pain is not simply a "symptom" of the injuries, but a significant cause of additional medical problems: pain causes people to breath and cough less deeply/often, which increases the risk of collapsing little parts of the lung. These collapsed areas often lead to complications, which can increase the risk of death. In addition, the higher the amount of pain in the weeks following the fracture, the higher the risk of developing persistent chronic pain that can last indefinitely. So, providing excellent pain control is very important for a variety of reasons. Various nerve blocks can greatly decrease pain, but even the longest acting are measured in hours or days, and not the weeks and months for which rib fracture pain can last. Therefore, opioids - "narcotics" - are the most common pain control method provided to patients; but they frequently do not provide enough pain control, have undesirable side effects like nausea and vomiting, and are sometimes misused, which can lead to addiction or overdose.

A prolonged nerve block lasting multiple months from a single treatment may be provided by freezing the nerve using a process called "cryoneurolysis." With cryoneurolysis and ultrasound machines, a very small "probe" may be placed through anesthetized skin and guided to the target nerve to allow freezing. The procedure takes about 5 minutes for each nerve, involves little discomfort, has no side effects, and cannot be misused or addictive. After 2-3 months, the nerve returns to normal functioning. We have completed a small study suggesting that a single cryoneurolysis treatment holds great promise to provide potent short- and long-term pain relief following chest injury with rib fractures.

The ultimate objectives of the proposed research study are to determine if temporarily freezing the nerves that go to fractured ribs will decrease short-term pain, opioid use, physical and emotional dysfunction, and long-term pain. The proposed research study will include patients with one to six fractured ribs. A probe will be inserted through an anesthetized portion of skin in the back and directed to the nerve that run underneath each fractured rib. Whether an active probe or a sham/placebo probe that does not freeze nerves is used will be determined randomly - like a flip of a coin. Neither the patients nor the investigators who follow patients will know which was used. This is to keep anyone from unconsciously affecting the study results and is called a "double blind" study. This process will be repeated for each of the fractured ribs using the same probe. Patients will be called for up to 1 year to collect information on their pain levels, opioid use, deep breathing ability, and multiple other factors.

The proposed study directly addresses the Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Areas "Non-Opioid Therapy for Pain Management" and "Trauma" by treating rib fracture pain with a cryoneurolysis procedure. Since cryoneurolysis is not an opioid, helps to control pain, has no side effects, is in no way addictive, and may be administered with a portable cryoneurolysis device, the following FY22 PRMRP strategic goal is addressed: "To develop and test pain therapies that will not

affect the cardiorespiratory system and cognitive abilities for use in trauma, battlefield, or resource-limited environments."

Within the United States, over 240,000 individuals experience chest injury with rib fractures, as have thousands of U.S. Service Members due to combat during recent conflicts.

Opioids are currently the most common pain control method but are associated with significant risks such as addiction and abuse. In contrast, cryoanalgesia has no addiction potential, produces no side effects, and does not influence thinking whatsoever, permitting patients to return to productive lives. If the proposed study is successful, patients with rib fractures in the future will experience far less short- and long-term pain, problems breathing and coughing, lung complications, and opioid use. The latter would decrease the risk of opioid misuse, overdose, dependence, and diversion (selling to others). Cryoneurolysis is a low-cost, low-risk procedure easier to administer than a local anesthetic-based peripheral nerve block. Since cryoneurolysis is already cleared by the U.S. Food and Drug Administration to treat pain, adoption of cryoanalgesia would be immediate and wide-ranging since there are few good pain control alternatives currently available. Importantly, injured Warfighters could be provided with excellent pain control without the use of opioids, allowing safer evacuation and transportation from forward operating bases to distant medical centers.

Proposal Title:	Role of Immune Cell-Podocyte Crosstalk in the Pathogenesis of APOL1-FSGS and Proteinuric Kidney Disease
Log Number:	PR221145
Current PI Name:	Shuta Ishibe
Award Number:	HT9425-23-1-0440
Current Contracting Organization:	Yale University
Current Performing Organization:	Yale University
Web Approval Date:	10-03-2023

Nearly 600,000 Americans suffer from end-stage kidney disease (ESKD), a failure of kidney function that necessitates either dialysis or a kidney transplant. Medicare expenditures for ESKD exceed $28 billion annually. The vast majority of ESKD in the United States is due to glomerular disease – a process in which the filtering component of the kidney is damaged, resulting in a severe loss of protein in the urine known as nephrotic syndrome, a Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Area.

While Americans of African ancestry make up 13% of the United States and Veteran population, they account for 35% of kidney failure. This large health disparity is partly attributed to the carriage of risk alleles in APOL1 (referred to G1/G2 risk alleles), which was discovered a little over a decade ago. Unfortunately, those with APOL1 risk variants manifest with Focal Segmental Glomerulosclerosis (FSGS) (FY22 PRMPP Topic Area), which is the most common form of nephrotic syndrome. However, limited mechanistic understanding of this disease process has hindered novel treatments, especially due to the lack of suitable animal models for study.

In our current research, we have made a mouse model that expresses human APOL1 risk alleles. Upon stimulation with an inflammatory agent, interferon, these mice develop excessive protein loss in the urine and kidney features that resemble human FSGS. Using data in humans, we have also identified for the first time that cells of the immune system, in addition to kidney cells, may play a profound role in this disease process. We hypothesize that specific immune cells in individuals with G1/G2 APOL1 release much more interferon which damages the kidney filtration barrier, resulting in accumulation of misfolded proteins.

Our first aim will study immune cell to podocyte (cells that line the kidney filtration barrier) crosstalk. The second aim will determine how excess interferons damage the podocyte using our novel mouse models expressing all APOL1 variants.

Successful completion of this grant may lead to identification of novel mechanisms and thus druggable targets, leading to potential weapons in the fight against the progression of FSGS to ESKD.

Proposal Title:	Role of Immune Cell-Podocyte Crosstalk in the Pathogenesis of APOL1-FSGS and Proteinuric Kidney Disease
Log Number:	PR221145P1
Current PI Name:	Madhav Menon
Award Number:	HT9425-23-1-0441
Current Contracting Organization:	Yale University
Current Performing Organization:	Yale University
Web Approval Date:	10-03-2023

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Proposal Title:	Targeting LRH-1 to Treat Ulcerative Colitis
Log Number:	PR221150
Current PI Name:	Eric Ortlund
Award Number:	HT9425-23-1-0420
Current Contracting Organization:	Emory University
Current Performing Organization:	Emory University
Web Approval Date:	05-18-2023

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) that currently affects 900,000 Americans. UC is an immunological condition, characterized by chronic inflammation of the bowel. UC patients experience chronic inflammation that leads to severe ulcers, and a large number of physiological problems. Several Food and Drug Administration (FDA)-approved medications are available for IBD; however, these drugs often fail to sustain long-term disease remission due to loss of response, immunogenicity, and worsening of the disease. Each of these approved treatment strategies have significant limitations, and our goal is to develop a safe oral medication that effectively treats UC. To achieve precise control of inflammation in the gastrointestinal (GI) tract, we are developing a small molecule therapeutic that operates through a new mode of action. Here, activation of native anti-inflammatory machinery will restore intestinal health. Liver receptor homolog-1 (LRH-1) is a phospholipid-activated nuclear hormone receptor that is expressed in the intestinal epithelium, where it maintains epithelial integrity and protects against inflammatory damage through multiple mechanisms. In our preliminary studies, we have discovered that an effective small molecule LRH-1 agonist reverses inflammation and disease in a commonly used mouse model of UC. The objective of this study is to optimize our LRH-1 small molecule agonists to reduce intestinal inflammation in models of UC.

Proposal Title:	Metabolic Interventions to Mitigate Acute and Chronic Bone Disease
Log Number:	PR221151
Current PI Name:	Robert Tower
Award Number:	HT9425-23-1-0534
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	10-03-2023

Fiscal Year 2022 Peer Reviewed Medical Research Program (FY22 PRMRP) Investigator-Initiated Research Award Focus Area to be addressed: This project aligns with the PRMRP Focus Area to: (1) understand the mechanisms underlying the pathobiology of associated musculoskeletal disorders, (2) develop strategies for improved care at point of injury to prevent musculoskeletal disorder onset, (3) develop and test strategies to increase quality of life or halt/slow disease progression, including regenerative medicine approaches and biologics for associated musculoskeletal disorders, and (4) understand correlations between nutrition and disease susceptibility. Specifically, we propose to validate an early dietary modification protocol/standard practice guideline and therapeutic intervention to prevent the most common complication of extremity trauma that limit return to duty: heterotopic ossification (HO). HO is the pathologic formation of extra-skeletal bone within soft tissues which occurs in patients with severe trauma and significantly limits return to duty given its cause of chronic pain and limitation of prosthetic use. This proposal has been developed to specifically address HO prevention after extremity trauma and treatment. The goal of this proposal is to validate a new standard practice guideline for a dietary protocol and a metabolite intervention to prevent posttraumatic HO in patients with severe trauma such as is seen with orthopedic and blast-related injuries.

With dramatic improvements in survival from combat-related blast injuries due to tourniquet use, we have witnessed a concomitant increase in patients with debilitating injuries which drastically diminish quality of life. Of the nearly 15,000 battle injuries suffered in Operations Iraqi Freedom/New Dawn and Enduring Freedom, over 50% of those injuries were extremity injuries. Of these wounded Soldiers with extremity injuries, over 60% of them will go on to develop HO. HO also causes significant disability in hundreds of thousands of civilians and Veterans with joint arthroplasties, amputations, and orthopedic injuries. For example, over 80% of patients with fractures and revision joint replacements will develop HO. As a result of forming bone outside of the skeleton, HO causes severe chronic pain, open wounds, and limited range of motion.

Current treatment strategies address HO after its development with surgical excision. However, surgery is unable to restore range-of-motion, which has often been chronically limited due to HO, cannot address chronic pain, and causes prolonged wounds with poor healing. After excision, patients often develop recurrence within the original tissue bed, which necessitates re-excision, or continues to cause the original signs and symptoms. Though several prophylactic medications have been previously trialed, all have negative side effects, and all fail to target the causative signaling mediators that lead to HO. We offer a paradigm shift in the prevention and management of HO through an easily implemented dietary protocol and a metabolite-based therapeutic with minimal adverse effects to improve our precision treatment of combat casualties with extremity trauma in a prolonged field care (PFC) scenario.

Potential clinical applications, benefits, and risks: This proposal is designed to be translatable and simulates real-world PFC trauma and management that patients may expect to receive. First, we use clinically relevant models of trauma-induced HO that are broadly applicable to combat-wounded military personnel and to civilians with significant trauma. Secondly, we utilize an approved metabolite (itaconate) and diet strategy; this method is highly translatable, cost-effective and easily implemented. Thirdly, this proposal addresses

duration of treatment by selecting a short time period of treatment to minimize cost, improve adherence, and avoid adverse consequences. The combination of these techniques makes this proposal an important preclinical study that lends itself to establishing key data necessary to push forward definitive clinical trials.

Projected timeline and expected patient-related outcomes: In this proposal, we plan to rapidly deploy our optimized dietary protocol and metabolite-based treatment interventions. In the first 24 months we will validate our dietary protocol to prevent HO in our proven extremity trauma models and determine the duration of time needed to use this diet. In the last 24 months, we will determine the effectiveness of metabolite-based therapy needed to inhibit HO. Upon completion of this proposal's aims, we will validate the key role of these pathways in human patient tissues.

Short- and/or long-term impact on patient care and/or restoration of function: This proposed research will significantly improve current occupational therapy and pharmacologic treatment strategies available to all patients who are at risk of developing HO. Through this proposal we will improve our understanding of the role of diet and specific metabolites that stimulate HO. This proposal will lead to a novel targeted therapy and a nutrition standard practice guideline to prevent HO.

Proposal Title:	Metabolic Interventions to Mitigate Acute and Chronic Bone Disease
Log Number:	PR221151P1
Current PI Name:	Benjamin Levi
Award Number:	HT9425-23-1-0535
Current Contracting Organization:	Texas, University of, Southwestern Medical Center at Dallas
Current Performing Organization:	Texas, University of, Southwestern Medical Center at Dallas
Web Approval Date:	10-03-2023

Fiscal Year 2022 Peer Reviewed Medical Research Program (FY22 PRMRP) Investigator-Initiated Research Award Focus Area to be addressed: This project aligns with the PRMRP Focus Area to: (1) understand the mechanisms underlying the pathobiology of associated musculoskeletal disorders, (2) develop strategies for improved care at point of injury to prevent musculoskeletal disorder onset, (3) develop and test strategies to increase quality of life or halt/slow disease progression, including regenerative medicine approaches and biologics for associated musculoskeletal disorders, and (4) understand correlations between nutrition and disease susceptibility. Specifically, we propose to validate an early dietary modification protocol/standard practice guideline and therapeutic intervention to prevent the most common complication of extremity trauma that limit return to duty: heterotopic ossification (HO). HO is the pathologic formation of extra-skeletal bone within soft tissues which occurs in patients with severe trauma and significantly limits return to duty given its cause of chronic pain and limitation of prosthetic use. This proposal has been developed to specifically address HO prevention after extremity trauma and treatment. The goal of this proposal is to validate a new standard practice guideline for a dietary protocol and a metabolite intervention to prevent posttraumatic HO in patients with severe trauma such as is seen with orthopedic and blast-related injuries.

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Proposal Title:	A Phase 2A, Randomized, Double-Blind, Placebo-Controlled Study of Single or Repeated Intravenous Administration of UC-MSCs in Ischemic Cardiomyopathy (CATO)
Log Number:	PR221163
Current PI Name:	Roberto Bolli
Award Number:	HT9425-23-1-0109
Current Contracting Organization:	University of Louisville Research Foundation, Inc.
Current Performing Organization:	University of Louisville Research Foundation, Inc.
Web Approval Date:	05-03-2023

This proposal addresses the Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Area of Cardiomyopathy and the Strategic Goal of treatment, including the Goal of “developing less-invasive treatment technologies.” We will study ischemic cardiomyopathy (ICM), which is a progressive deterioration of heart function caused by myocardial infarctions (heart attacks), resulting in heart failure. ICM is the most common form of cardiomyopathy and accounts for approximately half of all cases of heart failure. It is a common, lethal, disabling, and expensive condition. The number of patients with heart failure in the U.S. has reached epidemic proportions (~6 million) and continues to escalate as the population ages. The 5-year survival for those admitted to the hospital with heart failure is ~50%, worse than many cancers. These patients experience a high burden of morbidity and mortality and consume major health care resources, including frequent, recurrent hospitalizations. The burden of heart failure is disproportionately greater among minorities. There is, therefore, an unmet need to develop novel therapies for heart failure that are efficacious, safe, scalable, cost-effective, non-invasive, and thus widely applicable. This is the objective of the proposed project.

Several studies have shown that cell-based therapy using mesenchymal stromal cells (MSCs) holds great promise as a new approach to produce improvements in heart function in patients with heart failure due to ICM. If these effects can be clinically established and optimized, there is enormous potential for improving clinical outcomes for millions of patients. There is substantial scientific and public interest in cardiac regenerative or reparative cell therapy strategies, based on preclinical and early phase 1/2 clinical studies. Although most studies to date have used MSCs isolated from adult tissues, recent evidence indicates that MSCs obtained from the umbilical cord (which is collected after caesarian delivery of a baby) are superior to MSCs from adult tissues, since they are obtained from a much younger organism. Accordingly, we will use umbilical cord-derived MSCs (UC-MSCs).

Although cell therapy holds great promise, its development as a widely available clinical option is hindered by the invasive nature of current methods for cell delivery, which require a cardiac catheterization and make it very difficult or impossible to give repeated doses of cells. Giving repeated doses is important because cells disappear rapidly after they are transplanted and thus need to be replaced. To overcome this problem, we will use a less-invasive approach and inject the UC-MSCs by the intravenous route. Our approach is based on recent evidence suggesting that intravenous cell therapy is effective in patients with heart failure and that repeated doses are more effective than a single dose. Accordingly, the proposed clinical trial is a prospective test of these important therapeutic modalities. The study will be a phase 2a, randomized, double-blind, placebo-controlled, multicenter trial that will test whether intravenous injection of UC-MSCs produces beneficial effects in patients with heart failure caused by ICM, and whether four repeated doses have greater

therapeutic efficacy than a single dose. The primary outcome will be assessed using cardiac magnetic resonance imaging to measure cardiac function at 12 months after the first treatment.

We expect that patients receiving four doses of UC-MSCs will have better heart function than those receiving four doses of placebo, and that the improvement in heart function will be greater after four doses of UC-MSCs than after one dose. This outcome would demonstrate that intravenous delivery of cells is beneficial and that repeated cell doses are superior to a single dose. Such an outcome would have great importance because it would offer a new treatment option (intravenous injection of UC-MSCs) to millions of patients with heart failure who currently have few options and a grim prognosis. Compared with the invasive and expensive delivery techniques currently used, intravenous delivery is simpler, less expensive, and much safer; thus, it would greatly broaden the utilization, feasibility, and affordability of cell therapy, making it possible to use it as an outpatient procedure in almost every medical center and in almost every patient with heart failure while greatly reducing the complexity, cost, and risks of these treatments. The use of the intravenous delivery route would make cell therapy widely available to almost all patients with heart failure; as such, it would not only improve the health of hundreds of thousands of patients, but also decrease the health care resources required for this chronic condition. Importantly, it would reduce disparities in health care for minorities who may have less access to expensive invasive treatments. The impact would be even greater among the military population, where myocardial infarction and heart failure are more common than in the general population. Accordingly, we expect that this clinical trial will have a major impact both for the treatment of heart failure and for the development of the field of cell therapy for heart disease and will pave the way for a larger pivotal phase 3 trial. The proposed trial is currently approved by the U.S. Food and Drug Administration (FDA) under Investigational New Drug (IND) # 18642 and is ready to start.

Proposal Title:	Development of an Innovative Biomaterial for the Minimally Invasive Embolization of Vascular Malformations
Log Number:	PR221177
Current PI Name:	Karen Dubbin
Award Number:	HT9425-23-1-0485
Current Contracting Organization:	Boston Scientific Neuromodulation Corporation
Current Performing Organization:	Boston Scientific Neuromodulation Corporation
Web Approval Date:	05-18-2023

Arteriovenous malformation (AVM) is an abnormal connection between an artery and a vein marked by a complex tangle of blood vessels. AVMs occur in both Service Member and civilian populations and can lead to serious complications, including uncontrolled bleeding or hemorrhage. Current methods, including endovascular coils, which are inserted inside the blood vessel are used to treat AVM. However, these methods have major drawbacks, including incomplete stoppage of blood flow, high cost, reliance on natural blood clotting within the patient, the difficulty of proper administration, and challenges with patient imaging after the procedure. Obsidio, Inc. is developing an innovative universal biomaterial designed for the treatment of vascular malformation. Specifically, Obsidio is developing a patent-protected shear-thinning biomaterial (STB) technology that is biocompatible, non-toxic, stable, durable, promotes blood clotting, adhesive, inexpensive, requires less specialized skills to handle and does not rely on the body's natural ability to coagulate blood. We are advancing STB development to serve as an effective and versatile treatment for AVM in a broad range of settings, including hospitals and health care centers with fewer resources and limited equipment. For this Peer Reviewed Medical Research Program (PRMRP) Technology /Therapeutic Development Award, our proposal relates to the Fiscal Year 2022 (FY22) cardiovascular health portfolio in the Topic Area of Vascular Malformations. This project addresses the PRMRP Strategic Goal to develop less-invasive treatment technologies for associated cardiovascular conditions.

Obsidio's scientists, Dr. Ali Khademhosseini and Dr. Rahmi Oklu, designed and developed STB. This material has unique physical properties that make it unique as compared to other available treatments, including its ability to completely block an AVM, its ready-to-use formula, and its versatility in various parts of the body. The rationale behind our research lies in abundant research showing that STB appears to be biocompatible and safe to use, while also enabling very rapid obstruction of blood flow at the affected site. Using animal studies, it was demonstrated that STB can effectively stop blood flow without the downsides observed with other treatment options. Together, these studies showed that STB may be a safer and more effective treatment for AVM.

Based on these achievements, we propose to further develop STB as a treatment for AVM. Our first objective is to optimize the formulation of STB for use in hospitals by ensuring that the formula works with readily available microcatheters, which hospitals use to treat AVM. Our second objective will confirm that STB is biocompatible and safe for use using a panel of tests recommended by the Food and Drug Administration (FDA). These include ensuring that STB does not cause cell damage or mutations, is safe for the bloodstream and whole body, does not cause fever or allergic responses, and is safe to have in the body for an extended period of time. Our third and final objective is to confirm STB is effective in animals using Good Laboratory Practice (GLP) animal studies in preparation for Investigational Device Exemption (IDE) submission to the FDA, which will enable us to translate our preclinical findings into clinical applications. Ultimately, this proposed work will advance STB closer to clinical trials and to the market.

Proposal Title:	Translational Development of a Microneedle Patch SARS-CoV-2 Ferritin Nanoparticle Vaccine
Log Number:	PR221322
Current PI Name:	Louis Falo
Award Number:	HT9425-23-1-0427
Current Contracting Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Current Performing Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Web Approval Date:	05-06-2023

Emerging and re-emerging infectious diseases are serious threats that result in substantial impact on civilians and military Service Members, major socioeconomic burden, and potential cancellation of critical military operations with important national security implications. The Coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has demonstrated the unprecedented threat of emerging and re-emerging infectious pathogens, which is an ongoing challenge due to rapidly emerging new SARS-CoV-2 variants of concern. Most importantly, the emergence and re-emergence of infectious pathogens are inevitable as highlighted by the repeated outbreaks of coronaviruses (e.g., severe acute respiratory syndrome coronavirus and middle east respiratory syndrome outbreaks prior to COVID-19). These significant public health challenges posed by the long-term threat of emerging coronaviruses and by the likelihood of the repeated pandemics translate into a dire need for economically feasible and widely deployable solutions to effectively address these tremendous global health concerns for better pandemic preparedness.

Efficacious and safe vaccination strategies are a key element in pandemic preparedness programs. However, current vaccination strategies are suboptimal due to important challenges in the induction of broad and durable protection capacity, manufacturing, delivery, and logistics of existing vaccines, including COVID-19 vaccines, and these challenges reduce vaccine efficacy, acceptance, and coverage. As such, development of broadly effective, safe, economically feasible, patient-friendly, and widely deployable next-generation vaccination strategies is a rational goal for more effective, equitable, and sustainable immunization programs. The long-term goal of this project is to develop a next-generation vaccination strategy using readily translatable, plug-and-play platforms for safer, more effective, and more widespread immunization against emerging and re-emerging infectious pathogens. Thus, this project directly addresses the Fiscal Year 2022 (FY22) Portfolio Strategic Goal of developing and optimizing vaccine strategies, platforms, or compounds under the FY22 Peer Reviewed Medical Research Program (PRMRP) Topic Area of viral diseases for the FY22 Portfolio of infectious diseases.

Emerging experimental and clinical evidence supports the notion that the skin offers a readily accessible, highly immunoresponsive target that contains a rich density of antigen-presenting cells and immune-accessory cells for safe, efficacious immunization. Targeting vaccine components to the rich immune milieu of the skin improves vaccine-induced protective immune responses compared to traditional immunization routes (e.g., muscle or subcutaneous tissue). Importantly, the skin-targeted vaccine we are developing will be needle-free, painless, and capable of self-administration. It will also be temperature stable, enabling distribution and storage without refrigeration. Collectively, this vaccination strategy will enable more effective and sustainable global immunization campaigns for civilian and military programs compared to traditional vaccination strategies.

Skin vaccination using novel vaccine technologies, emerging vaccine adjuvants, and innovative vaccine delivery systems could enhance the magnitude, breadth, and longevity of vaccine-induced protective immune responses, reduce global health cost, and improve coverage. The main objective of this project is to

complete the translational development of a novel skin-targeted vaccine against emerging coronaviruses. We will (1) manufacture dissolving microneedle patches (MnPs, a novel vaccine delivery system) integrating SARS-CoV-2 Spike protein Ferritin Nanoparticle (SpFN, a novel vaccine technology) antigen and liposomal adjuvant (ALFQ, an emerging vaccine adjuvant) as a next-generation SARS-CoV-2 vaccine; (2) complete the preclinical evaluation of the safety, immunogenicity, protection efficacy, and translational correlates of this skin-targeted vaccine through animal studies and experiments on living human skin explants; and (3) develop and submit an Investigational New Drug (IND) application to enable the clinical development of this novel vaccine. The expected outcome of this project will be a next-generation vaccination approach, enabled by transformative plug-and-play vaccination platforms, to facilitate improved civilian and military immunization programs against SARS-CoV-2 variants and reduce the long-term threat of emerging coronaviruses. Ultimately, these novel platforms will be readily adaptable to develop efficacious, safe vaccination strategies against other existing and emerging pathogens. These next-generation vaccination platforms present tremendous advantages for civilian vaccination campaigns, for military immunization programs, and for vaccination in austere settings. These novel platforms will also make vaccines easier to transport and administer, without the need for needles, syringes, vials, trained medical workers, or cold-chain distribution, facilitating more effective and widespread military immunization programs, and increasing coverage for the global non-military population.

Proposal Title:	Genetically Dissecting Neural Circuits Underlying Anorexia Nervosa
Log Number:	PR221351
Current PI Name:	Benjamin Arenkiel
Award Number:	HT9425-23-1-0155
Current Contracting Organization:	Baylor College of Medicine
Current Performing Organization:	Baylor College of Medicine
Web Approval Date:	10-03-2023

Abnormal eating habits that drive excessive food seeking, or avoidance, can manifest as health-threatening and socially impactful eating disorders. Anorexia nervosa (AN) represents one of the most debilitating eating disorders with no effective cure and exhibits an array of core symptoms that include voluntary restrictive feeding, hyperactivity, and anxiety. In addition, AN frequently results from traumatic triggers and exhibits special penetrance in young females coinciding with estrogen surges, which is tightly associated with military personnel and their families. To date, the mechanistic understanding of AN has been greatly hindered by the lack of an effective animal model that faithfully recapitulates core symptoms in AN patients. Using a novel approach of chronic neuron activation, we have found that chronic activation of a group of hypothalamic neurons leads to core symptoms of AN, including voluntary restrictive feeding, anxiety, and frequent escape/jumping behaviors. Using multifaceted genetic, imaging, metabolic, and behavioral experimentation, we propose studies that will establish an effective model for AN research, and use this model toward better understanding AN. Such a model promises to help elucidate the critical, and yet unknown, circuit mechanisms by which hypothalamic neurons impact eating and associated emotional states and to identify pathophysiology responsible for AN development. Importantly, the proposed research will help reveal previously unidentified neural basis for the selective AN penetrance observed in young females and how AN is initiated by certain environmental triggers. Our results will also reveal key circuit bases for feeding abnormalities associated with AN, which often occurs in Service Members and Veterans, especially in young women encountering traumatic situations.

Proposal Title:	Defining Novel Cellular Circuitry and Communication in Fibrous Dysplasia
Log Number:	PR221358
Current PI Name:	Yingzi Yang
Award Number:	HT9425-23-1-0349
Current Contracting Organization:	Harvard University, Boston
Current Performing Organization:	Harvard University, Boston
Web Approval Date:	04-25-2023

Fibrous dysplasia (FD) is a severe skeletal disease where benign tumors cause bone deformities, fractures, and pain. This disease is caused by mutations in some cells in a protein called Galphas that changes levels of a signaling molecule called cyclic AMP. While it is known that high cyclic AMP levels cause problems in FD, exactly how this occurs remains poorly understood. In addition, we do not fully understand how bone becomes weak in FD with resultant fractures and bone pain. These knowledge gaps have hampered efforts of therapeutic development to treat FD patients or improve their health conditions. Here, we will study how high cyclic AMP levels cause disease in bone cells with FD lesions. We will use multiple, complementary approaches to better understand how downstream changes within cells in the cyclic AMP pathway drives fibrous dysplasia through molecular changes within bone cells bearing Galphas mutations. In addition, FD is a mosaic genetic disease where some cells bear the mutation, while many other cells do not. Mutation-bearing cells cause dramatic changes in nearby “normal” cells that do not bear FD mutations. We will study how normal and abnormal cells talk to one another using a novel approach to study inter-cellular communication. This work will identify new mechanisms through which rare mutation-bearing cells cause dramatic problems throughout the skeleton. Finally, we will test new therapeutic approaches in cutting-edge mouse FD models and confirm our discoveries in human samples from FD patients. The new information and treatment strategies developed here will benefit military personnel with FD and other related bone problems like bone pain, stress fractures, heterotopic ossification, and poor fracture healing.

Proposal Title:	Defining Novel Cellular Circuitry and Communication in Fibrous Dysplasia
Log Number:	PR221358P1
Current PI Name:	Marc Wein
Award Number:	HT9425-23-1-0350
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	04-25-2023

Fibrous dysplasia (FD) is a severe skeletal disease where benign tumors cause bone deformities, fractures, and pain. This disease is caused by mutations in some cells in a protein called Galphas that changes levels of a signaling molecule called cyclic AMP. While it is known that high cyclic AMP levels cause problems in FD, exactly how this occurs remains poorly understood. In addition, we do not fully understand how bone becomes weak in FD with resultant fractures and bone pain. These knowledge gaps have hampered efforts of therapeutic development to treat FD patients or improve their health conditions. Here, we will study how high cyclic AMP levels cause disease in bone cells with FD lesions. We will use multiple, complementary approaches to better understand how downstream changes within cells in the cyclic AMP pathway drives fibrous dysplasia through molecular changes within bone cells bearing Galphas mutations. In addition, FD is a mosaic genetic disease where some cells bear the mutation, while many other cells do not. Mutation-bearing cells cause dramatic changes in nearby “normal” cells that do not bear FD mutations. We will study how normal and abnormal cells talk to one another using a novel approach to study inter-cellular communication. This work will identify new mechanisms through which rare mutation-bearing cells cause dramatic problems throughout the skeleton. Finally, we will test new therapeutic approaches in cutting-edge mouse FD models and confirm our discoveries in human samples from FD patients. The new information and treatment strategies developed here will benefit military personnel with FD and other related bone problems like bone pain, stress fractures, heterotopic ossification, and poor fracture healing.

Proposal Title:	A Multimodal Nanotrap to Alleviate ARDS in Traumatic Injuries
Log Number:	PR221368
Current PI Name:	Juntao Luo
Award Number:	HT9425-23-1-0590
Current Contracting Organization:	New York, State University of, Upstate Medical University
Current Performing Organization:	New York, State University of, Upstate Medical University
Web Approval Date:	10-03-2023

Background: This proposal is to address the Peer Reviewed Medical Research Program (PRMRP) portfolio of Respiratory Heath with the Topic Areas of Respiratory Health, Trauma, and Sustained-Release Drug Delivery. The casualties with blast lung injury, abdominal trauma, or trauma with significant tissue loss are particularly at risk of developing sepsis and acute respiratory distress syndrome (ARDS). All these critical illnesses have the common feature of hyperinflammation. Trauma and infection cause tissue damage, releasing a lot of alarm molecules, which stimulate immune cells to produce signaling molecules, called cytokines to fight infection and manage tissue damage. However, if these alarming and dangerous molecules and bugs spread into blood circulation, immune reactions become overwhelmed and dysregulated, which leads to a "cytokine storm" as known in the severe COVID-19 patients and causes sepsis and multiple organ damage including ARDS. Thus, the control of both infection and hyperinflammation during early emergency treatment is critical to saving the life of Warriors with traumatic/blast injuries. Unfortunately, such approaches are still lacking even in the fully equipped civilian hospital, especially for hyperinflammation control. No effective treatment available for sepsis should antibiotics fail. The mortality rate for sepsis especially with ARDS remains high, about 18%-50% depending on the disease stage and comorbidity. Antibodies or steroid drugs for inflammation control all failed to reduce mortality of sepsis, because of the complex and unstable immune system in sepsis and ARDS patients.

Project Overview: We propose to fill this gap in emergency care by (1) developing clinical translatable blood purification therapy using our novel telodendrimer (TD) nanotrap (NT) resin to capture and remove the overflowing excessive sepsis-responsive molecules from the bloodstream, especially differentiating the pro-inflammatory and anti-inflammatory cytokines, thus controlling "cytokine storm" more effectively and preventing remote organ damage and ARDS. (2) TD NT can be incorporated into even small nanogels (~200 nm, about 1/1000 thickness of hair) as injectable or inhalable treatment to control inflammation directly in far-forward battlefield as emergency treatment. (3) In addition, potent antibiotics, e.g., polymyxin B and daptomycin as the last resort antibiotics (partly due to the toxicity) to overcome multidrug-resistant bacteria can be encapsulated in the immune-modulating nanogel for sustained drug release to control infection effectively and reduce drug toxicity. The goal of the study is to develop a translational multimodal TD NT platform approach to control both infection and inflammation as an emergency treatment for battle casualties with severe injury to prevent and treat sepsis and ARDS on the battlefield and continuously after evacuation in the field hospital.

Recently, our TD hydrogel resin treatment significantly improved the survival, and even cure of sepsis in combination with a moderate dose of antibiotics in severe septic mouse models induced with severe abdominal tissue injury and peritoneal infection (published in Nature Communications 2020). In order to translate such therapeutic efficacy into the clinic, (1) we will first collect a minimum amount of blood from sepsis patients from the clinic (two teaspoons per patient) to validate our TD NT resin in scavenging human septic molecules, and further optimize TD NT for targeting different groups of signaling cytokine molecules. Thus, TD NT resins with different adsorption profiles can be applied based on patient immune status for precise immune modulation. (2) We will further design and test TD NT nanogel for both antibiotics' encapsulation and immune modulation in vitro and in mouse models with severe sepsis with polymicrobial infection and traumatic injury to mimic battlefield casualties. (3) We will apply TD NT validated in human blood in hydrogel resin for hemoperfusion therapy in a clinical translatable pig sepsis-ARDS models induced

by ischemia/reperfusion and peritonitis infection to test the efficacy for ARDS prevention, which also mimics battlefield casualty. The Principal Investigator has assembled a multidisciplinary team with comprehensive research expertise, e.g., nanotechnology, pharmacology, microbiology, immunology, pathophysiology in sepsis and ARDS research, as well as the clinical expertise in sepsis treatment to ensure the smooth progression of the proposed research.

Impact: If successful, our TD NT therapy will transform the emergency treatment on far-forward battlefield and in the field hospital for the casualties with severe traumatic/blast injury to prevent sepsis and ARDS. It can be used to treat other critical illnesses, e.g., pancreatitis, complex cardiac surgery, and CAR-T cancer therapy.

Proposal Title:	A Phase 2 Randomized, Double-Blind, Placebo-Controlled Study of Oral Allopregnanolone (LYT-300) for the Treatment of Premutation Carriers with FXTAS
Log Number:	PR221394
Current PI Name:	Michael Chen
Award Number:	HT9425-23-1-0598
Current Contracting Organization:	PureTech Health LLC
Current Performing Organization:	PureTech Health LLC
Web Approval Date:	10-03-2023

The objectives of this study are to complete a randomized double-blind controlled trial of a new oral formulation of the endogenous neurosteroid, allopregnanolone (LYT-300), in individuals who have the Fragile X-associated Tremor/Ataxia syndrome (FXTAS) to assess the safety and benefit of LYT-300.

Our application targets the Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Area of Fragile X and the FY22 PRMRP Strategic Goal to develop and evaluate novel treatments, including research to repurpose existing drugs, for associated neurological diseases and psychological conditions. Our application also addresses development and testing of treatment strategies to manage symptoms and improve quality of life for these conditions. Fragile X refers to a group of disorders, including fragile X syndrome (FXS) and premutation or fragile X carrier disorders, that are caused by different numbers of repeated elements in the Fragile X Mental Retardation 1 (FMR1) gene. FXS and FXTAS are two very different disorders, one causing intellectual disability (ID) and autism and the other leading to neurodegeneration in otherwise normally developed aging individuals, respectively. FXTAS is the most serious of the fragile X spectrum disorders because it is a neurodegenerative disorder that typically begins in FMR1 premutation carriers when they reach the age of about 60. The symptoms of FXTAS include tremor in the hands with action or at rest, and balance problems (ataxia) which leads to frequent falling. Carriers are common in the general population, occurring in 1 in 150 to 200 women and 1 in 400 men, but FXTAS is often mistakenly diagnosed as Parkinson's disease. FXTAS also causes brain atrophy and white matter disease in the brain and for many patients there is cognitive decline that is sometimes misdiagnosed as Alzheimer's disease.

A previous clinical trial of IV-administered allopregnanolone showed that weekly infusion of the drug in individuals with FXTAS may improve deficits in executive function, learning, and memory. Effective administration of allopregnanolone has only been possible via intravenous infusion because when given orally almost no drug reaches the blood circulation due to processing by the liver. LYT-300 is designed to use the human body's lipid absorption pathways to bypass the liver and release potentially therapeutically effective amounts of allopregnanolone in the bloodstream. If this medication is helpful in FXTAS, it will likely be beneficial for other types of neurological disorders, and we expect that it could be widely used in the future.

This grant proposal includes a planning phase of 1 year to prepare for the trial and submit the U.S. Food and Drug Administration, Investigational New Drug, Institutional Review Board, and Human Research Protection Office applications. In the following 2-year clinical phase, we expect to enroll 50 individuals in a placebo-controlled, parallel-group clinical trial to determine safety, tolerability, and several measures of efficacy. A successful clinical trial in this indication will contribute to ultimate commercialization of LYT-300, which will help military Veterans and others who have FXTAS and other related neurological diseases.

Proposal Title:	Development of a Rapid, Non-Addictive, Intranasal Treatment for Headache Disorders
Log Number:	PR221462
Current PI Name:	Jonathan Beckwith
Award Number:	HT9425-23-1-0280
Current Contracting Organization:	OLFAX, LLC - Ashville
Current Performing Organization:	OLFAX, LLC - Ashville
Web Approval Date:	04-10-2023

The primary objective of the proposed research is to accelerate development and commercialization of Relaspen, a self-administered, intranasal nerve block that provides rapid acute migraine symptom relief without the harsh and addictive side effects of existing therapeutics. The proposed development project directly addresses the Fiscal Year 2022 Peer Reviewed Medical Research Program Neuroscience Topic Area of Non-Opioid Therapy for Pain Management and is responsive to the Strategic Goal: Develop and evaluate novel treatments, including research to repurpose existing drugs, for associated neurological diseases and psychological conditions.

According to the U.S. Centers for Disease Control and Prevention, 14.2% of U.S. adults report experiencing migraine symptoms within any given 3-month period, making it the second most disabling illness in the world. The prevalence of migraine in active-duty U.S. Armed Forces members has steadily increased year over year, and the Department of Veterans Affairs has reported that Veterans are more likely to experience migraine and headaches than the general U.S. population. There is strong evidence that the increased prevalence for military personnel is likely a direct result of their service. In addition to increased exposure to stressful combat situations, Service Members have higher rates of traumatic brain injury (TBI), concussion, and neck trauma, which are often followed by post-traumatic headache symptomology. For these individuals, migraine can take an immense toll on physical and emotional health, lead to missed or suboptimal training, and can reduce their “return to duty” rates. For Veterans, the debilitating physiological symptoms can have a broad-sweeping impact on quality of life, due to restrictions placed upon physical, emotional, occupational, academic, social, leisure, and family systems. There is no absolute cure for migraine, and 53% of active-duty Army Soldiers with migraine are prescribed last-line-of-defense butalbital and opioids within 1 year post-deployment.

Relaspen technology combines a hand-held, Posterior Nasal Cavity delivery system with silicon plate spray nozzle technology to accurately deposit a novel formulation of aerosolized liquid anesthetic directly into the upper-posterior nasal cavity – without the use of propellants – to efficiently block parasympathetic signals from the sphenopalatine ganglion (SPG) associated with headache disorders. The novel combination product is the commercial translation of an SPG block procedure, which has been used in primary, specialty, and ambulatory care settings to effectively abort symptoms of severe or intractable migraines for decades. Strong clinical evidence obtained through care of patients with diagnosed headache disorders supports Relaspen’s potential for high levels of efficacy (up to 26% more effective at eliminating pain than leading prescription medications), rapid symptom relief (81% pain relief in less than 20 minutes), and feasibility of self-administration verified by end users with proof-of-concept atomizer prototypes.

A complete pre-production development plan for Relaspen has been created based on industry standards (USP 601, ICH M3(R2)), regulatory guidance (21 CFR 320.1), and from our multidisciplinary development team’s clinical and industry-specific expertise. Specifically, Relaspen delivery device designs (single-button actuation, enclosure design, device positioning, storage, and safety features) will be developed through formative human factors evaluations that will bring together diverse groups of military personnel with

migraine, clinical care professionals, and key opinion leaders to obtain invaluable feedback on the Relaspen concept, prototype hardware, and their expectations for ease of use in an at-home environment. Prototypes will be manufactured, optimized, and validated through benchtop and in vitro performance testing (laser diffraction and surrogate nasal cast) to ensure localized drug deposition to the upper-posterior nasal cavity while meeting specifications for function and safety based on recommendations for drug delivery devices (21 CFR 320.1; USP 601). Finally, an optimized formulation of lidocaine hydrochloride solution will be prototyped and tested based on a target product profile (TPP) created to increase dwell time, reduce drain and inhalation, and match delivery device spray characteristics – culminating in a 4-week repeat dose intranasal toxicity study in two species (rats followed by dogs) with toxicokinetics. The proposed preclinical development and research program is carefully designed to directly result in Relaspen's next major commercialization inflection point of Investigational New Drug (IND) submission to the U.S. Food and Drug Administration (FDA).

If successful, Relaspen will be a single-use (multiple dose), prescription product for both adult and youth populations – providing a non-addictive therapeutic option for individuals who either do not respond to or are contraindicated for existing NSAID (non-steroidal anti-inflammatory drug), prescription, or emergency therapies. Long-term benefits of the proposed work will be a decrease in the number of opioids prescribed and administered to military personnel for severe or intractable migraines. More broadly, it will also indirectly reduce use of these highly addictive treatment options through fewer emergency department (ED) visits where opioids are the most common form of treatment (35% of ED visits).

Proposal Title:	Novel Centrifugal LVAD with Wireless Power Transfer and Antithrombotic SLIC Coating
Log Number:	PR221487
Current PI Name:	Lakshmi Dasi
Award Number:	HT9425-23-1-0663
Current Contracting Organization:	Georgia Tech Research Corporation
Current Performing Organization:	Georgia Tech Research Corporation
Web Approval Date:	04-10-2023

OVERALL PROGRAM

Heart failure (HF) occurs when the heart cannot pump enough blood and oxygen to the body. There are nearly five million Americans suffering from HF, with approximately 400,000 related deaths occurring each year. HF causes a significant public health care burden and greatly reduces the mobility, quality of life, and ability to work for the patient. Moreover, studies have found that Veterans have a much higher chance of being affected by HF, especially those suffering from traumatic experiences in combat. There is no medical treatment when HF reaches end stage, and the only option for many patients is invasive heart transplant surgery. But heart transplant is severely limited by the availability of donor hearts. To address this problem, scientists and engineers have invented a tiny implantable blood pump called Left Ventricular Assist Device (LVAD) to help the failing heart to pump blood to the body. However, due to the fast-spinning rotor damaging the blood cells, the patients are very likely to suffer from blood clotting, bleeding, infection, and stroke. Moreover, the LVAD can only be powered by an invasive driveline that runs through the body, causing serious infection problems that sometimes can only be treated with surgeries. Besides, living with a driveline greatly limit the mobility and the quality of life of the patient.

Through this work, the problems with the current LVAD will be addressed by a synergistic collaboration between four complementary research groups. We will first (Project 1, led by Dr. Lakshmi Dasi at Georgia Institute of Technology) reduce the blood damage by optimizing the LVAD geometry using advanced machine learning and with flexible blades and casing. The blood clotting problem near the inlet will be eliminated by an innovative stented design borrowed from prosthetic heart valve technology. Second (Project 2, led by Dr. Arun Kota at North Carolina State University), novel slippery coatings will be developed and applied to the LVAD surfaces to flight blood clot formation inside the device. Without clot formation, we can eliminate the possibility of associated pump failure. Third (Project 3, led by Dr. Cavallaro at Rice University), we will develop a wireless energy transfer system to power the implanted LVAD. By eliminating the driveline and associated infection risk, we will overcome one of the major limitations of LVAD. Lastly (Project 4, led by Dr. O.H. Frazier at Texas Heart Institute), we will convert the LVAD to a maglev drive system to further reduce the blood damage. The LVAD system will be thoroughly evaluated after implanting in large animals.

This innovative LVAD has the potential to profoundly change the way we treat HF. As the blood damage is greatly reduced and the clotting problem is eliminated, the pump can support patients for a very long time without subsequent surgeries to treat these side effects. With the elimination of the driveline, the LVAD is much less invasive and can greatly improve the quality of life. When the outcome of LVAD therapy is comparable to a heart transplant, millions of lives will be saved. This overall effort addresses the Topic Area and Strategic Goal outlined in Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP): Cardiomyopathy: Develop less-invasive treatment technologies for associated cardiovascular conditions. Toward the end of this grant period, the experimental LVAD will be evaluated in animal studies. The next step will move toward applying for regulatory approval and bringing it to the clinic. The LVAD

and the technology developed will be prioritized to use in military, Veteran, and public health. Intellectual properties and technological innovations developed from this grant, such as the wireless power transfer for medical devices and the anti-thrombotic coatings, can be translated to other military applications as well. The proposed project will have a significant impact on how we treat heart failure patients and benefit both our military and the U.S. public.

PROJECT 1 – Novel Design and Optimization to Improve Hemocompatibility and Reduce Thrombosis Risk

LVAD is a tiny blood pump used to treat end-stage heart failure. However, due to the fast-spinning rotor that destroys blood cells, patients often experience serious complications, including blood clotting, bleeding, and stroke. These problems can also be found in other blood circulating pumps used in civilian and military hospitals to support blood circulation. Also, blood clot is very likely to form near the inflow to the pump due to flow stasis there. Expensive re-hospitalization and invasive surgeries are often required to treat those devastating complications. Therefore, reducing the blood damage within the pump and eliminating flow stasis at the inlet are essential to improving the outcome of LVAD therapy and making it less invasive.

In this project, the problem will be addressed by several innovative design changes. First, the geometry of an LVAD will be optimized by state-of-the-art computer simulations and machine learning. It is expected the blood damage caused by the fast-spinning rotor will be reduced significantly. Next, the flow stasis problem will be eliminated by a novel stented inlet design that adapts to the shape of the patient's heart. Last, the blood damage of the LVAD will be further reduced by using novel flexible rotors and casings. This novel LVAD model will be thoroughly evaluated by advanced flow measurement techniques to prove the improvement. We hypothesize that after applying these innovative designs, the blood damage within the LVAD will be significantly reduced, and the risk of blood clotting at the inlet will be completely eliminated. Thus, we have addressed the Topic Area and Strategic Goal outlined in Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP): Cardiomyopathy: Develop less-invasive treatment technologies for associated cardiovascular conditions, as no invasive open-heart surgeries will be required to treat complications after the implantation.

The technological innovation will profoundly improve the hemocompatibility of LVADs and will lay a solid foundation for other innovations in the project. Together, they will pave the way for LVAD therapy to achieve similar outcomes when compared with cardiac transplants. The innovation will save millions of civilians and veterans and save U.S. taxpayers billions of dollars each year. More importantly, the technological achievement can be transferred to other blood circulating pumps as well, which are widely used in civilian and military hospitals to sustain blood circulation.

PROJECT 2 – Slippery Hydrophilic Anti-Thrombotic Coatings for LVADs

The leading cause of death in the United States is cardiovascular diseases, including heart attack, stroke, arrhythmia, and heart valve stenosis, all of which might eventually lead to cardiomyopathy and heart failure. A rapid increase in mortality due to cardiovascular diseases is specifically seen in military personnel due to their high-risk working environments. With the increase in cardiovascular diseases comes a need for cardiovascular devices. Thrombosis (undesirable blood clotting) has been identified as the major cause for device failure. While many studies have investigated surface modifications to reduce thrombosis, there is no clear and successful strategy to date. Almost all commercial blood contacting devices (LVADs, stents, heart valves, vascular grafts, extracorporeal membrane oxygenation and cardiopulmonary bypass pumps) still struggle with device related thrombosis. Through this project, we primarily aim to tackle thrombosis in LVADs by developing slippery hydrophilic (SLIC) coatings with unprecedented anti-thrombotic properties.

We hypothesize that our SLIC coatings can inhibit thrombosis due to the presence of ice-like hydration layer. The ice-like hydration layer can reduce friction between blood and the substrate, both in static and dynamic conditions, thereby preventing thrombosis. Indeed, our preliminary results confirm that SLIC coatings lead to unprecedented anti-thrombotic properties compared to even state-of-the-art coatings. We

aim to further maximize the anti-thrombotic response by preparing a wide range of SLIC coatings and evaluating their potential for thrombosis. The most promising SLIC coatings will be further iteratively optimized for novel LVAD designs in Project 1, compatibility with wireless power transmission in Project 3, and long-term, large animal studies in Project 4.

The proposed SLIC coatings have the potential to revolutionize blood contacting devices such as catheters, guidewires, dialyzers, oxygenators, heart-supporting systems, cardiac pacemakers, vascular grafts, stents, heart valves, etc., through their unprecedented anti-thrombotic properties. Further, the SLIC coatings can also lead to development of fouling-free coatings for contact lenses, chemical and biomedical sensors, food and beverage equipment, marine equipment, etc. We indeed foresee that our SLIC coatings will lead to anti-thrombotic blood contacting devices, thereby reducing dependence on direct care hospitals during military operations and contributing to the development of less invasive treatment technologies.

PROJECT 3 – Wireless Energy Transfer and Communication to Eliminate Risk of Driveline Infection

Patients with heart failure can benefit from an implanted pump that helps circulate blood to the body. However, the motor in the implanted pump, called a left ventricular assist device (LVAD), needs a significant source of power to operate. A regular non-rechargeable battery implanted to power the LVAD would not last very long. The alternative is to have a power cable or driveline connected to the implanted LVAD passing through the body and skin to an external rechargeable battery pack worn in a vest or belt. This type of power cable driveline that goes through the skin is essentially an open wound and a source of infection. The patient needs to be careful to clean the area where the driveline exits the body, and the power cable is inconvenient when dressing or bathing. The driveline cable decreases patient outcomes and quality of life for Veterans and other civilians and is the overarching challenge of this project. This project will develop a wireless energy transfer system, which is a less-invasive treatment technology and a Strategic Goal in the Cardiovascular Health Topic Area for improving medical health.

Wireless energy transfer is a topic of research and suitable for many medical applications. A rechargeable external battery and controller worn in a vest or a belt with a special radio transmitter antenna and an internal implanted receiver antenna will be used to transfer power. An internal rechargeable battery will then power the implanted LVAD. Performance data from the LVAD and the heart will then be transmitted to the external controller using a Bluetooth link. An improved design for the external and internal antennas will increase the transmission efficiency. The antennas will also be made of flexible material and contoured to the body to further improve comfort and efficiency. In addition to the study of the mechanical pumping of the LVAD, the electrical conduction behavior of the heart can be studied through the sensing data that will be transmitted to the external controller. This data can be used to develop digital signal processing and machine learning algorithms to modify and improve the LVAD calibration and control. Position and temperature sensors will also be added to internal and external antennas to monitor alignment and temperature during wireless power transfer in real time. These features in combination will improve the wireless energy transfer efficiency of the system and attain greater insight into the LVAD's impact on the heart that will lead to better long-term outcomes for both Veterans and civilians.

Wireless energy transfer can improve patient outcomes and quality of life. Wireless energy transfer also has the potential to power a large number of implantable biomedical devices for both civilian and military use. The proposed wireless energy transfer system can be adapted to multiple biomedical devices. The wireless energy transfer system developed in this project can also be used to power a body area network, which can lead to a new era in digital health care using coordinated multiple implanted devices and sensors.

PROJECT 4 – Drive System Design with Magnetic Levitation and In Vitro and In Vivo Validation

Heart failure is a progressively worsening disease in which the heart does not pump efficiently enough to supply blood to the body. Heart failure has a tremendous impact on patients, their families, the health care system, and our economy. LVADs—implantable pumps with rotational parts to transfer the blood from the

heart chamber into the body—have provided life-saving alternatives to transplant, but there are limitations and complications related to their use, which includes the formation of the blood clots, bleeding, and infections. Therefore, it is important to develop an LVAD that can significantly improve its hemocompatibility to the human cardiovascular system.

In order to reduce the bleeding chance when the blood passes through the rotational part of the pump, our research group proposed a non-contact bearing system that uses magnetic levitation to keep the stationary and rotational apart. This technology can be used to improve the performance of the LVADs by eliminating the contact points to reduce the chance of forming the blood clots and bleeding events. Our group will also test the pump in a continuous blood loop and animals in order to evaluate the overall performance of the pump. Evaluating the LVADs in the continuous blood loop prior to animal testing has clear advantages. Blood test can reduce the use of animals, reduce costs, save time, and provide more repeatable and controllable studies than animal testing. The proposed research work will produce a baseline for overall blood damage caused by the SLIC LVAD device early in the development process so that several improvements can be made afterwards.

To complete the proposed work, three aims are proposed, including: first, the design of the drive system with the maglev bearing system for the SLIC LVAD. A balance of motor gap, maglev system, and drive system performance will be studied in order to reduce the bleeding due to high rotational speed and/or small flow gap. Next, we will evaluate the SLIC LVAD device in a continuous blood flow loop. The overall device system will be evaluated by hydraulic performance, hemocompatibility testing, power consumption, and heat dissipation. Last, animal studies of the SLIC LVAD for both short and long periods to evaluate the overall device performance will be conducted.

Such maglev technology could be applied to much smaller size patients or even for child patients use. The maglev technology could also be applied to other direct blood-contact medical devices and/or devices that have rotary components to reduce the bleeding and the wear of the bearing system. Combined with the wireless energy transfer and communication, the wireless maglev drive system could potentially be applied for other medical devices that could not allow direct cable power transfer to the rotary components of the devices.

Proposal Title:	Biofabricated Patient-Specific Skin Gloves as a Personalized Therapy for Mitten Deformities in Epidermolysis Bullosa
Log Number:	PR221555
Current PI Name:	Hasan Abaci
Award Number:	HT9425-23-1-0487
Current Contracting Organization:	Columbia University Medical Center
Current Performing Organization:	Columbia University Medical Center
Web Approval Date:	10-03-2023

This project addresses the FY22 PRMRP Topic Area "Epidermolysis Bullosa" by developing wearable engineered skin grafts as an innovative technology for personalized and advanced skin replacement therapy. Recessive dystrophic EB (RDEB) is a severe type of Epidermolysis Bullosa (EB), in which recurrent blistering and scarring of the hands leads to fusion of fingers into a mitten-like deformity starting in early childhood. Engineered skin grafts made from RDEB patients' cells offer great clinical promise to treat the wounds and restore the function of the patients' hands and fingers. However, current skin grafts are flat tissue patches in generic shapes that have to be cut and wrapped around each finger individually, and sutured or bandaged together to sufficiently cover the wound area. This process significantly lengthens the surgery time and worsens the aesthetic and functional outcome of the procedure. In this project, we will challenge the prevailing paradigm in skin engineering by developing a 3D-bioprinting strategy to engineer wearable skin gloves that can be simply worn on the hands of RDEB patients with mitten-like deformities. Moreover, this new approach will generate both mechanically and functionally superior grafts compared to currently available engineered skin grafts. The wearable skin technology would have a transformative impact on the treatment of EB patients as well as the care of all types of extensive wounds caused by burns or severe impacts affecting the general population and military personnel, who are the greatest risk of non-fatal traumatic injuries.

Proposal Title:	Neuropeptide Modulation of Cerebral Blood Flow to Improve Neurological and Psychological Outcomes Following TBI in the Presence and Absence of Traumatic Stress
Log Number:	PR221566
Current PI Name:	Kelly Standifer
Award Number:	HT9425-23-1-0517
Current Contracting Organization:	Oklahoma, University of, Health Sciences Center
Current Performing Organization:	Oklahoma, University of, Health Sciences Center
Web Approval Date:	10-03-2023

Peer Reviewed Medical Research Program (PRMRP) Fiscal Year 2022 (FY22) Topic Area to be addressed: This proposal addresses the Neuroscience Topic Area of Trauma and is directed toward two strategic goals: (1) Foundational Studies and (2) Prevention. Foundationally, this project will examine a neuropeptide as a mechanism by which blood flow in the brain is immediately compromised following traumatic brain injury (TBI), with posttraumatic stress disorder (PTSD) and the combination of TBI+PTSD, such that brain tissue in the impact area, or in several brain regions with PTSD, function poorly due to the drop in oxygen levels (ischemia) that may stay reduced for many days. We will test our hypothesis that increased levels of the neuropeptide are responsible for reduced blood flow and subsequent problems with balance and coordinated movement, anxiety, pain, and memory loss, by treating with one of two different drugs that reduce the actions of the neuropeptide. Treatment will begin shortly after the TBI or traumatic stressor with the goal to prevent brain blood flow reduction and prevent or reduce appearance and severity of symptoms over the next 2 weeks.

Rationale: TBI and PTSD are major causes of disability for military personnel and Veterans as well as civilians. TBI is the leading cause of death and disability in young adults (

Objectives: We will use a rat model of TBI that produces mild and moderate TBI with physical symptoms related to injury severity (impaired balance and poor learning and memory, pain, and anxiety-like behaviors), that allows us to directly measure blood flow on injured and uninjured sides of the brain, and that easily can be combined with a model of PTSD. Our objectives are (1) to measure brain blood flow immediately after moderate TBI, PTSD or TBI/PTSD and then measure the ability of a new drug to reverse the injury-induced drop in blood flow; (2) Use a novel PET imaging agent to visualize areas of reduced blood flow throughout the brain over 8 days to compare the development of ischemia with the different types and combinations of trauma, and (3) to test two different NOP receptor blocking drugs, administered within 2-4 hr after injury and daily for 7 days, for their ability to prevent or reduce the symptoms produced by the trauma in male and female rats. Our study is specifically designed to test for differences in symptoms and drug response in males and females, because females may present with different types and severity of symptoms than males and are often not included in initial drug development studies. We also will study, for the first time, how the severity of TBI symptoms, ischemia, and blood flow disruption is altered in rats that were subjected to both TBI and PTSD.

Impact: There are no FDA-approved treatments for TBI, and only two for PTSD. We propose a new treatment for patients TBI or TBI+PTSD that may be given shortly after any type of TBI to prevent disrupted blood flow to the brain and the rapid and sustained impairments that follow. Benefits include identification of a new drug target and a drug to move forward for development to treat TBI and TBI+PTSD.

A similar drug with the same target (NOP receptor) was recently in a clinical trial for depression and has a good safety profile. With positive results from this project, perhaps Lilly would be persuaded to develop clinical trials with that drug for TBI.

Proposal Title:	Biomarkers of Sleep Loss
Log Number:	PR221578
Current PI Name:	Dragana Rogulja
Award Number:	HT9425-23-1-0447
Current Contracting Organization:	Harvard University, Boston
Current Performing Organization:	Harvard University, Boston
Web Approval Date:	05-18-2023

It is well known that quality sleep is integral for good health. Most of us have experienced the common consequences of only one night of poor sleep: tiredness, poor concentration, mental fog, and mood swings. But what happens when people sleep poorly for years and even decades? Studies have shown that poor sleepers tend to have higher risk for numerous serious illnesses such as diabetes, Alzheimer's disease, heart attack, stroke, and even some types of cancer. These findings are even more alarming when considering that approximately one-third of the adult U.S. population regularly experiences sleep disturbances. Military Service Members have an even higher rate of sleep problems, which continue even after returning home due to posttraumatic stress disorder (PTSD).

So why is chronic poor sleep so detrimental for health? We normally associate sleep with the brain, and it makes sense that not sleeping results in mental fog. But why would other parts of the body be affected by poor sleep? We think the cause might lie in an unexpected place – the gut. We recently discovered that sleep loss specifically causes cellular damage in the gut (and not in other organs) that directly and considerably reduces lifespan of sleep-deprived animals. On the other hand, animals can have a normal lifespan, even when sleep deprived, if this damage is prevented. Based on this, we think that humans might experience a similar phenomenon, which might lead to increased risk for many cardiovascular, neurological, and other disorders.

In this grant proposal, we describe steps we are taking to understand the connection between poor sleep and gastrointestinal health, colon cancer, as well as how gut damage may be associated with cognitive issues. We want to find noninvasive methods to detect biomarkers for gut damage to timely diagnose it and treat it before sleep loss leads to health decline. To our knowledge, no such approach has been described before. Our ultimate goal is to develop therapies that can offset the negative consequences of inadequate sleep. Considering that no such therapies currently exist, and considering how great the need for them is, we think that this work has the potential to be transformative.

Proposal Title:	Development and Validation of Innate Immunome-Targeted Therapy to Create Prosurvival and Organ-Protective Phenotype After Hemorrhage at the Point of Injury
Log Number:	PR221632
Current PI Name:	Yansong Li
Award Number:	HT9425-23-1-0382
Current Contracting Organization:	The Geneva Foundation
Current Performing Organization:	The Geneva Foundation
Web Approval Date:	05-03-2023

Severe bleeding (hemorrhage) post-trauma is the primary cause of death on the battlefield. Recent studies showed that 50% died of hemorrhage. In addition, bleeding is associated with 85% of potentially survivable deaths in the current conflicts. Approximately 90% of battlefield causalities die in the prehospital environment. The rate of death prior to evacuation increases from 20% with a 2-hour evacuation, to 26% with a 6-hour evacuation, and to 32% with a 24-hour evacuation. Of the increased deaths due to delayed evacuation, 62% are the result of hemorrhage. After arrival at a surgical facility and control of hemorrhage, casualties now enter a phase of care during which, according to a recent UK review, two-thirds of those who die do so because of causes other than exsanguination—to include unbridled inflammation and multi-organ failure (MOF). Mortality due to MOF after traumatic hemorrhage (TH) increases dramatically from 5% with single organ failure to 90% or more when at least four organ systems fail. The importance of better prehospital resuscitation strategies is highlighted by the fact that future combat casualty care scenarios suggest longer transport times and significant time delays in evacuation of casualties.

Trauma-induced tissue damage, hemorrhage-induced ischemia injury, and fluid infusion-induced reperfusion injury cause activation of two major immune cascades: damage associated molecular patterns (DAMPs) and complement cascade (ComC), which trigger early immune response and metabolic derangement, thus resulting in systemic inflammatory response syndrome (SIRS), endotheliopathy (ETP), and persistent inflammation/immunosuppression and catabolism syndrome (PICS), which contribute to early MOF and mortality. Secondary organ/tissue damage and PICS-induced sepsis after TH lead to further release of DAMPs and ComC, resulting in a vicious cycle with continued inflammation and immune activation. However, no current immunotherapy exists that directly addresses TH-induced MOF and mortality at prolonged field care (PFC) and prolonged damage control resuscitation (PDCR) scenarios, a serious unmet need.

Accumulating evidence suggests that early modulation of these two cascades may constitute the most effective therapeutic principle for the treatment of MOF and the improvement of survival after TH. Indeed, we have recently demonstrated that early inhibition of complement C5 by nomacopan or the inhibition of DAMPs by CX-01 significantly reduces inflammation, mitigates MOF, stabilize homeostasis, and improves survival in a rat model of TH at PFC and PDCR settings, suggesting that effective and timely resuscitation with nomacopan and/or CX-01 can attenuate morbidity and mortality after TH during PFC and PDCR. Recent reports indicated that PMX205 and ethyl pyruvate also repurposed drugs would act well in the combination with nomacopan and/or CX-01.

These four immune modulators are manufactured under Good Manufacturing Practices. They are clinical-stage drugs and currently investigated in phase 2-3 for non-trauma indications that will place us in a position to move toward the appropriate U.S. Food and Drug Administration (FDA) Investigational New Drug (IND)

application for phase 2 studies if this project proves to be successful. Furthermore, they are practical for transport and use on the battlefield; they can be carried in small vials, quickly reconstituted in small volumes of fluid, and administer by a single daily IM injection facilitating use in prehospital settings. Therefore, nomacopan, PMX205, CX-01, and ethyl pyruvate as a combinatorial therapeutic regimen in the prehospital setting may (1) reduce the weight and cube of resuscitation fluids and (2) reduce morbidity and mortality during PFC and PDCR.

The proposed preclinical studies should be completed within 4 years of the funding period. Comprehensively, the successful outcome of this project will not only make a significant impact on new knowledge and information of therapeutic management of trauma, to be translated for a new direction of trauma treatment, but also will provide important information to support the future filing of an IND for the treatment of traumatic hemorrhagic patients.

Proposal Title:	Quantified Coronary Artery Plaque and Outcomes (QUIET): WARRIOR Ancillary Study
Log Number:	PR221663
Current PI Name:	Carl Pepine
Award Number:	HT9425-23-1-0659
Current Contracting Organization:	Florida, University of
Current Performing Organization:	Florida, University of
Web Approval Date:	10-03-2023

The cardiovascular disease (CVD) burden, a major cause of mortality and morbidity among women, is increasing. Although emerging data document that most people with symptoms and or signs of chronic ischemic heart disease are women without obstructive coronary artery disease (INOCA), data are not available from large, randomized trials on an effective primary CVD prevention strategy for these women. The ongoing Department of Defense-funded WARRIOR trial is addressing this critical knowledge gap. However, the mechanisms responsible adverse outcomes in women with INOCA are unclear. The 2021 chest pain guidelines emphasize noninvasive testing utilizing coronary CTA, which provides the opportunity to evaluate mechanisms beyond presence/absence of obstructive plaque and calcification. Women participating in WARRIOR who underwent CCTA will provide an opportunity to better understand these mechanisms.

This project is designed to take the coronary CT images from WARRIOR and perform more sophisticated measurements of the plaque in the coronary arteries to determine if this information can be used to determine who is at the highest risk to go on to have a cardiac events.

Proposal Title:	Scalable Manufacturing and Toxicity Testing of Intranasal M2SR Influenza Vaccine
Log Number:	PR221664
Current PI Name:	Pamuk Bilsel
Award Number:	HT9425-23-1-0246
Current Contracting Organization:	FluGen Inc
Current Performing Organization:	FluGen Inc
Web Approval Date:	04-04-2023

Seasonal influenza virus infections are one of the most serious respiratory infections causing significant annual illness, hospitalizations, loss of life, and productivity in the U.S. and the rest of the world. Influenza virus infections circulate through human and animal populations year-round and while doing so they have a tendency to change over time. This tendency of the circulating virus to change is called antigenic drift. Occasionally, a new subtype of influenza virus to which the human population is unfamiliar will jump from infecting animals into infecting man. When this happens and this new virus starts to circulate rapidly and widely, it can cause an influenza pandemic. This new subtype jump from animals to humans is called an antigenic shift. Influenza virus shifts were responsible for the four influenza pandemics that occurred in the last century.

The primary way to protect against influenza infection is through use of the seasonal influenza vaccine. Current vaccines have three or four virus components making them trivalent or quadrivalent vaccines, respectively. The components of the seasonal influenza vaccine change each year in order to “match” the predicted three or four drift viruses. Currently licensed influenza vaccines are modestly effective (50%-60%) at preventing influenza infection. However, in a year where the vaccine viruses and a circulating drifted virus do not match, the effectiveness can be much less. For example, during the 2014-2015 influenza season the vaccine used in the U.S. was calculated to only be 23% effective. In addition, seasonal influenza vaccines do not offer any protection against other subtypes that can shift circulation into the human population and cause an influenza pandemic. When a shift like this happens, it can take up to 6 months to make a new vaccine, which is matched to the new virus subtype leaving the human population vulnerable to infection by the pandemic virus.

There is a clear need for better influenza vaccines to prevent seasonal influenza infections and to provide protection against a virus shift that could result in an influenza pandemic. An influenza vaccine that protects against drifted seasonal viruses and shifted viruses would be considered a universal influenza vaccine because it would offer protection against all types of influenza infection. FluGen has designed a new vaccine based on an influenza virus that acts like a normal flu virus but is unable to produce an essential viral protein, M2. The vaccine virus is called M2SR. It is able to infect cells but cannot spread from cell to cell or person to person, thus helping to build immunity against influenza but not spreading the disease. Animal studies with the M2SR vaccine showed protection from drifted and shifted influenza virus infections suggesting that M2SR could be a universal influenza vaccine. More importantly, human clinical trials evaluating M2SR vaccine have shown that in addition to being generally safe and well-tolerated, the M2SR vaccine can protect against infection with an influenza virus that does not match the vaccine. For these trials, only one vaccine component was tested. Flu vaccines have four components. Therefore, as next steps in the development of the M2SR vaccine, FluGen optimized the manufacturing process used for the single-component M2SR vaccine, for cost-effective production of the other three components of the vaccine. This includes how the virus is grown and purified away from manufacturing debris. In this proposal, manufacture of M2SR at a large scale is proposed for use in a toxicology study so that clinical trials can be conducted with the Quadrivalent M2SR, the eventual product to be licensed.

These proposed studies address Fiscal Year 2022 Peer Reviewed Medical Research Program Portfolio: Infectious Diseases, Respiratory Health; Topic Area: Viral Diseases and Respiratory Health; and Strategic Goal: Prevention.

The capacity to manufacture all four components of the quadrivalent M2SR vaccine, which has the properties of a universal influenza vaccine, will help it to move rapidly through development and toward U. S. Food and Drug Administration (FDA) approval. Approval of a universal M2SR vaccine will have tremendous public health benefits. Seasonal influenza kills tens of thousands of Americans annually with the very young and the elderly being the most affected. In addition, seasonal influenza infections cause a significant loss of productivity and hospitalization costing the U.S. economy billions of dollars. Influenza also has a devastating effect on readiness of our Warfighters because it can rapidly spread through units causing significant illness that requires medical attention. Finally, a universal M2SR could save hundreds of thousands to millions of lives by preventing or blunting the emergence of the next influenza pandemic through providing immunity in the population to a new virus subtype.

Proposal Title:	Enhancing the Deployability of the Sotair to Improve Ventilation and Survivability During Prolonged Field Care
Log Number:	PR221728
Current PI Name:	Prathamesh Prabhudesai
Award Number:	HT9425-23-1-0316
Current Contracting Organization:	SAFEBVM Corp.
Current Performing Organization:	SAFEBVM Corp.
Web Approval Date:	04-10-2023

Severe trauma is often accompanied by acute respiratory failure in which patients struggle to breathe. In such cases, artificial ventilation is used to provide oxygen to the lungs and remove carbon dioxide. While mechanical ventilators can provide consistent and safe ventilation, these machines are often heavy/bulky, expensive, require training, and rely on a power source, which can make them difficult to use for transport or in far-forward/austere settings without a reliable and consistent source of power. Therefore, manual resuscitators such as bag valve masks (BVMs) are often used in these cases, in which a provider squeezes a bag to deliver air into the patient's lungs. Unfortunately, research has shown that even well-trained providers are prone to overventilation, providing higher pressures, flow rates, and volumes than are safe for patients. Overventilation can be dangerous, resulting in numerous complications such as ventilator-induced lung injury and compromise to the cardiovascular system, which may increase the risk of death and prolong recovery.

To improve the safety of manual ventilation, SafeBVM has developed the Sotair, a small, lightweight, non-powered device that attaches to standard manual resuscitators and uses a custom automatic flow and pressure regulation valve to prevent dangerously high pressures, flow rates, and volumes. In this way, the Sotair virtually eliminates the delivery of breaths with pressures above commonly accepted "safe" guidelines. This is especially critical in the case of extended manual ventilation, such as may occur in some prehospital and battlefield situations. In particular, the Sotair could be used as a last resort to provide safe extended manual ventilation of military personnel during prolonged field care (PFC) should a mechanical ventilator not be available. To evaluate this possibility, this project will include the following: (1) environmental testing to ensure the proper operation of the Sotair under conditions likely to be encountered during military transport, storage, and deployment; development of a novel airflow sensors for field testing; (2) a PFC study in pigs, in which personnel will take shifts manually ventilating sedated pigs with or without the Sotair; (3) a field usability study in which usability feedback will be solicited from potential end users (medics, nurses, respiratory therapists, and doctors from all Service branches); development of military training materials; and (4) a human PFC performance study, in which we will determine how long personnel can perform manual ventilation on a lung simulator before performance degrades.

This project will investigate the ability of the Sotair to provide safer extended manual ventilation in austere settings. This project addresses the Peer Reviewed Medical Research Program Topic Area of Respiratory Health, Continuum of Care of Treatment, and Strategic Goal of developing improved fieldable devices to treat traumatic/acute lung injury in far-forward settings, including toolsets to enable correct airway placement, oxygenation in austere settings, or miniature and/or semi-automated ventilators. The Sotair is relevant to this goal as it is a fieldable device that decreases the conditions causing lung injury during ventilation and enables improved ventilation in far-forward, austere, and PFC settings.

Proposal Title:	Development of an RNA Therapeutic for the Treatment of Charcot Marie Tooth 1A
Log Number:	PR221745
Current PI Name:	Arthur Suckow
Award Number:	HT9425-23-1-0470
Current Contracting Organization:	DTx Pharma
Current Performing Organization:	DTx Pharma
Web Approval Date:	10-03-2023

This proposal from DTx Pharma addresses the fiscal year 2022 Peer Reviewed Medical Research Program (FY22 PRMRP) Portfolio Topic of Neuroscience, specifically the FY22 PRMRP Topic Area of Peripheral Neuropathy. The FY22 PRMRP Strategic Goal addressed in this application will be Treatment, namely, to develop and evaluate novel treatments, strategies or therapeutic targets, including research to repurpose existing drugs, for associated neurological diseases and psychological conditions and develop and test treatment strategies to manage symptoms and improve quality of life for those affected by associated neurological and psychological conditions.

Charcot-Marie-Tooth disease (CMT) encompasses a heterogeneous group of inherited peripheral neuropathies affecting both motor and sensory nerves. It affects 75,000 people in the United States and leads to muscle weakness and atrophy in the legs and arms, foot deformities and loss of sensation and numbness. The disease is slowly progressive and variable, and those affected may have difficulties with everyday activities and a shorter life expectancy. While the age of disease diagnosis varies, only 12.5% of the population is expected to be diagnosed before 14 years of age. Accordingly, it is expected that military Service Members will be diagnosed as the disease progresses during their military careers. Currently, there are no curative or symptomatic therapies have been approved for CMT. Current care consists of physical therapy, occupational therapy, and use of orthopedic devices to help patients cope with disability, along with medications for neuropathic pain.

The most common form of CMT is type 1A, which is an inherited disease affecting between 25%-40% of all CMT patients, and results from the duplication and overexpression of a specific gene. This gene, PMP22, plays an essential role in the formation and maintenance of the nerves and its overexpression causes degradation of the protective tissue surrounding these nerves, a process known as demyelination. These defects impair the transmission of electrical signals needed to drive muscle movement, resulting in peripheral neuropathies affecting both motor and sensory nerves. Several publications have demonstrated that suppression of the overexpressed gene can result in functional improvements in animal models of CMT1A, however, this success has not been translated into the clinic.

DTx Pharma has developed the FALCON platform that modifies already effective gene-targeting therapeutics to ensure their delivery to the correct tissues and cells and their retention in these tissues and cells for a sufficient amount of time, enabling them to act on gene overexpression by binding to and silencing them. In preliminary work, DTx Pharma screened over 60 drug candidates and identified a lead compound able to both silence PMP22 overexpression found in CMT1A and also able to reach and be retained in the nerve cells a sufficient amount of time to act on the gene overexpression prior to being broken down and flushed out of the system. In preliminary studies in mice, this compound, DTx-1252, was able to reverse disease and restore nerve function for 60 days following a single administration, without causing any adverse safety effects.

DTx Pharma is currently working to compile a regulatory package for DTx-1252 in order to commence clinical trials. The Aims proposed herein will help to confirm our ability to manufacture DTx-1252 for clinical use, identify appropriate dosing and schedule in animals that can later inform dosing in clinical trials, and fill in gaps in our knowledge around the duration of effect of the drug, options for alternative routes of administration, and potential consequences of over-suppressing the target gene. Critically, these data will allow us to see whether there is a clear and viable path to move DTx-1252 into clinical trials for CMT1A patients. This project will also serve as validation of the FALCON platform to enable the targeting to many tissues, including peripheral nerve.

By offering a treatment potentially curative of CMT1A, DTx Pharma aims to give renewed hope to those diagnosed with CMT1A to allow these individuals to live normal lives. Significantly, DTx recognizes the possibility that reversing demyelination, potentially via PMP22 suppression, could result in treatment for neuropathies beyond CMT1A, such as for diabetic neuropathy. The success of the proposed project will create the foundation for future research exploring the use of DTx-1252 or other FALCON-based PMP22 suppressor in diabetic and other neuropathies, thus the potential impact of this research could be tremendous.

Proposal Title:	Assessment of Eating Disorder and Comorbidity Risk and Resilience in a Nationally Representative Sample of Recent Military Enlistees
Log Number:	PR221802
Current PI Name:	Kelsie Forbush
Award Number:	HT9425-23-1-0310
Current Contracting Organization:	Kansas, University of, Center for Research Inc.
Current Performing Organization:	Kansas, University of, Center for Research Inc.
Web Approval Date:	04-09-2023

Eating disorders (EDs) affect people of all ethnicities and socioeconomic levels and occur across the lifespan. EDs are more common than previously believed and affect 13.1%-15% of young women and 3% of young men. EDs are one of the deadliest mental disorders, with standardized mortality ratios that are two times higher than mortality due to heavy smoking. In 2019, the Government Accountability Office (GAO) investigated the scope and impact of EDs in the military. The GAO's report stated that the military's ED screening does not accurately identify Service Members with an ED. The Department of Defense (DOD) did not have ongoing efforts aimed at preventing EDs among active-duty Service Members due, in part, to (incorrect) assumptions that the prevalence of EDs was low relative to other medical conditions. The GAO indicated that no existing screening tools for EDs were developed or tested in military-relevant populations but mentioned that, if successful, our team's 2018 Investigator-Initiated Award (#W81XWH-19-1-0207) could fill a critical need for DOD ED screening. Since publication of the GAO report, it has become exceedingly clear that EDs are a significant source of medical and psychiatric impairment among Service Members. The proposed research is, therefore, highly significant and impactful because our team successfully developed the first ED screening for use in Veterans, and we are poised to take the next step toward implementation of our screen within the DOD and Department of Veterans Affairs (VA) through additional testing in active-duty Service Members. Through our Fiscal Year 2018 Peer Reviewed Medical Research Program award, we created the Brief Assessment of Stress and Eating (BASE) for detecting EDs and related mental-health disorders. However, because some active-duty military Service Members have unique considerations that Veterans do not (e.g., the need to maintain a high level of physical fitness), additional testing of the BASE in military Service Members is needed. Thus, we propose to tailor the BASE for use in active Service Members, which is an important next step before implementation through the following specific aims:

Aim 1: Test the ability of the BASE to identify military Service Members who may have an eating, mood, anxiety, or trauma-related disorder compared to existing screeners.

Hypotheses: The BASE will be more accurate for identifying eating, mood, and anxiety (including trauma) disorders in military members than traditional screening instruments. Scores on the BASE will outperform existing screeners for predicting future psychosocial adjustment (i.e., impairment) in the first year of military service.

Aim 2: Identify factors that predict (or protect from) the development of an ED in military Service Members in their first 3 years of service.

Hypothesis 2a: History of dieting, unhealthy drive for muscularity, history of weight-based teasing or bullying, self-reported family history of an ED, high negative affect, perfectionism, and self-reported child sexual abuse at baseline will be significant pre-service predictors of ED onset.

Hypothesis 2b: Military sexual trauma (MST), serving in a theater of combat, combat-related trauma, disordered-eating behaviors designed to achieve military weight and personal appearance requirements, weight gain, and number of deployments will be significant active-duty predictors of ED onset.

Hypothesis 2c: Unit support and family support specific to military service will be significant negative predictors of ED onset.

Aim 3: Assess longitudinal course and patterns of comorbidity between EDs and internalizing and externalizing psychopathology in military members with EDs in their first 3 years of service.

Hypothesis: We predict that there will be significant bidirectional relationships among ED psychopathology, internalizing problems (mood, anxiety, and trauma symptoms), and externalizing problems (substance and alcohol use disorders) in military members over 3 years.

Secondary/Exploratory Aims: We will conduct qualitative interviews with 50 military Service Members who screened positive for an ED about perceived military cultural factors that lead to the development of an ED and body dissatisfaction. We will test lifetime prevalence and point prevalence of EDs, which will provide the first data on the prevalence of the full range of ED presentations in the military.

Military Relevance/Impact: Early identification and treatment of EDs will enhance military readiness because of Service Members improved physical and psychological health following treatment and could result in significant cost savings to military health care. The proposed research is, therefore, highly relevant to military health because it will provide data to support the implementation of the first military-specific ED screening tool in DOD and VA health care settings so that military members who have mental health issues can be more easily identified and referred to treatment to improve their psychological readiness to serve and be deemed world-wide qualified. Additional sources of impact include: (1) accurate and up-to-date prevalence estimates on the full range of EDs in military Service Members; (2) the first nationally representative longitudinal study to identify predictors of ED onset over the first 3 years of military service; and (3) improved understanding of military cultural factors that promote EDs, which will provide a critical first step toward prevention.

Proposal Title:	Development of Pharmacotherapies for the Treatment of Hydrocephalus and Associated Sequelae
Log Number:	PR221805
Current PI Name:	Bonnie Blazer-Yost
Award Number:	HT9425-23-1-0296
Current Contracting Organization:	Indiana University
Current Performing Organization:	Indiana University
Web Approval Date:	05-01-2023

OVERALL PROGRAM

The focus of our proposed research is to develop drugs that can be used to treat hydrocephalus. Hydrocephalus is a general term for excess cerebrospinal fluid (CSF) in the brain. Babies can be born with this or it can happen because of conditions such as traumatic brain injury (TBI), infection, or hemorrhage. There is even a form called idiopathic normal pressure hydrocephalus (iNPH) that arises from an unknown cause in the elderly. Thus, there are multiple forms of hydrocephalus. If too much CSF collects in the brain, then a person can develop symptoms from the increased pressure in the head such as pain, vision changes, sleep disturbance, and trouble walking. If left untreated, this can lead to cognitive changes and even death. Currently, the only effective intervention for hydrocephalus is brain surgery.

The type most applicable to active military personnel is called post-traumatic hydrocephalus or PTH. The incidence of PTH has been reported to be quite high in severe head injury patients. Injured Soldiers treated in the field may not have timely access to neurosurgical care and advanced diagnostic testing, leading to under-reporting of the condition. While injury-associated hydrocephalus may get better over time, the effects of the long-term damage remain unknown. Retired military personnel are more likely to be at risk for hydrocephalus that develops after a stroke or from the poorly understood condition called iNPH. The latter affects the elderly with symptoms that mimic other neurodegenerative diseases such as Alzheimer's and Parkinson disease. Young military families are more likely to be affected by childhood forms of the disease, most commonly caused by "brain bleeds" in premature births, spina bifida or trauma, all which result in chronic hydrocephalus. Civilian populations of similar ages are also affected by the multiple forms of hydrocephalus. Currently, the only long-term treatment is surgery. Shunts, the most common treatment require that a tube is implanted in the brain in order to drain the excess CSF from the brain to another part of the body. Unfortunately, shunts are prone to failure, blockage, and infection and require additional surgery when they malfunction. There are no long-term, effective drugs to treat hydrocephalus, and this represents a large, world-wide medical need. We are addressing this need by conducting studies to try to find safe and effective compounds that can be developed as drugs to treat all forms of hydrocephalus.

While distinct causes of hydrocephalus exist, there is extensive overlap in both acute and chronic symptoms. Common symptoms include disorientation, chronic pain, cognitive and executive function changes, vision and sleep disturbances, and difficulties walking normally. The similar clinical presentation, as well as the effectiveness of shunt placement for multiple types of hydrocephalus, suggests overlapping cellular and molecular mechanisms. Importantly, the overlap implies treatment strategies may show effectiveness regardless of initial cause.

The studies in these five proposed research projects are applicable to the Neuroscience Category – primarily to the Hydrocephalus Topic Area and secondarily to the Trauma Topic Area. Within these Topic Areas, the studies address the Strategic Goal of Foundational Studies by identifying underlying disease mechanisms and physiological processes that contribute to the development of all causes of hydrocephalus including from

traumatic brain injury (TBI). Importantly, the studies also address the Strategic Goal of Treatment because we have identified several novel treatments for hydrocephalus and will test these in preclinical models, including novel therapies to address disease progression, cognitive function, vision disturbances, chronic pain, and to improve quality of life.

The overarching challenge is to discover versatile, safe treatment options for use on the battlefield, athletic settings, and in hospitals of all levels in both rural and urban areas. The discovery of potential drug treatments would benefit patients with hydrocephalus of all ages, including our Service Members, their young children and/or older patients with normal pressure hydrocephalus. To address this challenge, our diverse team consists of experts in clinical care for hydrocephalus, preclinical drug development, neuroscience, and the pathophysiology of hydrocephalus and its associated sequelae such as acute and chronic pain as well as vision, sleep, and cognitive disturbances. Through collaborations between research teams at two universities, Indiana University and Johns Hopkins University, we have identified several potential compounds that are effective in treating various forms of hydrocephalus in rats and mice. These preliminary findings will now be extended to studies directed toward identifying the optimal drug or combination of drugs that would be effective for treating each of the types of hydrocephalus and to determine how these compounds act not only on the increased amount of fluid in the brain but also on common hydrocephalic side effects such as vision and sleep disturbance as well as walking and cognitive changes.

Research Plan:

Based on data from our combined research laboratories, we have identified three potential treatments that have been shown to decrease hydrocephalus in rat and mice models. These compounds are: Transient Receptor Potential, vanilloid 4 (TRPV4) antagonists, erythropoietin (EPO), and melatonin (MLT). All are candidates for further drug development, and we will study them alone and in combination. This research is designed to determine which drug(s) will be most effective against various forms of hydrocephalus and the resulting problems associated with the disease.

In many cases, the development of hydrocephalus happens in different stages – acute (right after the injury or cause), subacute (days later), and chronic (long-lasting effects). The compounds we are testing work together to reduce the production of CSF, decrease inflammation, and restore normal brain function and, therefore, this drug “cocktail” is a promising, novel approach with the potential to revolutionize treatment.

Relevance to Military Health:

People of all ages, including military Service Members, are at risk for hydrocephalus. TBIs suffered by our military personnel, especially those who have served in Iraq and Afghanistan, have increased the numbers of those living with PTH. Specifically, two-thirds of current and former military Service Members who suffered moderate to severe TBIs are at risk of developing PTH. Further, thousands more could develop hydrocephalus as a result of their injuries but remain undetected and undiagnosed as a result of improper or ineffective screening. Further, many children and family members of Service Members also suffer hydrocephalus from preterm birth or infant TBI.

The most common form of surgical treatment is a CSF shunt to divert the excess CSF from the brain to another body cavity for absorption. After insertion, shunts are prone to malfunction or infection at any time over the lifespan, and additional surgery is often required. Thus, an individual with a shunt must live reasonably close to a hospital with neurosurgical care available at all times. Additionally, the person is always at risk for the family and job disruption caused by shunt problems, and medical complications associated with urgent surgeries and long hospitalizations. Shunt insertion and revision surgeries remain the most common surgery performed by pediatric neurosurgeons. Thousands of people in the U.S. live with CSF shunts, and the need for an effective, non-surgical treatment cannot be over-emphasized. Avoiding the necessity of shunt placement and shunt malfunction would also decrease the burden on the military health

system by decreasing the number of military Service personnel and their family members who require access to immediate neurosurgical care for shunts. Worldwide, most people do not have access to timely, safe neurosurgery, and shunts. They suffer and die from lack of treatment. Thus, medical treatment to prevent hydrocephalus could save tens of thousands of lives a year and is applicable to active duty and retired military personnel and their families as well as the civilian population.

PROJECT 1: Comprehensive Drug Testing

Project Leaders: Bonnie Blazer-Yost, Ph.D., Shenandoah Robinson, M.D., Lauren Jantzie, Ph.D., Teri Belecky-Adams, Ph.D.

In Project 1, all of the Project Leaders will collaborate to test of the potential drugs of interest (Transient Receptor Potential vanilloid 4 [TRPV4] antagonists and erythropoietin [EPO] plus melatonin [MLT]) individually and in combination (triple therapy) in two types of rat and mice models. The two types are acquired and genetic preclinical models of hydrocephalus. Acquired means that the animals are not born with the disease but acquire it by some injury such as traumatic brain injury, stroke, or infection. Genetic means that the animals have a defect that they are born with that causes the hydrocephalus. In the human population, genetic defects that cause hydrocephalus are rare. However, the genetic animal models are useful for experimental studies because they can tell the scientists a lot about the development of the disease because the genetic defect is known.

Based on our preliminary data, we hypothesize that many of the changes that take place in the brains of humans or animals with hydrocephalus are the same regardless of the type of hydrocephalus or its initial cause. We arrived at this hypothesis because many of the symptoms and side effects of the disease are similar regardless of cause. It is the similarities that we are attempting to understand in order to ultimately treat multiple forms of the disease. Some examples of the similarities in the brain are imbalance in fluid and electrolytes and the presence of inflammatory responses, both of which we are targeting in our studies.

In Project 1, we will systematically test all individual drugs and drug combinations in all models where they have not been previously tested. This will provide important information for all investigators so they can hone their studies to the most efficacious treatments in responding models. The teams at Indiana University (IU) will conduct the treatment of two different genetic models; the teams at Johns Hopkins will conduct the treatment on two acquired models, post-traumatic hydrocephalus (PTH) and post-hemorrhagic hydrocephalus (PHH). All the models will be analyzed by magnetic resonance imaging (MRI) at the Johns Hopkins.

Because of the importance of inflammation, we will also collect cerebrospinal fluid (CSF) and blood and look for chemicals called cytokines to determine if these are increased. There are many types of cytokines, some cause inflammation and some seem to prevent it. We will conduct an in-depth analysis of the identity of the cytokines that are increased in each of the types of hydrocephalus. This can provide important information about the inflammatory response and even the biochemical pathways involved in the response. If similar cytokines are found in multiple models, these analyses will substantiate our hypothesis that all forms of hydrocephalus have similar types of pathological changes in the brain and will provide additional targets for drug development.

At the conclusion of these studies, we will have accomplished the most comprehensive comparison of models of hydrocephalus that has been completed by a collaborative research group to date. The data will not only inform our subsequent studies but will be a substantial addition to the scientific literature regarding the characteristics and similarities of various models.

PROJECT 2: In Vivo and In Vitro Studies of Components of Fluid/Electrolyte Balance in the Choroid Plexus Epithelium and Astrocytes

Project Leader: Bonnie L. Blazer-Yost, Ph.D., Fellow of the American Physiological Society, Professor of Biology, Indiana University–Purdue University Indianapolis with joint appointment in Anatomy, Physiology and Cell Biology, Indiana University School of Medicine.

In Project 2, we will conduct in-depth studies using both a genetic rat model and an acquired rat model of post-hemorrhagic hydrocephalus (PHH). We will also perform studies in an immortalized cell line of the choroid plexus epithelial cells. This cell line is of human origin and represents the cells in the brain that are responsible for the production of cerebrospinal fluid (CSF).

Salt and water balance are exquisitely sensitive to factors to that are secreted in response to trauma, injury, and pressure – notably signaling molecules called cytokines and other inflammatory mediators. Defining the cellular mediators involved, the site of production and the site of action are all important components of rational drug development.

There are two major parts to Project 2. First, we will look at changes in the amounts and cellular expression of two proteins that are known to be involved in salt and water balance in the brain. Both the native choroid plexus inside the brain and the isolated cell line contain a protein called transient receptor potential vanilloid 4 or TRPV4 for short. This is a protein that allows electrolytes like sodium and calcium to enter the choroid plexus cells. We have found that a compound that blocks the activity of this channel protein called a TRPV4 antagonist prevents the development of hydrocephalus in a rodent model and, therefore, these TRPV4 antagonists are one of the compounds we are studying as part of our therapeutic cocktail to treat hydrocephalus. Aquaporins are proteins that actually transport water and are found in several different types of cells in the brain. They too play an important role in salt and water balance in various parts of the brain. Changes in the amount of cellular localization of TRPV4 and aquaporins will be explored during hydrocephalic development and subsequent drug treatment with TRPV4 antagonists, erythropoietin plus melatonin (EPO+MLT) treatment or a cocktail of the three drugs in a genetic model and, for comparison, the PHH model. The other aspect of this project is to use a human choroid plexus cell line to study potential cytokine production by the choroid plexus epithelium, which also produces CSF and then to study the effect of cytokines on salt and water balance controlled by the choroid plexus epithelial cells and the effect of our proposed drug treatment on this balance.

In the brain, the choroid plexus that produces the CSF is a complex tissue. Cell lines provide isolated cells, which are ideal for mechanistic studies from biochemical pathway analysis to drug testing. We have previously used a cell line to study the effect of cytokines and other inflammatory mediators on TRPV4-stimulated movement of salt across the cells. This movement is important because when the body needs to produce a fluid like CSF, it moves salts and water followed by osmosis. TRPV4 activation stimulates salt movement into the CSF. Using the cell line and a technique called electrophysiology, we found that select pro-inflammatory cytokines modulate the TRPV4-mediated changes in salt movement. In addition, by an unknown mechanism, TRPV4 seems to be involved in cytokine production. We will use the isolated cells to study both the production of cytokines by the choroid plexus cells and their responses to those cytokines. We will also use electrophysiology to determine how the other two components of our proposed therapeutic cocktail, EPO+MLT, alter the responses of the isolated cells to salt movement stimulated by TRPV4 activation.

In summary, the outcomes of the experiments in Project 2 contribute to the preclinical studies necessary to establish TRPV4 antagonists in conjunction with EPO and MLT as viable components of drug therapy for hydrocephalus and to characterize the multiple effects that these compounds have in the brain. The studies will use both animal models and an established cultured cell line to examine important proteins involved in salt and water balance in the brain. The studies will also explore the site of production of cytokines as well as their effect on the cells that produce CSF. The studies are meant to provide depth to our understanding of the mechanism of action of the drugs in the brain but also to set the stage for a triple therapy that can be used to treat multiple forms of hydrocephalus.

PROJECT 3: Preclinical Studies of Traumatic Brain Injury Leading to Post-Traumatic Hydrocephalus and Chronic Pain

Project Leader: Shenandoah Robinson, M.D., Professor, Departments of Neurosurgery, Neurology and Pediatrics, Johns Hopkins University School of Medicine.

Hydrocephalus, or “water on the brain,” describes the accumulation of cerebrospinal fluid (CSF) within the brain. Hydrocephalus can arise from many different causes and affects people across the lifespan from newborns to older adults. The most common cause of hydrocephalus that affects military Service Members and Veterans is post-traumatic hydrocephalus (PTH), which typically arises a few weeks after a moderate to severe traumatic brain injury (TBI). If too much CSF collects on the brain, then a person can develop symptoms from the increased pressure in the head and can even die. Currently, the only effective intervention for hydrocephalus is brain surgery.

The most common form of surgical treatment is a CSF shunt to divert the excess CSF from the brain to another body cavity for absorption. After insertion, shunts are prone to malfunction or infection at any time over the lifespan. Additional surgery is often required and is common. Thus, an individual with a shunt must live reasonably close to a hospital with neurosurgical care available at all times. Additionally, the person is always at risk for the family and job disruption caused by shunt problems, and medical complications associated with urgent surgeries and long hospitalizations. Thousands of people in the United States live with CSF shunts, and the need for an effective, non-surgical treatment cannot be over-emphasized. Worldwide, most people do not have access to timely, safe neurosurgery and shunts. They suffer and die from lack of treatment. Thus, medical treatment to prevent hydrocephalus could save thousands of lives a year.

For this proposal, we combine our experience from clinical practice and our scientific laboratories. Data supports that inflammation in the brain is a major contributing factor to the development of hydrocephalus and its side effects. Specifically, we will focus on how inflammation catalyzes the development of PTH after a TBI. Using clinically relevant animal models, we will discover the connection between TBI and changes to specialized cells in the brain that make CSF, drive CSF flow, and ensure CSF drainage and reabsorption system. We will then test how PTH changes the response to sensory stimuli, the development of chronic pain, and alters brain circuits responsible for perceiving pain. We will use specialized pain assessments and look at brain cells that are part of pain networks using specific types of brain imaging, including magnetic resonance imaging (MRI) and microscopy. Finally, we will test how medications known to reduce inflammation enhance recovery after hydrocephalus. These medications are called erythropoietin, melatonin, and a transient receptor potential vanilloid 4 (TRPV4) channel blocker. Erythropoietin and melatonin are natural brain hormones, and TRPV4 channels are important to regulating salt and water flow through the brain. Together with our collaborators in Projects 1, 3, 4, and 5, we test how these medications when used individually as a single drug, or as a combination therapy, normalize the brain environment and help optimize CSF flow and reabsorption. We expect that using these medications can reduce injury to the entire brain, including those parts in control of sensing and transmitting pain signal, executive function, and memory.

In summary, completion of these studies will transform how we approach the treatment of hydrocephalus acquired from brain insults such as trauma, alleviate chronic pain, and improve the lives of thousands of military Service Members, Veterans, their family members affected by hydrocephalus, and as well as civilians. Our goal is to develop a medical drug treatment to prevent people from developing hydrocephalus after brain injury.

PROJECT 4: Chronic Inflammation and Functional Recovery After Posthemorrhagic Hydrocephalus

Project Leader: Lauren Jantzie, Ph.D., Associate Professor, Departments of Pediatrics, Neurology and Neurosurgery, Johns Hopkins University School of Medicine.

Hydrocephalus, or the collection of cerebrospinal fluid (CSF) in the brain, most commonly occurs in children and adults after a brain bleed. This bleeding and fluid buildup can be extremely serious and life threatening. Unfortunately, the only way to treat hydrocephalus is to alleviate pressure on the brain by inserting a medical device called a shunt. This requires neurosurgery and complex, expensive medical care. Shunts are not a perfect treatment or cure for hydrocephalus. They are unreliable and can sometimes break or become infected. This is not desirable for any person, let alone military Service Members, Veterans, or their beneficiaries. Accordingly, in this project, and in all the other projects part of this collaborative, our team is dedicated to discovering a safe, medical treatment for hydrocephalus. One that does not rely on surgery, but rather, is based in treatment with existing pharmaceutical therapies.

Brain bleeds can happen to any person and at any time in life. We know that inflammation during pregnancy can predispose a baby born too early, or prematurely, to a specific kind of hydrocephalus. Similarly, in adults, a serious infection throughout the body called sepsis can accelerate the transformation of a brain hemorrhage to hydrocephalus. Here, we will use this knowledge to understand how blood products breaking down in the brain cause inflammation. We will focus on how certain blood metabolites activate the immune system in the brain and the body. We will also study how immune system activation changes the circulation of CSF in the brain.

As part of our project, we predict that drugs that are anti-inflammatory may be beneficial in treating hydrocephalus occurring after a brain bleed. Erythropoietin (EPO) and melatonin (MLT) are naturally occurring, anti-inflammatory hormones that we will test as non-surgical therapies. We will try EPO and MLT alone and together as part of a drug cocktail with a TRPV4 antagonist, or a medication that changes CSF production and balance in the brain. We expect that this combination of drugs will decrease toxic factors in the brain and increase protective ones, thereby preventing the development of hydrocephalus after a brain hemorrhage.

Military Service Members, Veterans, their beneficiaries, and civilians desperately need a safe therapy for hydrocephalus that does not involve neurosurgery or complex medical care. The personal, familial, health care, and societal cost of hydrocephalus is immense. Thus, we designed this project to addresses multiple areas in Neuroscience, Hydrocephalus, Trauma and Treatment Topic Areas and Strategic Goals. Specifically, research on a non-surgical treatment would reduce the need for shunt revision surgery. The drugs that we are testing are also designed to address at the complications of hydrocephalus, including the brain fog, memory problems, thinking difficulties, and pain that commonly occur with hydrocephalus after a brain bleed.

In conclusion our team, and all the investigators in this collaborative, are devoted to transforming the care of people with hydrocephalus and improving science, medicine, and lives by discovering a safe, medical treatment for all forms of hydrocephalus.

PROJECT 5: Vision and Sleep Disturbances in Hydrocephalus

Project Leader: Teri Belecky-Adams, Ph.D., Associate Professor, Department of Biology and Director of the Neuroscience Program, Indiana University–Purdue University Indianapolis.

Just as in hydrocephalus, blinding disorders can be inherited, acquired from injuries that occur during a person’s lifetime or a combination of both. In the proposed work, my laboratory in collaboration with the three other laboratories, will be examining the effects of hydrocephalus on vision and the sleep/wake cycle. As has been pointed out by my colleagues, hydrocephalus refers to a group of conditions wherein there is an increase in the amount of fluid that surrounds your brain. This fluid provides nutrients, acts like a cushion for the brain, and removes toxins and waste products from the brain tissue. When there is too much of this fluid, it creates pressure on the tissues as well as makes it difficult for the fluid to move around and perform its normal functions.

Many patients that have hydrocephalus have problems with their vision, including double vision, blurriness, and loss of vision. Loss of vision can lead to a loss of the light signal that is sent to the brain to control the sleep/wake cycle, resulting in sleep disturbances. The loss of vision results from the increased pressure of the liquid surrounding the brain. In case of the visual system, increased pressure occurs on the optic nerve, where nerve cells send processes to connect with other regions of the brain. In fact, the pressure is so great that it actually triggers swelling in the eye. This condition is known as papilledema and can be a highly destructive process if not addressed right away.

The papilledema occurs at a region of the eye, the optic disc, that is particularly vulnerable to injury. In addition to the cell processes that carry information out of the eye, the optic disc also has blood vessels that move into or out of the eye. All of these are injured when papilledema is not treated immediately. The only way to treat papilledema right now is by surgical means, either by placing a shunt to drain excess CSF or by surgically poking holes into the area surrounding the optic nerve to release some of the pressure. This is costly, time consuming, and, of course, much more dangerous than if there were a pharmaceutical treatment for reducing the pressure. Furthermore, for those serving in the military, there might not be access to surgical treatments immediately. Moreover, even if a hospital is available, for traumatic brain injury, early intervention may be key to survival and better recovery. Hence, the driving force for this proposal is to find animal models that will (1) allow scientists to test safe and effective pharmaceutical treatments and (2) accurately depict the disease process of hydrocephalus and papilledema, so that we mechanistic studies can be performed. In the end, determining the mechanism of a disease will lead to more effective treatments.

We will also be testing whether the loss of vision might also be tied to disturbances to sleep. The brain uses cues from the visual system in order to synchronize with the day/night cycle. When some of the neurons necessary for communicating that there is light to the brain are lost, or part of the brain the receives that information is damaged, patients will have sleep disturbances. My laboratory will determine if loss of vision or a loss of the part of the brain that gets light cues has been damaged by hydrocephalus and determine if the proposed treatments will alleviate the sleep disturbances.

Until now, there have been few model systems that accurately reflect the disease condition of papilledema. Teaming up with the investigators in this proposal will allow us to investigate more closely what occurs in eyes of each model system so that we can offer these models to the vision community. We will also be using the models to determine whether the proposed treatment will also alleviate papilledema, thus reducing the loss of vision and potentially sleep disturbance experienced by so many patients with hydrocephalus.

Proposal Title:	Preclinical Testing of a TRPV4 Antagonist for the Treatment of Hydrocephalus in a Porcine Model
Log Number:	PR221811
Current PI Name:	Bonnie Blazer-Yost
Award Number:	HT9425-23-1-0401
Current Contracting Organization:	Indiana University
Current Performing Organization:	Indiana University
Web Approval Date:	04-30-2023

The focus of our proposed research is to develop drugs that can be used to treat hydrocephalus. Hydrocephalus is a general term for excess cerebrospinal fluid (CSF) in the brain; babies can be born with this or it can happen because of conditions such as traumatic brain injury (TBI), infection, or hemorrhage. If too much CSF collects in the brain, a person can develop symptoms from the increased pressure in the head, with pain, vision problems, walking abnormalities, and cognitive changes that, if left untreated, can result in death. Currently, the only effective intervention for hydrocephalus is brain surgery. Shunts, the most common treatment, require that a tube is implanted in the brain in order to drain the excess CSF to another part of the body. After insertion, shunts are prone to malfunction or infection at any time over the lifespan, and additional surgery is often required. Thus, an individual with a shunt must live reasonably close to a hospital with neurosurgical care available at all times.

There are no long-term, effective drugs to treat hydrocephalus, and this represents a large, world-wide medical need. We are addressing this need by conducting studies to try to find safe and effective compounds that can be developed as drugs to treat all forms of hydrocephalus.

Our challenge, therefore, is to discover versatile, safe drugs for use on the battlefield, athletic facility or in geriatric and pediatric clinical wards. To address this challenge, we have identified a type of compound, called TRPV4 antagonists, that are effective in treating hydrocephalus in several rat models of hydrocephalus. The next step in the progression to treatment in humans is to test the compounds in a large animal model. We have formed a collaboration with a scientist who developed a pig model of hydrocephalus due to brain bleeds. We will use this model to test whether a form of TRPV4 antagonists that can be taken by mouth decreases their hydrocephalus.

Relevance to Topic Areas: The proposed studies are applicable to the Neurosciences Portfolio Category and primarily to the Hydrocephalus Topic Area. Since it is our hypothesis that TRPV4 antagonists will be effective for the treatment of multiple forms of hydrocephalus, these studies are also applicable to the Topic Area of Trauma since many trauma patients experience post-traumatic hydrocephalus. The proposed studies address two of the Strategic Goals, namely, to conduct Foundational Studies and also the Treatment Goal due to the translational nature of the proposed preclinical studies.

Applicability and Impact of the Research: People of all ages are at risk for hydrocephalus, including military Service Members. Traumatic brain injuries suffered by our military personnel, especially those who have served in Iraq and Afghanistan, have increased the numbers of those living with post-traumatic hydrocephalus (PTH). The incidence of PTH has been reported to be as high as 86% of severe head injury patients. Injured Soldiers treated in the field may not have timely access to neurosurgical care and advanced diagnostic testing, leading to under-reporting of the condition. Even if the hydrocephalus resolves in time, there may be long-lasting effects such as pain, vision changes, walking problems, and even personality changes.

Retired military personnel are more likely to be at risk for hydrocephalus that develops after a stroke or from a poorly understood condition called idiopathic normal pressure hydrocephalus. The latter affects the elderly with symptoms that mimic other neurodegenerative diseases such as Alzheimer's and Parkinson disease. Young military families are more likely to be affected by childhood forms of the disease, most commonly caused by "brain bleeds" in premature births, spina bifida, or trauma, all which result in chronic hydrocephalus. Civilian populations of similar ages are also affected by the multiple forms of hydrocephalus.

In summary, the proposed studies, if successful, would have the long-term outcome of developing a drug to treat hydrocephalus. It is our hypothesis, based on our research results to date, that this type of drug would be useful in treating multiple types of hydrocephalus. The proposed experiments build on our previous research and represent the next steps in drug development. The final outcome would be a better quality of life for both military and civilian personnel who have hydrocephalus.

Proposal Title:	Dual Orexin Antagonist Treatment to Prevent the Neurobehavioral Sequelae of TBI
Log Number:	PR221869
Current PI Name:	Rama Maganti
Award Number:	HT9425-23-1-0200
Current Contracting Organization:	Wisconsin, University of, Madison
Current Performing Organization:	Wisconsin, University of, Madison
Web Approval Date:	03-23-2023

Traumatic brain injury (TBI) is a common problem in civilians and military personnel alike, with over 1.5 million new cases each year and over 5 million people living in the U.S. currently with TBI-related disability. Consequences of TBI can not only lead to disability but also cause a societal and economic toll. TBI leads to neurological consequences including sleep disturbances, posttraumatic epilepsy (PTE), cognitive impairment as well as mood and anxiety disorders (depression and posttraumatic stress disorder - PTSD), which we collectively termed “neurobehavioral sequelae.” All the sequelae are much more common in military personnel compared to civilians. Moreover, all the sequelae are interrelated, as sleep and epilepsy as well as sleep and mood disorders have a bidirectional relationship where they exacerbate each other. Similarly, cognitive disturbances are common after TBI but also common due to epilepsy as well as mood and anxiety disorders. A major gap in clinical care and research is lack of a complete understanding into mechanisms and molecular targets for treatment of sequelae of TBI. The long-term vision of this application to foster preventive therapies for the sequelae of TBI.

In the prior study, we hypothesized that treatment with sleep aids immediately after TBI will restore sleep disturbances and prevent posttraumatic epilepsy. We recorded EEG (electroencephalogram) for sleep-wake patterns and seizures periodically for 3 months during and after a month-long treatment with two sleep aids. The first acts on GABAA receptors in the brain (THIP) and a second acts as a blocker of the receptors of a brain hormone orexin, which is responsible for maintaining wakefulness and vigilance during the day (dual orexin receptor antagonist or DORA). We found that TBI resulted in sleep disturbances and PTE. Contrary to our hypothesis, treatment with DORA but not THIP, restored the sleep homeostatic process but also suppressed PTE. In a mechanistic study in brain slices, we found that TBI impaired GABAergic inhibition (a balance of excitation and inhibition is required normally) in a brain region important for seizures (dentate gyrus) and DORA treatment restored it. We also found phenotypes of depression and PTSD in mice that were subjected to TBI compared to sham injury. The experimental design for the current proposal was generated from this prior work in a Peer Reviewed Medical Research Program (PRMRP) Idea Development Award (PR161864).

Based on the above data, in this PRMRP Expansion Award application, we will perform a confirmatory preclinical study where, following TBI, we will determine if chronic treatment with a sleep aid DORA will mitigate the development of some or all the neurobehavioral sequelae. We will test the hypothesis in a mouse model, where following TBI or sham injury, mice will be treated daily with DORA for 2 months while we perform EEG recordings for sleep-wake pattern and seizure analysis. Towards the end of the treatment, the same animals will undergo behavioral studies for depression, PTSD, and learning/memory. At the end of 2-month-long treatment, animals will be sacrificed for recordings in brain slices from regions that are relevant for sleep disturbances, epilepsy, and mood/anxiety disorders to understand the mechanisms. To further establish the hypothesis, we will also perform comparative study where we will examine sleep parameters, seizures, and behavioral tests after treatment with a drug that activates orexin receptors in the brain (Orexin agonist).

The work in this PRMRP Expansion Award is novel, using comprehensive methodology that can foster a clinical trial in humans with TBI to repurpose currently U.S. Food and Drug Administration (FDA)-approved sleep aids (orexin receptor 1 and 2 or dual orexin antagonists) as a novel therapy. The data from this preclinical study can support clinical studies in military personnel, Veterans, as well as civilians. Preventing the neurobehavioral sequelae can reduce TBI-associated disability as well as health care utilization. In military personnel, a neuroprotective therapy could improve mental resilience in occupational environments that require vigilance upon return to duty after a TBI.

This PRMRP Expansion Award application addresses the Fiscal Year 2022 (FY22) Topic Area of Sleep Disorders. It also addresses several of the strategic areas listed in FY22 program announcement including: (a) identify mechanisms underlying neurological diseases and psychological conditions associated with TBI; (b) develop early intervention strategies; (c) improve objective biomarkers for the association of sleep disturbances to other consequences of TBI and (d) develop novel therapeutic strategy by repurposing currently FDA-approved drugs. Our strategy will not only be effective for military personnel and Veterans and also civilians, but also improve their quality of life as well as to reduce long-term health care utilization.

Proposal Title:	Improving Surgical Outcomes with Early Physical Therapy After Anterior Cervical Discectomy and Fusion
Log Number:	PR221916
Current PI Name:	Kristin Archer
Award Number:	HT9425-23-1-0300
Current Contracting Organization:	Vanderbilt University Medical Center
Current Performing Organization:	Vanderbilt University Medical Center
Web Approval Date:	04-04-2023

Anterior cervical discectomy and fusion (ACDF) is the most common surgery for cervical spine degenerative conditions at both civilian and military health centers. Over the last three decades, there has been a substantial increase in the rate and associated costs of cervical spine surgery. Despite the increased utilization, up to 40% of patients report persistent pain and disability as well as dissatisfaction following ACDF surgery, and up to 20% of patients in the military have difficulty returning to duty and meeting criteria for a successful outcome. Opioid use has also been found to be high with up to 20% of patients reporting chronic opioid use at 1 year after cervical spine surgery. Physical therapy (PT) has been found to be beneficial for improving pain, disability, and function in patients with chronic neck pain. In particular, specific exercises that strengthen the deep neck muscles and improve neck mobility appear to be effective for reducing long-term pain and disability. While research efforts have found evidence for the benefits of PT in patients with chronic neck pain, little work has been done to study the effects of PT after cervical spine surgery. Patients with chronic neck pain have been found to have moderate to severe muscle and range of motion (ROM) deficits prior to surgery, which then progress during the acute postoperative period. Early PT has the potential to address these deficits and halt or slow the progression of muscle degeneration. Therefore, it is critical that rehabilitation specialists have an evidence-based PT program that can be delivered after ACDF, especially during the early postoperative period, in order to improve outcomes that are relevant and meaningful to both civilians and Service Members.

This 4-year application is directly aligned with the Fiscal Year 2022 Peer Reviewed Medical Research Program Topic Area of Musculoskeletal Disorders and the Strategic Goal for this topic focused on the development and testing of strategies to increase quality of life or halt/slow disease progression for associated musculoskeletal disorders. The purpose of our proposed multicenter randomized controlled clinical trial is to understand the benefits of early postoperative PT in patients undergoing ACDF surgery at civilian and military health centers. PT within the first 3 months of spine surgery for chronic neck and/or arm pain has the potential to improve short- and long-term outcomes related to disability, pain, function, opioid utilization, and military readiness. In addition, attending early postoperative PT may increase muscle strength and endurance and neck ROM, which will help halt/slow the progression of muscle degeneration and deficits found in this patient population after surgery.

This study will include 76 patients with a cervical degenerative condition, such as cervical stenosis, spondylosis, and degenerative spondylolisthesis, that undergo a 1- or 2-level ACDF procedure. Patients will be recruited from a civilian academic medical center and two military treatment facilities and randomized to either (1) early postoperative PT (2 weeks – 10 weeks after ACDF) or (2) delayed postoperative PT (12 weeks – 20 weeks after ACDF). The first year of the project will focus on finalizing study procedures and the PT interventions, the second and third years will involve patient recruitment and delivering the PT interventions, and the fourth year will focus on collecting patient outcomes up to 1 year after ACDF surgery. Results from the proposed study will provide an evidence-based PT intervention focused on specific neck exercises that can be immediately integrated into rehabilitation care in the civilian and military setting. This study will also provide critical data on the timing of PT after spine surgery for chronic neck and/or arm pain.

Our structured PT program with home exercises will provide individuals with a greater chance of restoring function, returning to work/duty, and participating in social roles. In the long term, our PT intervention lays the groundwork for targeted PT programs that can be delivered safely after surgical intervention and provide providers and patients with a non-pharmacologic treatment option.

Proposal Title:	Clinical Study of Sustained-Release Implant for Trauma Repair
Log Number:	PR221938
Current PI Name:	Luis Alvarez
Award Number:	HT9425-23-1-0693
Current Contracting Organization:	Theradaptive, Inc.
Current Performing Organization:	Theradaptive, Inc.
Web Approval Date:	10-03-2023

This work directly addresses the Peer Reviewed Medical Research Program (PRMRP) Topic Area Orthopaedic Medicine - Sustained Release Drug Delivery. We specifically address the following PRMRP Strategic Goals:

Prevention - Develop strategies for improved care at point of injury to prevent musculoskeletal disorder onset

Treatment - Develop and test strategies to increase quality of life or halt/slow disease progression, including regenerative medicine approaches and biologics for associated musculoskeletal disorders.

Our work targets the treatment of spinal disc degeneration that commonly occurs as a result of injury or trauma from training or combat. Spinal disc degeneration can also be non-impact related, and it affects a large number of active military and veterans. The disc degeneration condition is painful and can be treated vertebral spinal fusion. By improving the success rate and enabling faster fusion rates for spinal fusions, it could be possible to increase the readiness to deploy of active military affected by this pathology. Bone morphogenetic protein-2 (BMP2) is the most potent bone-inducing agent identified to date. However, its use has suffered setbacks due to serious safety concerns. We have developed a variant of BMP2 called AMP2 that binds very tightly to implant materials. Using this technology, we have created resorbable implants that are surface-coated with AMP2 that retain the biologic at the implant site over the extended period of time required to achieve a lasting therapeutic effect. This permits precise delivery of bone-healing bioactivity that eliminates the risk of off-target effects. The release of AMP2 from the implant is synchronized with implant resorption, thus producing robust bone formation. We used this technology to develop a product called OsteoAdapt SP, the first biological device of its kind. We have validated OsteoAdapt SP in models of long-bone repair in rodents, goats, and sheep (vertebral interbody application) and demonstrated superiority over the current best treatments available.

Objective: Our objective is to complete Feasibility clinical studies of OsteoAdapt SP to demonstrate safety for utilization in single level TLIF spinal fusion procedures. This will enable Pivotal clinical studies in order to gain U.S. Food and Drug Administration approval with the goal of making this treatment available to Service Members in need.

Clinical Impact and Relevance to Military Health: The single biggest impact of our product is to increase readiness and accelerate return to duty. The immediate impact it can offer to patients and healthcare providers is a faster bone healing rate, resolution of degenerative disc disease (DDD), higher resolution of non-fusions, shorter length of stay, lower infection rates and better quality of life. The long-term impact is a significant cost saving for the entire healthcare system. Patients with non-healing bone injuries ("non-unions") or with lower back pain from DDD have been found to be more likely to use various types of surgical care, inpatient care and outpatient physical therapy than those without non-unions. Non-Unions and DDD surgery often require revision surgeries to achieve fusion if they fuse at all, resulting in lifelong pain and disability. In addition, patients are much more likely to be prescribed pain medications, especially strong

opioids and had longer length of opioid therapy than patients without non-union. Improved methods to heal critical fractures and degenerative disc disease by promoting active bone regeneration will produce better outcomes and improve the health of our Service Members and Veterans.

Proposal Title:	Pivotal and Diverse Roles for Matricellular Proteins in Musculoskeletal Disorders
Log Number:	PR222005
Current PI Name:	Kurt Hankenson
Award Number:	HT9425-23-1-0327
Current Contracting Organization:	Michigan, University of
Current Performing Organization:	Michigan, University of
Web Approval Date:	05-01-2023

OVERALL PROGRAM

Topic Area and Strategic Goals:

This proposal addresses the Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Areas: “Orthopaedic Medicine: Musculoskeletal Disorders (related to acute and chronic bone conditions and injuries) and “Arthritis.” It also addresses the following four FY22 PRMRP Strategic Goals: (1) understand mechanisms underlying the pathobiology of associated musculoskeletal disorders; (2) develop strategies for improved care at point of injury to prevent musculoskeletal disorder onset; (3) develop and test novel and improved intra-articular treatments for joint injuries; and (4) develop and test strategies to increase quality of life or halt/slow disease progression, including regenerative medicine approaches and biologics for musculoskeletal disorders.

Background: Injuries to the arms and legs – the extremities – are very common for our military personnel and civilians. Indeed, these extremity injuries are the most common battlefield injuries and also occur in non-deployed military personnel during training. Most extremity injuries affect the musculoskeletal system. These injuries are painful, result in loss of function and use, and result in expensive medical costs. Our project will investigate four different types of musculoskeletal disorders: (1) bone injury (fracture), (2) amputation (the loss of digits or limbs), (3) joint injury eventually causing degenerative joint disease also known as arthritis, and (4) the abnormal formation of bone in soft tissue following trauma (a disease condition called heterotopic ossification). These conditions affect military and civilian in the short-term (acute) immediately following injuries and over a much longer time course, spanning decades or a lifetime (chronic).

Summary of Proposed Studies: Our goal is to study the role that certain molecules – proteins – play in the progression of these various disorders. The molecules we will study are called “thrombospondins.” There are two molecules – thrombospondin 1 and thrombospondin 2 – that are “siblings.” They are very similar in their structure, but interestingly, the proteins are produced by different types of cells and are present during different periods of healing. In the first aim of this study, we plan to dissect how these two molecules function differently in these various injury conditions by studying mice that have been genetically engineered to lack either one or both of these proteins in certain cell types only at certain times post-injury (conditional modification). We will use the most advanced innovative nucleic acid sequencing techniques to explore the specific cells that express the proteins and the impact that the absence of either thrombospondin 1 or 2 genetically has on the injury responses. Additionally, we will examine a suite of new drug therapies that target the function of the thrombospondins by either preventing their production or by activating or blocking their interactions with other proteins.

The Program includes scientists from the University of Michigan, the University of Texas Southwestern (UTSW), and Vanderbilt University. In addition to the four projects focused on the aforementioned

disorders, we also will develop two research hubs (one at Vanderbilt and one at UTSW) to enable the success of our research.

Impact: Our study will provide an in-depth new understanding of traumatic injury responses. By studying thrombospondins, we will gain new insights into how to enhance bone healing, promote the regrowth (regeneration) of amputated digits and limbs, prevent the development of arthritis, and prevent the formation of bone that forms in soft tissue. Ultimately, we will characterize a novel set of therapies. These will be validated in mouse models, and at the end of the study we will be ready to take therapeutics to next steps in the process of drug and pharmaceutical development.

PROJECT 1

Topic Area and Strategic Goals: The Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Topic Area addressed by Project 1 is Orthopaedic Medicine: Musculoskeletal Disorders (related to acute and chronic bone conditions and injuries). The FY22 PRMRP Strategic Goals addressed by this proposal are: (1) understand mechanisms underlying the pathobiology of associated musculoskeletal disorders; (2) develop strategies for improved care at point of injury to prevent musculoskeletal disorder onset; and (3) develop and test strategies to increase quality of life or halt/slow disease progression, including regenerative medicine approaches and biologics for musculoskeletal disorders.

Background: Bone fractures are common for both military and non-military individuals. Injuries to the arms and legs represent over one-half of all combat-related injuries, and in Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF), bone injuries (fractures) represented 26% of all the injuries to legs and arms. Beyond our soldiers in the military, long-standing problems because of bone injuries affect hundreds of thousands of Americans each year and cost billions of dollars. Ten to fifteen percent of fractures fail to heal with initial surgical treatment, which adds tremendously to the burden of these injuries. Additional personal psychosocial “costs” associated with reduced employment and mobility are harder to calculate but are surely staggering.

To date, there has only been a single drug approved by the U.S. Food and Drug Administration for promoting repair of leg bone fractures. That drug, bone morphogenetic protein (BMP) is potent, but it can only be delivered surgically, not all patients respond, and it can cause excess unregulated bone production, at abnormal sites, termed heterotopic ossification (which is being studied in Project 4). Thus, there is a significant unmet clinical need to develop new fracture repair drugs.

Our work has shown that a particular family of molecules, proteins, termed thrombospondins (TSP), specifically, TSP 1 and 2, are among the most upregulated genes expressed following bone injury. In mice that have been genetically modified to have no TSP2, there is enhanced bone repair. TSP1 is abundant immediately after injury, and our data suggest that it facilitates formation of the fracture callus, which provides a template for new bone formation during the final phases of fracture repair. TSP2 expression peaks at ~day 7 when blood vessels invade the fracture callus. TSP1 is also abundant during this phase, and both proteins inhibit new blood vessel formation and proliferation of the cells that form cartilage and bone. These variable roles for TSP1 and TSP2, based on when they are present in the callus, need to be better understood. The overall goal of this project is to leverage the positive roles of TSP1 during the immediate/early phrases of fracture healing, and to limit the antiangiogenic and antiproliferative functions of TSP1/2 during the intermediate and final phases of healing.

Summary of Proposed Studies: In Aim 1, we will study fracture healing in mice where production of TSP1, TSP2, or both can be inhibited with temporal and spatial control. These experiments will allow us to more fully understand the contributions that TSP1 makes during the early events of fracture healing and to determine the degree to which TSP1/2 limit blood vessel formation and new bone growth later in the healing process. In Aim 2, we will test new drugs that target TSP1 and TSP2. Specifically, we will study fracture healing in mice administered TSP1 activating molecules at the time of injury to promote callus formation

and in mice administered TSP1/2 inhibitors after the fracture callus has begun to develop to allow enhanced blood vessel formation and bone formation. In contrast to BMP, these drug formulations can be injected and do not need to be surgically applied directly to the fractured bone.

Impact: Successful completion of Project 1 will demonstrate that targeted manipulation of TSP1/TSP2 biology is a viable therapy for enhanced fracture repair. We believe that the targeting of modulatory proteins, such as the thrombospondins can enhance healing without the deleterious effects of growth factor delivery strategies and that modifying modulatory proteins is more suitable for systemic targeting.

PROJECT 2

Topic Area and Strategic Goals:

This project addresses the needs outlined in the Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP)Strategic Goals Address by this proposal: (1) understand mechanisms underlying the pathobiology of associated musculoskeletal disorders; (2) develop strategies for improved care at point of injury to prevent musculoskeletal disorder onset; and (3) develop and test strategies to increase quality of life or halt/slow disease progression including regenerative medicine approaches and bio- logics for musculoskeletal disorders (limb regeneration).

Background: Although many studies are aimed at improving bone healing, no major breakthroughs have led to regeneration – or regrowth – of lost tissue after amputation. Musculoskeletal injuries represent one of the most common forms of trauma observed on the battlefield. While previously fatal, improved battlefield care now means more traumatic forms of injury, especially extremity trauma, can be survived. As a result, over two million individuals are living with limb loss in the United States. Beyond the desire to promote tissue regeneration, understanding the body's own natural regrowth processes could improve acute care of patients with extremity trauma and decrease the need for amputations, and improve the long-term care and prosthetic technologies available to people living with limb loss. To reach our long-term goal of promoting skeletal regeneration, we need a more detailed and in-depth understanding of how natural limb regeneration occurs. In mice, the very tips of the toes will grow back after amputation. Our preliminary data show that two protein molecules, thrombospondin (TSP) 1/2, are highly abundant in the regenerating tissue at a critical transition point where the cells stop dividing to fill in the missing part of the toe and begin forming new bone. Thus, TSP1/2 may serve as a signal to the cells responsible for regeneration to switch to the next phase in the process, and therapeutic modulation of TSPs may serve as a promising new strategy to enhance limb regeneration. The central goal of this project is to determine the efficacy of TSP1/2 modulation to enhance the bodies inherent regenerative potential following amputation.

Summary of Proposed Studies: Two specific aims have been designed to determine the roles of TSP1/2 in regeneration using the mouse digit tip amputation model. First, using mouse models that have been genetically manipulated to eliminate the TSP1 and TSP2 genes, we will determine the functional consequences of deleting TSP1 and/or 2 from the regenerating digit. This gene deletion will be carried out at different healing phases to understand the role of TSPs throughout the regenerating process. Next, we will attempt to regulate TSP signaling therapeutically to determine whether modulation of TSP activity could serve as a potential clinical intervention to help promote skeletal tissue regrowth after injury.

Impact: Implementation of improved battlefield medicine practices has resulted in increased survival following severe musculoskeletal trauma. As a result, new methods are needed to regenerate large musculoskeletal injuries following trauma. This project aims to understand the body's intrinsic regenerative potential to promote severe injury repair and to inform future works developing engineered approaches to large-scale injuries not able to regenerate without medical interventions. While preferentially beneficial to Service men and women who suffer from severe extremity trauma, understanding the regenerative process will have broad implications in regenerative medicine for active-duty members, Veterans, and civilians alike.

PROJECT 3

Topic Area and Strategic Goals: This proposal addresses the Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP)Topic Areas: “Orthopaedic Medicine: Musculoskeletal Disorders (related to acute and chronic bone conditions and injuries) and “Arthritis.” It also addresses the following four FY22 PRMRP Strategic Goals: (1) understand mechanisms underlying the pathobiology of associated musculoskeletal disorders; (2) develop strategies for improved care at point of injury to prevent musculoskeletal disorder onset; (3) develop and test novel and improved intra-articular treatments for joint injuries; and (4) develop and test strategies to increase quality of life or halt/slow disease progression, including regenerative medicine approaches and biologics for musculoskeletal disorders.

Background: Osteoarthritis (OA) is a joint disease that causes the gradual breakdown of the tissues in joints responsible for absorbing shock necessary for normal daily activities such as walking and running. OA is one of the most common causes of disability worldwide, and it affects both the civilian and military population. In the U.S., approximately one in three adults has OA. Major joint injuries, such as knee dislocations or ligament tears, lead to a subtype of OA known as post-traumatic osteoarthritis (PTOA). PTOA patients suffer from extensive pain and disability for decades. Because of the extreme physical demands of military activities, military Service Members have a much higher rate of joint injury, and therefore, also a higher rate of PTOA development. This makes PTOA a major problem in both active military Service Members and Veterans. Unfortunately, there are still no treatments that stop or reverse the disease. Current management of PTOA involves pain killers, which don’t address the actual disease process but just dull pain, and long-term use of pain killers is known to cause kidney and liver damage. The main limitation in developing effective PTOA treatments is the absence of knowledge about what actually causes PTOA. Our studies will address this gap in knowledge by describing a set of molecules thought to regulate blood vessel formation. In the case of PTOA, the increased formation of blood vessels in the tissues of the joint is considered a negative consequence of joint injury because these blood vessels promote tissue breakdown via inflammation. Therefore, stopping the formation of blood vessels after joint injury is a possible way to treat joint injuries immediately after they occur. In order for an effective treatment that can stop blood vessel formation to be developed, more knowledge about the biological processes causing blood vessel formation is needed. Our proposal will study two proteins, thrombospondin 1 and thrombospondin 2, which, based on our data, are promising candidates as regulators of blood vessel formation and inflammation.

Summary of Proposed Studies:

In Aim 1, we will first use a mouse model of joint injury. Joint injury will be induced in mice that are genetically modified to lack either thrombospondin 1, thrombospondin 2, or both. If PTOA is more severe in these genetically modified mice, we can conclude that the thrombospondins are important in preventing PTOA, which would confirm that new drugs targeting them are a promising way to treat PTOA. Importantly, no previous study has described this. In these studies, we will also use technology to measure thousands of genes at once, allowing us to thoroughly understand the biological impact of removing thrombospondin 1 or 2 from the mice.

In Aim 2, we will test new drugs that target the biological processes involving thrombospondin 1 and 2 in their ability to slow down PTOA progression after joint injury. These new drugs will be modifications of existing drugs that have been shown to effectively target these biological processes, and our modification will enhance the drugs’ ability to reach the injured knee joint when injected into the blood stream. Getting drugs into a knee joint without directly injecting them into the joint is known to be very challenging, and we have rigorous preliminary data that shows that the modification can increase the amount of drug that reaches the knee joint.

Our experiment will test whether the drugs targeting thrombospondin 1 and 2 are effective in making PTOA less severe after joint injury. Our long-term goal is to be able to inject patients that just experienced a joint

injury with these new drugs quickly after injury so that the biological processes causing PTOA development are blocked or slowed down. This would hopefully mean that there is less severe long-term PTOA, decreasing the need for long-term pain killer use and joint replacement surgery.

Impact: As there are currently no treatments for OA/PTOA that actually stop the disease, our studies have the potential to have a large impact on OA patients by providing a more effective pain-relieving therapy while also blocking disease progression. Current patients have to receive multiple steroid injections into their joints over several years, but these injections only provide short-term pain relief and don't stop disease progression. If our studies are successful, we may be able to offer patients a therapy that not only addresses pain but also stops disease progression. This, in turn, should lead to less overall pain and disability in the long term, since PTOA patients generally have to live with this disease for several decades.

PROJECT 4

Topic Area and Strategic Goals: This project addresses the needs outlined in the Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP)Strategic Goals Address by this proposal: (1) understand mechanisms underlying the pathobiology of associated musculoskeletal disorders; (2) develop strategies for improved care at point of injury to prevent musculoskeletal disorder onset (heterotopic ossification); (3) develop and test strategies to increase quality of life or halt/slow disease progression (heterotopic ossification), including regenerative medicine approaches and biologics for musculoskeletal disorders.

Background: Over the past decade, improved personal protective equipment and medical support has reduced combat fatalities substantially among wounded Warfighters with extremity injuries. As a result, survivors are more likely to present with severe trauma to their extremities that will need multiple reconstructive surgeries or amputation during their recovery creating a substantial challenge to return to duty. Of the nearly 15,000 battle injuries suffered in Operations Iraqi Freedom/New Dawn and Enduring Freedom, over 50% of those injuries were extremity injuries. Additionally, clinicians have been concerned by the fact that over 60% of these patients go on to form abnormal bone within the soft tissue of their injured limbs. This condition, known as heterotopic ossification (HO), causes pain, loss of mobility, and often requires additional surgeries to remove the rock-hard tissue that has replaced their fat and muscle. For example, over 80% of patients with fractures and 100% of patients with revision joint replacements will develop HO. As a result of forming bone outside of the normal skeleton, HO leaves patients with severe chronic pain, open wounds, and limited range of motion. This proposal has been developed to specifically address HO formation in cases of extremity trauma such as is seen with military-related, orthopaedic, civilian, and Veteran-related injuries and surgeries. The overall goal of this proposal is to target thrombospondin signaling pathways using novel biomaterials to prevent trauma-induced HO while minimizing treatment duration to enhance recovery and return to duty.

Summary of Proposed Studies: Proper healing involves several stages that are each required but need to be resolved before the next step can occur. Immediately after injury immune cells drive inflammation, which attracts cells – called progenitor cells – that will help repair the injury. In our own studies, we find specific immune cells must be present at the injury site for regeneration to occur. These immune cells interact with factors and progenitor cells present at the injury site, causing them to become more regenerative in nature. We have designed injectable materials that can be used to inhibit the amount of TSP1/2 that cells make and others that block or activate TSP1/2 interactions with other molecules. We hypothesize that these biomaterials can be designed to program immune and progenitor cell phenotype. Furthermore, with the correct cues, we can improve tissue repair to mitigate HO.

In the short term (next 2-3 years), we hope to determine the optimal timing and gene deletion strategy to mitigate HO, including the cues necessary to alter immune and progenitor cells to promote proper

differentiation of these cells. To accomplish this, we will use a proven HO model. In the long term (3-5 years), our goal is to complete additional preclinical studies, apply for U.S. Food and Drug Administration (FDA) approval, and perform clinical studies to move this therapy into the clinic.

Impact: This proposal is directly relevant to the health care needs of military Service Members and Veterans. The experiments proposed within this application will provide proof of principle for a new approach to mitigate HO. In the long term, this approach may have additional relevance for other types of musculoskeletal injuries resulting from extremity trauma or equivalent injuries in the civilian population.

Proposal Title:	Optimization and Evaluation of Photo-Responsive Microneedle Arrays for Sustained Ocular Drug Delivery
Log Number:	PR222011
Current PI Name:	Kuen-Ren Chen
Award Number:	HT9425-23-1-0179
Current Contracting Organization:	Washington State University, Pullman
Current Performing Organization:	Washington State University, Pullman
Web Approval Date:	03-23-2023

Eye disorders and vision loss are costing America over $140 billion every year. As of 2014, 1.3 million Americans are blind, and that number is estimated to rise to 2.2 million Americans in 2030. The leading cause of blindness in the working-age population is diabetic retinopathy, and age-related macular degeneration is the leading cause of irreversible vision loss among people aged 50 and older. In the U.S., the prevalence of diabetic retinopathy and age-related macular degeneration is 7.7 and 2.1 million, respectively. The numbers are continuously growing due to the aging society and growing diabetic populations.

Treatment of a variety of eye diseases requires a localized drug delivery to the eye, which is challenging due to several barriers, such as tear dilution and fluid circulation in the eye. Eye drops and oral drugs are not very effective because only a small portion of the drug can eventually reach the retina of the eye. Direct injections are usually required to achieve the desired drug concentration in the eye. However, direction injections are very invasive and put patients at risk of a number of side effects. Moreover, repeated injections (e.g., monthly) are often required. It is very inconvenient for patients who have eye diseases to travel to the eye clinic frequently. The frequent visits also cost the health care system, including the Veterans Health Administration significantly. A less invasive, long-lasting, and cost-saving drug delivery device is desired.

In the previously funded project, we demonstrated the feasibility of a self-adhesive microneedle array that steadily releases the drug over a 4-week period. The goals of this project are (1) to improve the performance of the microneedle by extending the period of drug release up to 24 weeks and further easing the microneedle removal process, and (2) to evaluate the safety and treatment efficacy of the microneedle in support of launching clinical trials.

This project is strongly related to two of the Fiscal Year 2022 Peer Reviewed Medical Research Program Topic Areas: Sustained Release Drug Delivery and Diabetes within the Portfolio Category - Nutrition and Metabolism. We will focus on addressing the Strategic Goal of Treatment: Develop and test strategies to decrease the burden of treatment regimens.

This project will improve the treatment of age-related macular degeneration and diabetic retinopathy. If successful, we can reduce the frequency of treatment as well as the burden on patients, their families, and the health care system, reduce the cost of drugs with a more efficient way of delivery, achieving the same therapeutic effect with less amount of drug, and achieve a better patient outcome. The immediate patient population, including diabetic retinopathy and age-related macular degeneration, that could be impacted by the proposed device is about 10 million people in the U.S., which require 120 million intravitreal injections annually. This project will also advance controlled and sustained drug release using microneedle, which sees many applications for drug delivery to wet surfaces in human body due to its self-adhesive nature, such as oral and nasal cavities or ligament.

Proposal Title:	A Stem Cell-Based Therapy for Recessive Dystrophic Epidermolysis Bullosa Delivered with a Spray-On Skin Device
Log Number:	PR222029
Current PI Name:	Dennis Roop
Award Number:	HT9425-23-1-0303
Current Contracting Organization:	Colorado, University of, at Denver
Current Performing Organization:	Colorado, University of, at Denver
Web Approval Date:	04-27-2023

Current therapy for patients with epidermolysis bullosa (EB), a group of rare inherited skin blistering diseases, is primarily limited to wound care. EB is caused by genetic mutations in structural proteins of the skin and sentences those afflicted to a life of severe pain and disability due to constant blistering and scaring, and in some cases, early death. This is especially true for severe autosomal recessive dystrophic EB (RDEB), which is caused by mutations in the COL7A1 gene, encoding for the production of Type VII Collagen (Col7), a structural protein that anchors the outer layer of the skin, the epidermis, to the dermis. The technological breakthrough that allows adult skin cells to be reprogrammed into immature induced pluripotent stem cells (iPSCs) now offers the possibility of developing a permanent corrective therapy for RDEB without the risk of immune rejection. Specifically, skin cells can be biopsied from a patient suffering from RDEB and then “reprogrammed” into iPSCs. The iPSCs can then be grown outside the body, genetically corrected, differentiated into new skin stem cells, and then administered back to the same patient as an autograft. The main hypothesis of this proposal is that epidermal cells and dermal cells derived from gene corrected RDEB iPSCs when grafted onto wounds will adhere tightly and provide long-term wound closure. The objective of this new research effort is to continue to develop and improve our iPSC-based therapy for RDEB as proposed in our in our original application. There are two major changes proposed in our current application. We originally proposed to differentiate genetically corrected RDEB iPSCs into epidermal (keratinocytes) and dermal (fibroblasts) cells using a standard protocol where iPSCs are grown in culture as a single layer (in one dimension). However, cells derived by this method failed to form stable human skin when grafted onto mice. We recently discovered that the differentiation of genetically corrected RDEB iPSCs under conditions that allow growth in three dimensions results in the formation of skin organoids (miniature pieces of human skin). Furthermore, epidermal and dermal cells isolated from skin organoids consistently form stable skin when grafted onto mice. Thus, our new application proposes to use skin cells isolated from human skin organoids. Our previous award, proposed to deliver epidermal and dermal cells derived from genetically corrected RDEB iPSCs to RDEB patients as composite skin grafts, comprised of epidermal and dermal cells. However, the time required to generate composite skin grafts is lengthy and consequently expensive due to the long period of use of a current Good Manufacturing Practice (cGMP) manufacturing facility. Therefore, our new application is proposing to deliver genetically corrected iPSC-derived skin cells using a “spray-on-skin” delivery system developed by AVITA Medical that is currently approved by the U.S. Food and Drug Administration (FDA) for treating severe burns. The use of the “spray-on-skin” delivery system will not only decrease the time to patient application, but also greatly reduce the cost compared to growing composite skin grafts. It may also produce superior outcomes for RDEB patients due to the lower risk of inflammation and scarring.

A major goal of our current application is to adapt the production of organoid-derived keratinocytes and fibroblasts to manufacturing conditions required by the FDA and to generate a set of safety and efficacy data to fulfill regulatory requirements for FDA approval of a clinical trial for RDEB. Therefore, this application addresses Fiscal Year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Portfolio Category: Internal Medicine and the FY22 PRMRP Topic Area: Epidermolysis Bullosa. The FY22 PRMRP Strategic Goal is Treatment: to develop and test therapeutics or dressings that enhance wound healing. iPSC-

based strategies similar to the one proposed in this application, whereby patient-specific iPSCs undergo genetic modifications for subsequent differentiation into target cell types for transplantation, can be applied to virtually any other currently incurable genetic disease. However, unlike other genetic diseases, EB, and especially RDEB, may represent an ideal platform to initially test an iPSC-based therapy due to the orphan nature of EB and its severity. Furthermore, the skin is an ideal target tissue to initially test an iPSC-based therapy: it is readily accessible, easy to monitor, and if an adverse event should occur, the affected cells could be easily excised. Therefore, if successful and proven to be safe in a clinical trial for EB, the iPSC-based therapy could then be easily expanded to genetic diseases affecting internal organs. We also predict that the iPSC-based therapy for EB could be used to treat military Service Members who develop severe blistering following exposure to vesicants, such as sulfur mustard, or who suffer from burns over a large portion of their body. In addition, iPSC-based therapies could also be applied to accelerate wound repair in military personnel who experience acute injuries or in older Veterans with chronic wounds.

Proposal Title:	Combination Nitazoxanide and Auranofin Treatment for Anaplastic Thyroid Cancer
Log Number:	RA220009
Current PI Name:	Electron Kebebew
Award Number:	HT9425-23-1-0625
Current Contracting Organization:	Leland Stanford Junior University, The
Current Performing Organization:	Leland Stanford Junior University, The
Web Approval Date:	07-10-2023

Anaplastic thyroid cancer is a rare (incidence of one to two cases per million persons per year) and deadly cancer. No current treatment regimen results in long-term shrinkage of the cancer and thus, no current treatment cures patients or prolongs their life. Given this, we have used high-throughput drug screening of already clinically approved or investigational drugs to identify candidate new drugs that could be repurposed for anaplastic thyroid cancer treatment. Because single drug treatment for most cancers is not effective long-term, we also used combination drug screening of those compounds that had high activity for killing anaplastic thyroid cancer cells from our high throughput drug screening studies. One of the combination with the highest activity of killing anaplastic thyroid cancer cells in preclinical models was combination nitazoxanide and auranofin treatment. This combination treatment also altered the protein levels of two molecules (glutathione Peroxidase 4, heme oxygenase-1) in anaplastic thyroid cancer cells. What remains unknown are the preclinical safety, optimal doses of combination nitazoxanide and auranofin treatment, and whether the two molecules could be used for determining treatment response. Thus, this proposal directly addresses a Focus Area for fiscal year 2022 Rare Cancers Research Program, as it will evaluate a new combination treatment strategy (combination nitazoxanide and auranofin treatment) and the idea of repurposing of drugs in use for other indications for a rare cancer (anaplastic thyroid cancer).

In this proposal, we will evaluate the safety and anticancer activity of combined nitazoxanide and auranofin treatment in preclinical models of anaplastic thyroid cancer and will determine if two molecules are associated with response to combination nitazoxanide and auranofin treatment in preclinical models of anaplastic thyroid cancer. The rationale for performing these experiments is our promising preliminary data, and that (1) its successful completion will significantly contribute to the translation of this novel treatment combination into the clinic for patients with anaplastic thyroid cancer, and (2) allow the selection of cancers that could be treated with this regimen and could help determine early treatment response. We anticipate the project will take 2 years to complete and that it will lay the foundation for moving this treatment to the clinic quickly for patients with anaplastic thyroid cancer. This is because both drugs have been in clinical use for decades for rheumatoid arthritis (auranofin) and parasite infections (nitazoxanide) and their effect and toxicity, which are minimal when used alone, is well known in the human body.

Upon successful completion of the proposed research, we expect to have shown dose ranges that could be safely used for optimal anticancer treatment and confirm the accuracy of the two molecules that can predict treatment response in comprehensive preclinical models that mimic the aggressiveness of anaplastic thyroid cancer. This contribution will be significant because it will represent an important next step toward establishing a new treatment for a rare and deadly cancer. Further, our findings will allow this combination treatment strategy using nitazoxanide and auranofin to be rapidly translated to the clinic to conduct clinical trials in humans with anaplastic thyroid cancer. Moreover, combined nitazoxanide and auranofin treatment for other rare cancers could also be considered in the future based on the results from this research proposal, which would be an advance for rare cancer research.

Proposal Title:	A Human Pluripotent Stem Cell-Based Approach to Identifying the Drivers of Pulmonary Carcinoid
Log Number:	RA220012
Current PI Name:	Huanhuan Chen
Award Number:	HT9425-23-1-0636
Current Contracting Organization:	Chicago, University of
Current Performing Organization:	Chicago, University of
Web Approval Date:	07-10-2023

One of the rare types of lung cancer, called pulmonary carcinoid, is composed of the cells with properties of pulmonary neuroendocrine cells (PNECs). There is no big improvement in treatment and prognosis of this cancer during the last 4 decades, and this reflects our limitation in understanding the disease mechanism. The research progress has been hindered by several barriers: (i) Due to a low frequency of pulmonary carcinoid, it is difficult to obtain clinical tumor tissues for in-depth research. (ii) Few cancer cell lines or disease models have been established for robust and reliable mechanistic studies. As a result, no definitive molecular markers or genetic drivers have been identified and little is known on its targets of therapy.

Thanks to increasing information about the development of human lung organ, the availability of methods for differentiating human stem cells in a controlled fashion, new means for isolating thousands of single cells, tools for analyzing their genes and gene expression patterns rigorously, and cell engineering and gene editing methods for making cancer-causing mutations in living cells. Now we are able to produce the PNEC cells in abundant amounts from human pluripotent stem cells (hPSCs), and convert the normal PNEC into pulmonary neuroendocrine tumors, such as small cell lung cancer. Using the similar strategy of subjecting the PNECs with the genetic factors that are commonly found in pulmonary carcinoid, we expect the cells will undergo transformation into cancer cells and form lung carcinoid-like tumors in mice. The cancerous cells and tumor model will be then characterized on the features of pulmonary carcinoid at morphological and genetic levels.

In this project, we will use the novel hPSC-derived PNEC model to effectively and efficiently test the role of the recurrent genetic mutations in causing the tumor initiation and progression. If successful, our study is expected to provide fundamental knowledge about pulmonary carcinoid and facilitate the identification of new prognosis markers or therapy targets.

Proposal Title:	Defining Genetic Vulnerabilities in Pancreatic Neuroendocrine Tumors That Rely on the Alternative Lengthening of Telomeres Pathway
Log Number:	RA220027
Current PI Name:	Christopher Heaphy
Award Number:	HT9425-23-1-0819
Current Contracting Organization:	Boston Medical Center
Current Performing Organization:	Boston Medical Center
Web Approval Date:	10-03-2023

Pancreatic neuroendocrine tumors (PanNETs) are a heterogeneous group of endocrine tumors that vary in clinical presentation, natural history, and prognosis, thereby making them a challenging disease to treat. While the majority of cancers maintain telomere lengths by expressing the enzyme telomerase, a subset of cancers maintains telomere lengths via another mechanism, termed Alternative Lengthening of Telomeres (ALT). ALT is cancer-specific and ALT telomeres are defined by dramatic cell-to-cell telomere length heterogeneity, increased chromosomal alterations, and increased replication stress. Our group previously identified a strong correlation between ALT-positive PanNETs and mutations in either ATRX or DAXX. While ALT is present in about 10% of all cancers, the prevalence skyrockets in certain cancer types, including gliomas, sarcomas, and PanNETs. Specifically, for PanNETs, ALT is present in 21%-31% primary tumors and then is greatly enriched in metastatic lesions (71%). PanNETs commonly escape current therapeutic strategies and can have a poor overall survival. Importantly, ALT can be readily detected in tissue samples, and we have previously demonstrated that the presence of ALT is associated with poor outcomes in patients. In summary, ALT (i) is cancer-specific, (ii) can be readily identified in patient samples, (iii) is associated with specific loss-of-function somatic mutations, and (iv) is enriched in aggressive PanNETs. Therefore, we believe ALT is an attractive target in PanNETs that could be exploited for novel therapeutic development to improve the clinical management of patients.

We have identified oxidative phosphorylation as the most significantly altered pathway in ALT-positive PanNETs. Oxidative phosphorylation is a metabolic process that takes place in the mitochondria and creates energy for the cell. However, too much oxidative phosphorylation leads to an accumulation of reactive oxygen species within the cell that can cause DNA damage. Damage to telomeric DNA is thought to be a significant factor contributing to activation of the ALT pathway in cancers. Therefore, we hypothesize that increased oxidative stress may not only contribute to the development of ALT, but could be exploited therapeutically in the treatment of ALT-positive PanNETs. Thus, our proposal will directly address two of the fiscal year 2022 Rare Cancers Research Program Focus Areas. Specifically, for PanNETs, we will "identify disease-defining molecular pathways, cell context, and microenvironment," as well as "identify novel therapeutic strategies."

Our overarching hypothesis is that the underlying molecular mechanisms unique to ALT can be exploited by forcing intolerable levels of oxidative stress, thereby allowing therapeutic targeting of a substantial proportion of primary and metastatic PanNETs. Thus, using a combination of human tissue-based and cell line-based approaches, we will: (i) Determine whether oxidative phosphorylation and mitochondrial DNA content are increased in ALT-positive PanNETs, (ii) Validate the presence of mitochondrial dysfunction and oxidative stress in PanNET tissues using a combination of immunohistochemistry and immunoprecipitation, and (iii) Evaluate the mechanistic and therapeutic efficacy of GPX4 inhibition, a peroxidase essential for antioxidant defense, to sensitize ALT-positive PanNETs. In the study proposed here, we will generate the necessary data needed to test this innovative hypothesis in PanNETs, facilitating the identification of new therapeutic leads for the clinical management of patients with ALT-positive PanNETs, which currently lack effective therapies. The data evaluating the response of these cells to intolerable levels of oxidative stress

will generate strong support to further test whether inhibition of these pathways is effective as individual, or in combination, treatment modalities in ALT-positive PanNETs. Although clinical trials are underway to test the efficacy of telomerase inhibitors in the treatment of cancer, there are currently no therapies that specifically target ALT. To address this translational roadblock, we have created a collaborative, multidisciplinary research team at Boston Medical Center and Boston University School of Medicine to try and improve the clinical management of patients with ALT-positive PanNETs.

Proposal Title:	Transcriptional Regulation of Heterogeneous Populations in Choroid Plexus Carcinoma
Log Number:	RA220049
Current PI Name:	Haotian Zhao
Award Number:	HT9425-23-1-0628
Current Contracting Organization:	New York Institute of Technology, Inc.
Current Performing Organization:	New York Institute of Technology, Inc.
Web Approval Date:	06-15-2023

The choroid plexus (CP) in each brain ventricle consists of a fibro-vascular core encapsulated by epithelium that differentiates from roof plate progenitors. Although neoplasms of the CP are rare, they mostly occur in childhood and comprise up to 20% of brain tumors in children under 1 year of age. CP papilloma is typically benign and can be resolved successfully through surgical resection, whereas malignant CP carcinoma (CPC) is poorly understood and highly lethal, with few treatment options. Even with treatments that include surgery, chemotherapy, and radiation, CPC usually responds poorly. CPC is prone to recurrence and metastasis, with only ~ 40% of children still alive 5 years after diagnosis. In addition, survivors often suffer from debilitating long-term treatment effects, including intellectual disability and increased cancer risk. Despite the dire consequence of CPC, knowledge of the molecular and cellular underpinnings of CPC is limited, severely hampering the development of therapies that can specifically suppress tumor growth without damaging the developing brain.

Accurate experimental models for rare cancers such as CPC will help to gain knowledge of CPC pathogenesis and facilitate search for more effective and safer therapies. The Principal Investigator's laboratory generated multiple mouse models of CP tumors driven by molecular defects commonly identified in human diseases. The proposed studies will utilize these animal models to address one Focus Area: the biology and etiology of CPC. The overarching theme of this application arises from the discovery that molecular mechanisms of CP differentiation are critically involved in CPC development. The proposed studies will not only illustrate the molecular details of the specification and differentiation of the CP, but significantly advance knowledge of CPC biology. Results from these studies will help to identify promising targets for innovative anti-tumor therapy and hopefully lead to successful clinical trials that will eventually improve outcome for patients with CPC.

Sex-determining region Y-box 2 (SOX2) is a transcriptional regulator of stem cell pluripotency. In human cancers, dysregulated SOX2 is associated with poor survival. SOX2 promotes proliferation, survival, invasion/metastasis, cancer stemness, and drug resistance. Indeed, SOX2 is highly expressed in CP epithelial progenitors, CP tumors in humans and mice, while loss of Sox2 significantly decreases the growth of CP tumor in animal models. Human CP tumors also frequently display aberrant expression of critical transcriptional regulators of roof plate/CP differentiation, including LIM homeodomain transcription factors LMX1A, and orthodenticle homeobox 2 (OTX2). Accordingly, CP tumors in animal models exhibited dysregulation of these transcriptional programs. Therefore, Sox2, Lmx1a, and Otx2 may represent novel anti-cancer targets in CPC.

In aim 1, we will determine the mechanisms of SOX2 and LMX1A functions and interactions in CP tumor. Gain- or loss-of-function studies will be conducted to examine the role of SOX2 in CP tumor cells. The effect of alterations in SOX2 expression on LMXA levels, and the role of LMX1A in SOX2 signaling will be evaluated. The global landscape of SOX2 and LMX1A binding at regulatory elements in CP tumors will be characterized. Integrated analysis will identify potential downstream targets of SOX2 and LMX1A and provide insights into regulatory mechanisms of SOX2 and LMX1A in tumor-initiating cells in CPC. The

tumorigenic potential of Lmx1a/Sox2 population will be investigated through gain- or loss-of-function genetic and transplantation studies. Sox2 will be inactivated in Lmx1a progenitors in CPC, while Lmx1a /Sox2 cells derived from CPC will be transplanted into recipients to define the cellular origin of CPC. In addition, oncogenic signals will be targeted to Lmx1a/Sox2 progenitors in an inducible manner to drive CPC development after birth. In aim 2, OTX2 binding at regulatory sequences across genome in CP tumors will be characterized, and downstream targets of OTX2 will be identified. Otx2 will be inactivated in CPC to determine its role in CP tumor progression. These studies will provide crucial insights into the regulatory landscape of CPC initiation and progression, identify cell populations with distinct role in tumorigenesis. Knowledge of the epigenetic basis of the heterogeneity in CPC will facilitate the identification of candidate targets for eliminating tumor cell populations.

The short-term (3-5 years) goal of these studies is to leverage the knowledge of molecular mechanisms governing CP lineage properties for better understanding of the biology of CPC. The investigation of the interaction of oncogenic signals with CP lineage mechanisms will identify potential vulnerability in CPC that can be used for safer and more effective treatments. The animal models will also provide a platform for preclinical evaluation of potential therapeutic compounds. The long-term (5-10 years) goal of this project is to gain significant insight into the biology and etiology of CPC and bring drugs into the clinical pipeline that could stop or slow the growth of CPC. These efforts will help to lead us to a future (beyond 10 years) when children will not only survive CPC, but lead a prosperous life after successful treatment.

Proposal Title:	NUAK-1, a Novel Target for Appendiceal Cancer Treatment
Log Number:	RA220084
Current PI Name:	Andrew Lowy
Award Number:	HT9425-23-1-0757
Current Contracting Organization:	California, University of, San Diego
Current Performing Organization:	California, University of, San Diego
Web Approval Date:	10-03-2023

Background: Mucinous adenocarcinomas of the appendix (MAC) constitute a rare type of cancer that affects 1-2 people per million each year in the U.S. Relatively little is known of the specific biology of these cancers. The treatment of choice is surgery, but many patients are not candidates for surgery, because their cancer is too advanced or aggressive. These patients instead receive chemotherapies approved for colorectal cancer, but these frequently have only limited efficacy in MAC.

This application will address two listed Focus Areas of Rare Cancers: "Biology and Etiology" and "Therapy." Aim 1 proposes research predominantly centered on achieving a better understanding of MAC biology. Here, we will study whether a protein, NUAK-1, which is present in much larger amounts in MAC than normal appendix is important for MAC cells to divide, survive, and spread. Studies proposed in Aim 2 will be directed at testing the basis for a new therapeutic approach to treating MAC, hence will center on the Therapy Focus Area. We will test specific inhibitors (specifically, the drugs HTH-01-015 and WZ4003) and will also test the efficacy of "knockdown" with a particular type of RNA (called shRNA, for "short hairpin ribonucleic acid") that inhibits gene expression and can be directed to inhibit expression of just one specific gene of choice (in this case, the NUAK-1 gene; see below). Other drugs we plan to test include palbociclib (which inhibits part of the cellular machinery controlling cell replication), MRTX1133 (a drug that inhibits one of the "driver mutations" that is thought to engender carcinogenesis in MAC and other cancers), and TAK-981 (a first-in-class inhibitor of two entirely different enzymes called sumo activating enzymes 1 and 2). All of these drugs are experimental in MAC, and will be tested for the first time in MAC in this study.

Scientific Objective and Rationale: We have identified NUAK-1 as a gene that is expressed at high levels in MAC samples compared to normal appendix. We know that NUAK-1 has important roles in various cellular functions, many of which are relevant to cancer growth. NUAK-1 also promotes tumor growth and spread in some cancers, but has never been investigated in MAC. Since NUAK01 is overexpressed in MACs, we hypothesize that this could constitute a new therapeutic target in MAC. We will test that in this study.

Applicability of the Research: This research will help those afflicted with MAC, which have no successful treatment options beyond surgery (where this is still possible). We plan to help these patients in two broad ways: (1) by contributing to a deeper understanding of the biology of MAC; and (2) by testing the basis for a new treatment of MAC that involves inhibiting NUAK-1. Since this protein is expressed at high levels in MAC, we hypothesize that blocking this will constitute a new, effective treatment for MAC. This can be accomplished either by blocking NUAK-1 function or by reducing expression, and we will test both here.

Potential Clinical Applications: We don’t yet know whether this strategy will work. There are two possibilities: (1) that NUAK-1 expression is increased in MAC as a result of cancer, rather than as a cause of cancer; and (2) increased NUAK-1 expression is directly required for either tumor development, metastasis, or both. We predict that the second of these two possibilities will be true, hence the therapy should work as planned. The next steps would include detailed toxicity studies to make sure the drugs we will test do not cause undue harm to patients with MAC. Other studies would include pharmacokinetic/pharmacodynamic studies, which are technical studies critical to establish the correct dose that elucidates drug distribution in the body, how long it lasts, how it is excreted, and other features of drug biology. Here, we will mainly focus only on drug efficacy. Risks include potential toxicity, which might even preclude use of the drug if severe

enough. However, we note that these drugs are currently being evaluated for use in other conditions, and we will rely partly on these studies to guide us.

Projected Time to Achieve a Clinically Relevant Outcome: This is difficult to determine with any precision, since there are many variables that can affect this. However, our best estimate is that by the end of this study, we will have a very good idea of what will work, and what will not. From there, given the U.S. Food and Drug Administration regulatory process for rare diseases (which is markedly accelerated compared to common disease that have a high prevalence), we envision a fairly short pathway to the clinic, perhaps as short as just a few years.

Likely Contributions of the Study to Advancing Rare Cancer Research: If we are successful, our research will provide a new therapeutic that will effectively treat MAC. This will represent the first real hope for patients suffering from MAC.

Proposal Title:	Targeting the Novel Long Noncoding RNA PRKCQ-AS1 for Cancer Therapy
Log Number:	RA220088
Current PI Name:	Tao Liu
Award Number:	HT9425-23-1-0714
Current Contracting Organization:	New South Wales, University of
Current Performing Organization:	New South Wales, University of
Web Approval Date:	08-30-2023

Cancer is the most common cause of death from disease in children. Neuroblastoma is a solid tumor in early childhood. Despite multimodality therapy, the majority of high-risk neuroblastoma patients die of the disease, and survivals suffer from long-term disabilities due to adverse side-effects from chemo- and radiotherapy at the early age. There is an urgent need to better understand its tumorigenesis and to identify novel therapies.

The Focus Areas: (1) Therapy, and (2) Biology and Etiology

In this project, we aim to define the novel non-protein-coding RNA, PRKCQ-AS1, as a critical driver of neuroblastoma, to identify small molecule compound inhibitors of PRKCQ-AS1 and to demonstrate their anticancer efficacy. Successful completion of this project will provide a novel targeted therapy for potential clinical translation in patients with the rare but deadly neuroblastoma.

Proposal Title:	Small Molecule Inhibitor Screen Against the Histone Demethylase JMJD1C to Develop a Targeted Therapy for Acute Myeloid Leukemia
Log Number:	RA220100
Current PI Name:	Nan Zhu
Award Number:	HT9425-23-1-0695
Current Contracting Organization:	Versiti Wisconsin, Inc.
Current Performing Organization:	Versiti Wisconsin, Inc.
Web Approval Date:	10-03-2023

Our research will develop new drugs for Acute Myeloid Leukemia. This falls within the "Therapy" Focus Area of fiscal year 2022 Rare Cancers Research Program.

Acute myeloid leukemia is an aggressive cancer from the bone marrow, where blood is made. There are many subtypes of leukemia. One subtype is "mixed lineage leukemia (MLL)-rearranged leukemia." In this type, an important gene breaks and is then connected wrongly to other genes. These "rearranged" genes lead to cancer-causing proteins that drive leukemia. MLL-rearranged leukemia makes up over 70% of infant leukemia and 10% of all leukemia. Patients with MLL-rearranged leukemia have worse survival rates compared to other leukemia subtypes. Current drugs to treat this disease are toxic. Even when current drugs work at the start of treatment, the leukemia comes back more than half of the time. All these problems show the need for better drugs. We want to find new drugs.

Our previous research showed that a protein called "JMJD1C" is required for leukemia. There is more of this protein in MLL-rearranged leukemia compared to other subtypes. Lab mice with leukemia that do not have this protein survive longer. Stopping this protein with new drugs might help human leukemia patients. We are looking at a small unique region of the protein where we think new drugs will work well. We anticipate it will take approximately a decade to develop a drug that works in this way.

Our work contributes to rare cancer research by developing a new drug for MLL-rearranged leukemia. In the future, this drug could work on its own or in combination with current drugs.

In summary, we focus on blood cancer in general and MLL-rearranged leukemia in particular. We want to discover drugs that treat this disease in new ways.

Proposal Title:	A Novel Immuno-Oncology Pipeline for FGFR2 Fusion-Positive Cholangiocarcinoma
Log Number:	RA220126
Current PI Name:	Daniela Sia
Award Number:	HT9425-23-1-0641
Current Contracting Organization:	Icahn School of Medicine at Mount Sinai
Current Performing Organization:	Icahn School of Medicine at Mount Sinai
Web Approval Date:	07-27-2023

The overall scientific objective of this project is to identify and validate more effective immunotherapeutic strategies able to improve the outcome of patients with intrahepatic cholangiocarcinoma (iCCA). With an incidence of 1-2 cases per 100,000 people and steadily increasing mortality rates, iCCA is a rare but devastating type of liver cancer. Currently, the only potential cure for iCCA is to diagnose the cancer at an early stage when it can be removed surgically. Unfortunately, early diagnosis is extremely difficult since iCCA generally presents with mild and unspecific symptoms. Therefore, the majority of patients are diagnosed at advanced stages when the only option is a combination of chemotherapy plus immunotherapy, with 95% of patients dying within 5 years, highlighting the urgent need to improve the therapeutic arsenal for this disease. Oncogenic fusions involving the FGFR2 gene occur in up to 20% of all iCCA patients and positive results from pivotal phase 2 clinical studies have confirmed that they are promising therapeutic targets for FGFR2-positive iCCA patients. Despite this success, initial clinical responses are modest and short-lived due to complex mechanisms of resistance. Therefore, the design of combination strategies able to enhance the extent of initial responses in FGFR fusion-driven iCCA patients remains of the utmost importance.

Checkpoint inhibitors (ICIs) are immunotherapeutic drugs that boost the patient's own immune response by blocking key "checkpoint proteins" that prevent immune cells to kill the tumor. While blockade of the checkpoint protein PD1 or its ligand PD-L1 has shown promise in many solid cancers, including iCCA, only a small fraction of patients respond. Emerging evidence suggest that specific oncogenic pathways, including FGFR signaling, can promote immunosuppression and thereby, compromise antitumor immune responses and sensitivity to ICIs. If inhibition of oncogenic FGFR fusion signaling could reduce the immunosuppression and evasion mechanisms, combining FGFR inhibitors with ICIs could overcome the limitations of each strategy alone. The rationale of this study is that by understanding how FGFR2 fusions impact immune cell composition and functions, we will be able to design novel immunotherapy-based combination regimens that enhance initial clinical responses in FGFR2 fusion-driven iCCA. In pursuing this project, we seek to establish and characterize a novel series of immunocompetent iCCA murine models driven by FGFR2 fusions (Research Method Focus) and use them to thoroughly investigate the immunoregulatory role of FGFR gene fusions (Biology Focus) as well as identify promising immunotherapeutic combinations (Therapy Focus). We expect that the discoveries made through this project will impact the design of forthcoming clinical trials in FGFR2 fusion-driven iCCA patients.

Overall, the results of our efforts will provide the benefit of improving the outcome of a large fraction of patients with this cancer and bringing new drugs to these patients faster than ever as we estimate that the timeframe for translating results obtained in this project into clinical results can be of around 3 years. There are no risks to patients that are directly attributable to the studies proposed in this application. Should new drugs be tested, however (which is beyond the scope of this application), these agents could lead to unwanted toxicities or side effects. As noted, however, such clinical studies are not a direct component of this application.

The ultimate applicability of this research will be widespread, and could improve the outcome for patients with iCCA, including Veterans and the U.S. population suffering from this disease. Indeed, despite being a rare cancer, iCCA disproportionately impact U.S. Veterans, their families, and other military beneficiaries. This is in part due to the fact that the prevalence of risk factors associated with iCCA (i.e., viral hepatitis, cirrhosis, obesity) is higher among U.S. Veterans. Furthermore, Veterans who served in Southeast Asia risked exposure to liver fluke infections that strongly predispose to iCCA. The likely contribution of this effort will be to create new treatment options for patients with advanced iCCA, thus transforming the outlook for both U.S. Veterans and the general public who are afflicted by this devastating disease. Further, the establishment of novel experimental models for mechanistic and translational studies will dramatically facilitate rare cancer research by contributing to advance our currently insufficient understanding of this devastating disease and accelerate treatment development in the near future.

Proposal Title:	Alternative Splice Variant-Derived Neoantigen-Targeted Immunotherapy in Diffuse Hemispheric Glioma, H3 G34-Mutant
Log Number:	RA220179
Current PI Name:	Anthony Wang
Award Number:	HT9425-23-1-0616
Current Contracting Organization:	California, University of, Los Angeles
Current Performing Organization:	California, University of, Los Angeles
Web Approval Date:	07-12-2023

This proposal seeks to develop a new mode of immunotherapy targeting a rare brain tumor type that afflicts children and young adults. We utilize techniques (long-read RNA-seq) to develop a new, more robust computational method (IRIS2) to identify targets for immunotherapy that are unique to this tumor type (diffuse hemispheric glioma, H3 G34-mutant). This tumor is a particularly ideal candidate for such an approach in that the mechanisms by which this tumor develops involves RNA dysregulation, which IRIS2 is made to explore.

We have shown that designing immunotherapy against targets identified by an earlier version of IRIS can direct the immune system to effectively identify and kill this particular type of tumor with striking efficacy. We propose to expand upon these early studies to develop immunotherapy against this and other cancer types.

The potential impact of our success will be relevant to active-duty Service Members, Veterans, and the family of Service Members, including their children, by studying a cancer that kills young, healthy boys and girls, with an eye toward developing a clinical trial to offer a better treatment. Moreover, the relevant findings will provide a basis upon which to explore similar strategies in other brain tumor types.

Proposal Title:	Immunogenicity and Targeting of Shared Neoantigens in Adenoid Cystic Cancer
Log Number:	RA220201
Current PI Name:	Luc Morris
Award Number:	HT9425-23-1-0716
Current Contracting Organization:	Sloan Kettering Institute for Cancer Research
Current Performing Organization:	Sloan Kettering Institute for Cancer Research
Web Approval Date:	08-30-2023

Background on Adenoid Cystic Carcinoma: In this project, we seek to develop new therapies for adenoid cystic carcinoma (ACC). ACC is a rare cancer, with approximately 1,600 new cases diagnosed and 1,200 deaths recorded annually in the United States. ACCs usually arise in the salivary glands of the head and neck, and less commonly affect the trachea, lung, breast, and other sites. Both men and women are affected. There are no known risk factors for the development of ACC, although salivary cancers have been strongly linked to Agent Orange herbicide exposure among Vietnam War Veterans.

Most ACCs are treated with surgery and radiation therapy, but over 50% of patients experience cancer recurrence. In a majority of these cases, cancer recurrences are not able to be successfully treated. At this stage, most patients receive chemotherapy, although there is no clear evidence that any chemotherapy drug is effective. In recent years, cancer immunotherapy has shown great promise; unfortunately, in contrast to some other cancer types, immunotherapy drugs have only been effective in a very small percentage of patients with ACC. The goal of our multidisciplinary ACC research team is to develop new, more effective immunotherapies to treat metastatic ACC, which is otherwise an incurable cancer.

Objectives and Rationale: The rationale behind this project is that most ACC tumors contain “non-self” signals (neoantigens) that result from alterations inside the tumor. Neoantigens can be detected by the immune system. Sometimes, these neoantigens result from a gene mutation in the tumor. In other cases, neoantigens may also come from gene fusions (a combination of parts of two different genes), an alteration that happens to be found in most ACC tumors. Our early data suggest that many ACCs have neoantigens that result from a gene fusion; however, further work is needed to translate these findings into new therapies for patients. Our objective is to identify the neoantigens in ACC that can be recognized by T cells, especially the set of neoantigens that are shared among patients. Such shared neoantigens would be particularly amenable to the development of standardized “off-the-shelf” immunotherapies based on T cells programmed to identify and kill cells expressing these signals.

FY22 RCRP Focus Areas: Our project addresses components of all three Focus Areas of the fiscal year 2022 Rare Cancers Research Program:

Biology and Etiology: We will identify new targets in ACC tumors that can stimulate T cell attack.

Research Model: We have developed a new approach to screen tumors for immune reactivity in mice, as well as a collection of ACC patient-derived tumor models that have been grown in mice and can be used for drug treatment studies. These new models will be shared with the research community.

Therapy: We will establish the feasibility of targeting neoantigens in ACC, a new immunotherapy approach that has the potential to improve upon current drugs that have minimal effectiveness.

Applicability to Patients with ACC and Other Rare Cancers: This research will provide unprecedented insight into a common genetic event in ACCs, as well as identifying whether this common genetic event represents an Achilles' heel that can be targeted with immunotherapy. The findings of this project will facilitate development of a new neoantigen-targeting treatment strategy for ACC which, if successful, would be the first effective therapy for this highly lethal cancer.

In addition, there are a number of other rare cancer types that share many biological similarities with ACC, including several types of pediatric cancers and sarcomas that have comparable genetic profiles and low response rates to immunotherapy. We anticipate that the techniques and findings of our study will have applicability to these other cancer types as well.

During the 3-year timeline of this project, we expect to generate clear, actionable data in support of the most feasible treatment strategies for ACC. The immediate next steps would be clinical trials of the most effective therapeutic approaches.

Proposal Title:	Novel Combination Therapies for Anaplastic Thyroid Cancer
Log Number:	RA220221
Current PI Name:	Marie-Claude Hofmann
Award Number:	HT9425-23-1-0675
Current Contracting Organization:	M.D. Anderson Cancer Center, University of Texas
Current Performing Organization:	M.D. Anderson Cancer Center, University of Texas
Web Approval Date:	07-27-2023

Background and Objectives: Anaplastic thyroid cancer (ATC) is a rare form of thyroid cancer and is one of the most aggressive tumors known, with an annual incidence rate of 0.18/100,000 and a survival rate of about 3-6 months after diagnosis. ATC happens when cells in the thyroid develop DNA mutations that make the cells multiply very rapidly. The cells form a tumor that grows to invade nearby tissue and can spread (metastasize) to the lymph nodes in the neck. ATC cells do not respond well to radiotherapy or chemotherapy. However, recent therapies with drugs called kinase inhibitors have been very successful to make tumors shrink and easier to remove surgically. Unfortunately, responses to the drugs are short-lived due to the emergence of additional DNA mutations during treatment. There is, therefore, the need to identify novel molecules produced by the resistant cells that we could target with different drugs. We have found a new molecule called p38/MAPK14 in resistant cells and a drug that can possibly inhibit it, called ralimetinib. This project will evaluate if p38/MAPK14 is truly responsible for resistance and test the efficacy of ralimetinib in combination with other drugs in cultured ATC cells and in mice developing ATC. If successful, these results will justify future clinical trials. We believe that our study has the potential to create new treatment options for patients with ATC or other aggressive tumors.

Focus Area: This proposal addresses the vision and mission statements of the fiscal year 2022 Rare Cancers Research Program, which are (1) to improve outcomes for patients with rare cancers through discovery, (2) to expand knowledge across the cancer landscape, and (3) to enable clinically impactful discoveries for the benefit of Service Members, Veterans, and/or the American public.

Patient Population: ATC affects patients of both sexes. In one of our recent studies investigating 87 ATC patients, the median age was 65 years (range 35-88 years). The clinical course of ATC is characterized by rapid and invasive local tumor growth, frequent distant metastases, and fatal outcomes. Therefore, ATC requires rapid diagnosis and prompt treatment.

Potential Clinical Application: Ralimetinib is currently in clinical trials for glioblastoma. As mentioned above, if treatments of our ATC mouse models are conclusive, the data will justify a future phase 1 trial with ATC patients. Adverse reactions to ralimetinib exist such as hepatitis, rash, and nausea. However, because we are testing synergistic interactions between ralimetinib and other Food and Drug Administration-approved drugs, we will use lower doses of the combination constituents, which will hopefully reduce adverse reactions.

Time Until Clinically Relevant Outcome: We expect to present the complete set of data within 3 years.

Contributions for Rare Cancer Research: Mutations found in ATC are very similar to those found in melanoma, which accounts for only 1% of all skin cancers diagnosed in the United States but causes most of the deaths from skin cancer. We therefore believe that our study has the potential to create new treatment options not only for ATC patients, but also for patients affected by aggressive tumors such as melanoma.

Proposal Title:	Identifying Novel Compounds for Treatment of pdChordoma and Other SMARCB1-Deficient Rare Tumors
Log Number:	RA220232
Current PI Name:	Sanjay Malhotra
Award Number:	HT9425-23-1-0796
Current Contracting Organization:	Oregon Health and Science University - Portland
Current Performing Organization:	Oregon Health and Science University - Portland
Web Approval Date:	10-03-2023

This proposal focuses on a group of extremely rare tumors with a common driver of tumor growth - loss of the SMARCB1 gene, also known as INI1. These tumors include poorly differentiated chordoma, atypical teratoid/rhabdoid tumor, epithelioid sarcoma, epithelioid malignant nerve sheath tumor, epithelioid schwannoma, extrarenal and renal rhabdoid tumors, extraskeletal myxoid chondrosarcoma, myoepithelial carcinoma, ossifying fibromyxoid tumor, and renal medullary carcinoma. These tumors are very rare, occurring in no more than 1 in 1 million people each year, many occur in young children, and most have very poor survival. None have effective medical therapies available.

Our National Cancer Institute group runs a natural history study at the National Institutes of Health Clinical Center for very rare solid tumors in children, teens, and adults. This study follows patients with different rare tumors to see what their challenges are and how they respond to the treatments they receive. We also collect tumor tissue to look at what genes are mutated in tumors and to make new research tools. Through a partnership with the Chordoma Foundation, a chordoma advocacy group, we are learning about patients with poorly differentiated chordoma as well as conventional chordoma that doesn’t lose SMARCB1. By interacting with participants in our study, we are gaining expertise in theses diseases. We have opened a treatment trial for patients with mutations in the SMARCB1 gene that can test drugs in the different tumor types with SMARC1 loss listed above.

In this proposal, we are testing our hypothesis that there are drugs that will treat tumors with SMARCB1 loss even if the tumors are different types. Oregon Health Sciences University has technology to test thousands of drugs on tumor cells. Through a collaboration between the National Cancer Institute and Oregon Health Sciences University, we will address all three of the Focus Areas for the Rare Cancer Research Program.

First, we will make new research models by putting a fluorescent gene into chordoma cells. By making the cell nucleus glow, we can more easily count cells using machines to measure fluorescent light. This can make our drug screens more exact and lets us look at whether drugs stop cells from dividing or kills them. We will also make chordoma cells that either turn SMARCB1 on or off in tumor cells that still have it. These cells can be used by researchers interested in studying SMARCB1 function. Because these cells can be used for many different types of experiments, we will share them with researchers through the Chordoma Foundation biobank.

Secondly, we will use the chordoma cells that turn on or off SMARCB1 to learn how loss of SMARCB1 leads to tumor formation. SMARCB1 is part of a protein complex that sits on DNA and changes which genes are expressed. We will look at how changing SMARCB1 expression changes the DNA and which genes are turned on or off. There is also evidence that when SMARCB1 is lost, there are changes in genes that control how cells get nutrients they need to grow (called nutrient transporters). If we can understand this process, we may be able to starve tumor cells so they can’t grow. Because the biology of poorly differentiated chordoma hasn't been studied, what we learn about how loss of SMARCB1 changes molecular pathways will help us develop new ideas for treating poorly differentiated chordoma.

Finally, we will use our new cell lines to test which drugs can block cell growth or cause cell death. The team at Oregon Health Sciences University will test both U.S. Food and Drug Administration (FDA)-approved drugs for potential drug repurposing as well as compounds that have not yet been developed into drugs but may have specific activity against tumors that have lost SMARCB1. We will take drugs and other compounds that inhibit chordoma cells with SMARCB1 loss and test them in mouse models of chordoma, as well as other type of tumor cells that have lost SMARCB1. We will test atypical teratoid/rhabdoid tumor, renal rhabdoid tumor, epithelioid sarcoma, and renal medullary carcinoma. If tumor cells from other tumor types with SMARCB1 loss become available, we will test them as well.

By the end of the 3-year grant period we expect to know if there are candidate FDA-approved drugs that could be repurposed to treat poorly differentiated chordoma specifically or tumors with SMARCB1 loss generally. Because several members of our team are expert in developing treatment trials for rare tumors, we would test candidate drugs in patients as soon as possible. If we do not find any candidates in the FDA-approved drugs, but find candidates in the other compound library, the team at Oregon Health Sciences University can use chemistry to make the compound more drug-like and work toward developing a new drug, using additional funding sources.

Our study will advance rare cancer research by finding candidate precision medicine therapies for tumors of unmet need and shed light on how loss of SMARCB1 causes tumors. This could lead to new and more effective ways for treating these tumors.

Proposal Title:	Bench-Bedside-Bidirectional Investigation of a New Combinatorial Immunotherapy Strategy to Treat Platinum-Resistant Penile Cancer
Log Number:	RA220233
Current PI Name:	Xin Lu
Award Number:	HT9425-23-1-0613
Current Contracting Organization:	Notre Dame, University of
Current Performing Organization:	Notre Dame, University of
Web Approval Date:	09-15-2023

Scientific Objective and Rationale: In 2022, it is estimated that penile cancer will comprise 2,070 new cases and 470 deaths for American men. Despite being rare, the incidence of penile cancer has increased in developed countries, including the United States. Over the past 6 decades, and the largest increase was noted in men less than 65 years of age. A disease of geographic disparity, penile cancer can constitute up to 10% of male malignancies in some African, Asian, and South American areas. Penile cancer is highly morbid, often resulting in devastating disfigurement, and only half of the patients survive beyond 5 years. Very limited basic and clinical research has been devoted to the understanding and treatment of this rare but lethal disease. Currently in the clinic, surgery, radiation, and conventional chemotherapy are the only treatment options. For aggressive penile cancer that is resistant to platinum-based chemotherapy, there are no further treatment options. Therefore, there is an urgent need to better understand and develop effective therapies for platinum resistance penile cancer.

Our research goals will be achieved through close collaborations between cancer biologists and medical oncologists. Our research objective is to develop an innovative therapy strategy that employs the combination of molecularly targeted therapy and cutting-edge immunotherapeutics to treat platinum resistance penile cancer. We have developed the critical experimental tools that enable us to explore the idea of treating penile cancer with combined targeted and immunotherapy for the first time. These tools include the first genetically engineered mouse models of penile cancer, with which we will be able to test therapies in animals with intact immune system. Our choice of targeted therapy for penile cancer is drugs known as poly(ADP-ribose) polymerase (PARP) inhibitors. PARP is an enzyme that is part of the cellular machinery responsible for the repair of damages, either spontaneously occurring or externally induced, to the DNA – the genetic materials of the cell. If a cell has mutations in genes involved in the DNA damage repair, the cell is rendered sensitive to the killing by a PARP inhibitor. In our recent genomic studies of human penile cancer, we identified that 23.5% cases of penile cancer harbored mutations in these genes. For the cases that do not have mutations in DNA repair genes, they are potentially still sensitive to PARP inhibitors, because PARP inhibitors can themselves induce DNA damage. The damaged DNA can then be sensed by the cells, and as a result, the cells will send signals to the immune system as if the cells are invaded by DNA-based viruses. This pathogen mimicry response can be exploited for cancer treatment through combining PARP inhibitor and immunotherapy (in this case, immune checkpoint inhibitors that won the Nobel Prize in Physiology/Medicine in 2018).

Based on this rationale, we will first evaluate the effect of three PARP inhibitors that are approved by the U. S. Food and Drug Administration to treat other cancers (olaparib, niraparib, and talazoparib) on both mouse and human penile cancer cells, and we expect these drugs are active to stimulate the pathogen mimicry response. Next, we will test the antitumor efficacy of PARP inhibitor niraparib and immunotherapy anti-PD1 antibody, as single therapy or in combination, in our animal models of penile cancer. Finally, a highlight of our study (the "bench-bedside-bidirectional" approach) is that we will validate the biomarkers for the drug effects from PARP inhibitor and immune checkpoint inhibitor using the collected penile cancer specimens from NCT05526989. This clinical trial (the Principal Investigator is Dr. Chahoud) is the largest multicenter

clinical trial that enrolls only patients with chemotherapy-relapsed penile cancer to receive the combination of immune checkpoint inhibitor therapy (anti-PD1 antibody Dostarlimab) and targeted therapy (PARP inhibitor niraparib). Both the animal models and the clinical samples will be examined for biomarkers of pathogen mimicry response and immune cell changes with cutting-edge technologies.

Focus Area(s): Our research project aims to identify combination therapy strategies to treat chemoresistant penile cancer; thus, it mainly targets the fiscal year 2022 (FY22) Rare Cancers Research Program (RCRP) Focus Area – Therapy. Our project conducts the preclinical/co-clinical studies in novel mouse models; thus, it also addresses the FY22 RCRP Focus Area – Research Model. The bench-bedside-bidirectional nature of our study predicts high potential benefits to patients.

Applicability of the Research: Our research is expected to provide the key insight to help improve the survival and wellness of all the patients with refractory penile cancer in the United States and worldwide. By aligning the preclinical studies and the clinical trial of combined PARP inhibitor and immunotherapy in penile cancer, we expect to reveal the benefits and risks of this combination in a highly translational manner. The clinical impact is expected to be imminent, because the knowledge gained from this study can be used to interpret the result from the clinical trial NCT05526989 and guide the clinical management and future clinical trials on penile cancer. The study will also advance the understanding of the tumor biology of rare squamous cell carcinomas in general.

Proposal Title:	Leveraging Reactivation of Tissue-Resident Memory T Cells in the Tumor Microenvironment for Oncolytic Virotherapy
Log Number:	RA220248
Current PI Name:	Jianfang Ning
Award Number:	HT9425-23-1-0448
Current Contracting Organization:	Minnesota, University of, Twin Cities
Current Performing Organization:	Minnesota, University of, Twin Cities
Web Approval Date:	05-10-2023

Scientific Objective and Rationale

A brain cancer is a disease that occurs in brain where malignant cells grow fast and form a mass of abnormal tissue. Brain cancers are the leading cause of cancer death in United States (U.S.) active-duty military. Glioblastoma multiforme (GBM) is the most common and aggressive brain cancer in adults. GBM is a rare cancer with an incidence of 3.21 per 100,000 persons in the U.S. It causes around 12,000 new cases, but 10,000 deaths each year. GBM remains invariably lethal. Nearly all GBM patients die within 2 years of diagnosis. There is no effective treatment for GBM so far.

Immunotherapy is a type of cancer treatment that helps to boost the immune system and enables it to effectively fight cancer cells. Immunotherapy is bringing great promise to several types of cancers and is being actively pursued for GBM treatment. However, so far it has not benefitted GBM patients, largely due to (1) lack of enough immune cells within the tumor itself that can kill the GBM cells and (2) inactivation of immune cells mediated by the GBM cells. Significant efforts are now underway to develop the means of increasing and activating immune cells in the GBM tumors.

The human body encounters many different viral infections throughout its life, such as flu and COVID-19. Tissue-resident memory T (T) cells are a group of special immune cells that can be developed and remain long-term after an infection has been eliminated by immune response in the body. Those memory T cells permanently reside in different tissues, including tumors, throughout the entire body. T cells can remember a previous infection and quickly become activated to fight a past infection upon reexposure to a previously encountered virus. Importantly, the reactivated T cells can recruit and activate other immune cells, and make them join the fighting. Recent research found that T cells not only protect the body against viral infection, but also cancer development.

Our previous work showed that T cells specific to common human viruses, including flu, are present in patient GBM tumors and can be activated by viral peptides that are derived from a previously encountered virus. In mouse model, we found that applying viral peptides to reactivate T cells within GBM tumors is a highly effective means of immune activation that recruits more immune cells to GBM tumors and suppresses tumor growth. Our current work found that T cell-activating viral peptides significantly improve the antitumor efficacy of an oncolytic (or tumor killing) herpes simplex virus (oHSV) against GBM.

oHSV is a well-studied antitumor agent, engineered in the laboratory to selectively kill cancer cells without harming healthy cells. oHSV infects cancer cells and makes copies of itself until the cell bursts, causing cell death and the release of special danger signals that help to generate an immune response against the tumor. One oHSV has been approved in the U.S. for melanoma treatment and the other in Japan for brain cancer. Despite the progress in clinic, the efficacy of oHSV therapy is limited and needs improvement.

Given the common roles of T cells and oHSV in stimulating immune activation in tumor, our hypothesis is that T cell reactivation improves oHSV efficacy against GBM by promoting the antitumor immune response. Our objective is to develop a new oHSV-T cell based immunotherapy with translatable, clinical potential for human GBM by testing our hypothesis. We will validate this novel oHSV- T cell immunotherapy and understand how it works to kill the GBM tumor in our proposed research.

Focus Area: The proposed work addresses “Therapy”, one of the Fiscal Year 2022 Rare Cancers Research Program Focus Areas. The proposed research has the potential to innovate a therapeutic platform that meaningfully impacts the survival and quality of life for GBM patients, including those who are active-duty Service Members, Veterans, and other military beneficiaries.

Applicability of the Research: We will apply GBM as a cancer model to test our newly developed immunotherapy. GBM patients will be the most direct population our research can help. We anticipate to complete the proposed research within 3 years. If successful, given the safety profile of oHSV in clinic trials and the presence of T cells in GBM patient specimens, this research can be rapidly translated into a clinical trial and may improve the survival of GBM patients by using the new immunotherapy we develop in this research.

Proposal Title:	Epigenetic Mechanisms Mediating Resistance to Immunotherapy in Adrenocortical Carcinoma
Log Number:	RA220277
Current PI Name:	Kleiton Borges
Award Number:	HT9425-23-1-0726
Current Contracting Organization:	Children's Hospital, Boston
Current Performing Organization:	Children's Hospital, Boston
Web Approval Date:	10-03-2023

Adrenal Cancer (ACC) is a rare and aggressive tumor that originates in the adrenal gland. While there are currently no curative treatments for advanced ACC, immune therapy may ultimately prove effective, as it has for other cancers. Immune therapy is a type of cancer treatment that boosts the body's natural immune defenses to help fight cancer. It uses substances made by the body or in a laboratory to improve how the immune system works to find and kill cancer cells. Immunotherapy has shown encouraging efficacy in the treatment of a wide range of cancers. Unfortunately, early clinical trials of immune therapy in patients with ACC have shown poor treatment responses. What causes this remains unclear. The goal of this proposal is to use a new mouse model of ACC to discovery mechanisms that block immune therapy form working in patients with ACC.

Focus Area: Biology and Etiology

What types of patients will it help and how will it help them? This research will benefit patients with advanced forms of ACC who currently do not respond to immune therapy.

What are the potential clinical applications, benefits, and risks? This research may lead to the discovery of new ways that ACC, and also other cancers, avoid detection by the immune system. Understanding these mechanisms may lead to new treatments for patients with ACC and other tumors that currently do not respond to immune therapy.

What is the projected time anticipated to achieve a clinically relevant outcome? These studies could lead to new treatments for cancer within 5-10 years.

What are the likely contributions of this study to advancing rare cancers research? This study is likely to lead to the discovery of new mechanisms that allow cancers to avoid the immune system.

Proposal Title:	Epistatic Effects of Fcgamma (GM) and FcgammaR Genes on the ADCC of EGFR-Overexpressing Osteosarcoma Cells
Log Number:	RA220310
Current PI Name:	Janardan Pandey
Award Number:	HT9425-23-1-0504
Current Contracting Organization:	Medical University of South Carolina
Current Performing Organization:	Medical University of South Carolina
Web Approval Date:	06-15-2023

Fiscal Year 2023 (FY23) Rare Cancers Research Program (RCRP) Focus Areas: Biology and Etiology; Therapy

Currently available immunotherapies do not help patients with osteosarcoma. It is relevant to note that about two-thirds of the human population remains cancer free. Presumably in these people their own immune system eradicates precancerous lesions before they can develop into full-blown cancer. This suggests that a better understanding of how our natural immune system reacts to cancer cells is likely to enhance our ability to devise therapeutic strategies that would be beneficial to the majority of the patient population.

In this proposal, we will investigate the immunology of a protein called EGFR, which is overexpressed on osteosarcoma cells. Its overexpression correlates with worse survival. In some cancers, a gene of the immune system – called GM allotypes – influences natural antibody responses to EGFR and these antibodies are associated with enhanced survival. To further understand the mechanisms underlying the beneficial effects of anti-EGFR antibodies, we will characterize osteosarcoma specimens stored at Children's Oncology Group (COG) Biospecimen Bank. DNA will be used to type for GM genes and another gene of the immune system called FCGR. Plasma samples will be used to measure antibodies to EGFR. We will use statistical tests to determine whether people expressing particular genes are more or less likely to make antibodies to EGFR. Most importantly, we will determine whether a particular combination of these genes is more potent in killing osteosarcoma tumor cells.

Encouraging results from this Concept grant will lead to large-scale studies to conclusively show the influence of GM allotypes on natural immunity to EGFR. Results from these large-scale studies could lead to the development of immunotherapy for patients who already have osteosarcoma and also those who are likely to develop this malignancy. Results obtained here could potentially divide the population into naturally high or low responders to EGFR. Subjects with the high responder genotype are more likely to make therapeutic responses to EGFR-based vaccines (active immunotherapy). In individuals with the low responder genotype EGFR could be fused with appropriate adjuvants (immune enhancers) to overcome the genetic restriction in immune responsiveness. Our experiments involving cytotoxicity (killing) of tumor cells could identify the most potent combination of genes for killing osteosarcoma tumors. This knowledge would be instrumental in manufacturing antibodies for treating patients with osteosarcoma (passive immunotherapy).

Anti-tumor antibodies could be manufactured whose Fc (GM) region has high affinity for a given FCGR molecule expressed on immune killer cells. After determining their FCGR genotype, the patients could be given anti-tumor antibodies carrying the GM variant having the highest affinity for that FCGR molecule. It is also important that the patient and the therapeutic antibody share the same GM variant, thus preventing the generation of anti-allotype antibodies in the host, which could eliminate the therapeutic antibody from the system. Current approaches to immunotherapy do not take into account the inherent variability of GM genes. Designing antitumor antibodies – taking into account the natural variability in Fc (GM) and FCGR – that

have a high potential for killing tumor cells is commercially feasible and could be done in just few years. These markers are variable, but not too variable to make the manufacturing of antibodies commercially prohibitive. Only a few different kinds of antibodies need to be manufactured, and typing for the patient's FCGR genotype can be easily automated. For a disease with no cure, these efforts are worth trying. A patient-related outcome could be achieved within 2-3 years after the completion of the large-scale studies.

Proposal Title:	Albumin-Bound siRNA Therapeutics for Synergistic Targeting of KRAS and MCL-1L in Cholangiocarcinoma
Log Number:	RA220327
Current PI Name:	Craig Duvall
Award Number:	HT9425-23-1-0717
Current Contracting Organization:	Vanderbilt University
Current Performing Organization:	Vanderbilt University
Web Approval Date:	10-03-2023

Cholangiocarcinoma (CCA), or cancer arising from the bile ducts, is a rare but aggressive cancer that is diagnosed in around 9,200 Americans every year. Patients with CCA currently face limited treatment options when surgical removal of the tumor is not feasible. State-of-the-art chemotherapy and immune therapy regimens only result in typical patient survival of about one year. Recent advances have revealed mutations in these cancers' DNA - the blueprints of cancer cells – that contribute to CCA tumor growth and may be exploited to create new targeted treatments. However, many of these mutations do not have a corresponding U.S. Food and Drug Administration-approved drug therapy, including the majority of mutations in a cancer growth gene called KRAS. Furthermore, research suggests that blocking KRAS on its own is often insufficient, and combination therapies may be needed to unlock the full potential of KRAS-targeted treatment. A promising strategy that we have identified in cancer cell lines is to block both KRAS and a second target called MCL-1. MCL-1 allows cancer cells to evade self-destruction (apoptosis), so blocking it can result in increased cancer cell death. Blocking both targets simultaneously appears to be synergistic, meaning they work better together than would be expected based on how they perform individually. In this project, we propose to create a treatment regimen that simultaneously targets the combination of KRAS and MCL-1 by using small interfering ribonucleic acid (siRNA) therapeutics.

siRNA therapeutics use short, customized RNA molecules to eliminate perfectly matching messenger RNA inside cells, blocking the function of genes like KRAS that help the cancer grow. However, to use siRNA as cancer treatment, measures must be taken to keep it from coming out in the urine, guide it to the sites of cancer, and help it enter cancer cells. Our research group has pioneered siRNA modifications that enable "hitchhiking" on albumin, the most common protein in blood. Albumin normally remains in the bloodstream but is taken up by cancers that are scavenging for nutrients to drive their abnormal growth. Our innovative albumin-binding siRNA modifications therefore lead to longer blood circulation and better accumulation in the cancer compared to healthy organs. Using albumin-bound siRNA, we propose to (1) determine what biological factors are involved in the observed synergy between KRAS and MCL-1 treatment and (2) test the therapeutic potential and safety of this combination in animal models of CCA. This project will therefore address the "Therapy" Focus Area of the Rare Cancers Research Program.

Our ultimate goal is to establish new targeted treatment strategies that extend survival compared to conventional chemotherapy in KRAS-mutated CCA. This would most directly impact the roughly 22%-42% of CCA patients who have KRAS mutations, although the delivery technology could further be developed for other molecular targets in the future. We aim to have mouse model results regarding safety and effectiveness of this treatment strategy in the next year of this project, although several more years of preclinical development are anticipated to be necessary before direct human impact. In the interim, our biological findings regarding the interaction between different targets like KRAS and MCL-1 in CCA will help advance our understanding of how we can more effectively attack CCA from different angles to maximize the treatment effect.

Proposal Title:	Engineering an Effective CAR Treg Combination Therapy to Control VCA Rejection
Log Number:	RT220003
Current PI Name:	Megan Levings
Award Number:	HT9425-23-1-0626
Current Contracting Organization:	British Columbia, University of
Current Performing Organization:	British Columbia, University of
Web Approval Date:	09-14-2023

Rationale: Vascularized composite allotransplantation (VCA), including face, limb, and penile transplantation, is used to restore the appearance and function of patients with severe tissue loss. A limitation of current VCA approaches is that immunosuppressive drugs are used to stop the patient's immune system from rejecting the graft. These drugs have side effects, such as increasing the risk of cardiovascular and kidney disease, and globally suppress immunity, putting patients at risk of infection and cancer. Moreover, immunosuppression often fails to prevent rejection: >50% of patients have transient or permanent graft rejection. There is an urgent need to develop more effective and less toxic treatments to control immunity in VCA.

Our team is focused on harnessing one of the natural ways that the immune system uses to regulate itself to improve VCA outcomes. Specifically, a special type of white blood cell known as T regulatory cells (or Tregs) has a unique ability to prevent inappropriate immune responses. We know this function of Tregs can be applied in transplantation to promote long-term graft acceptance. We recently made a significant improvement in how Tregs can be used in transplantation by applying a cell engineering strategy involving the creation of a custom- designed protein called a chimeric antigen receptor (CAR). When CAR are expressed on Tregs, they are more potent and specific. However, we also know that CAR-Tregs on their own are not strong enough to control rejection, so we are studying how to use them with other types of immunosuppression that are less toxic than traditional forms. This work revealed the two exciting approaches that are the focus of this proposal: to combine CAR-Tregs with co-stimulation blockade and/or with a microparticle technology to improve their survival. Our combined expertise in CAR-Tregs, VCA immunotherapy, and immunosuppression positions us to make rapid progress in this highly innovative, yet clinically-feasible, approach.

Objectives: Our hypothesis is that, in combination with non-toxic immunosuppression, CAR-Treg therapy will be an effective way to minimize VCA rejection. Our objectives are to use mouse models of VCA to test whether CAR-Treg therapy prevents VCA rejection and to optimize combination therapies for a maximal efficacy.

Focus Areas: “Develop novel approaches for immune tolerance and “reduce immunosuppression toxicity.”

Patient Impact: This work will help make VCA accessible to patients who would benefit from transplant of tissues such as a face, arm, leg, and/or penis. In the past 15 years, ~40% of combat injuries sustained by Service Members involved severe extremity and craniofacial trauma. There are also many civilians who have accidents causing similar injuries. Many of these patients would benefit from VCA.

Clinical Applications, Benefits, and Risks: CAR-Treg therapy (with or without a novel immunosuppressive protocol) would be applicable in any patient undergoing VCA. The most likely first application would be in upper extremity (e.g., hand) transplantation since this is the most commonly performed form of this procedure, and hence there is the largest patient population. The benefit would be the opportunity to reduce

conventional immunosuppressive therapy and its associated side effects, as well as the overall risk of rejection. The main risk of CAR-Treg therapy is that it may not work as expected and lead to unwanted episodes of rejection. If this were to happen, then conventional immunosuppression could be quickly restored and, if deemed necessary, CAR-Tregs could be eliminated by use of T cell depleting drugs.

Time to Patient-Related Outcome: First-in-human trials of CAR Treg therapy are in progress, so by the end of this project (2026), there will be safety and efficacy data from these studies which can be used to support application in VCA. For combination therapy, some of our approaches use clinical-grade drugs and so could be readily implemented; others will require scale-up and more preclinical validation. Evidence of efficacy in the proposed studies would allow us to seek further funding to support the latter efforts.

Benefit to Patients/Families with Traumatic Injury: Improving the outcomes of VCA will change its benefit /risk ratio and make it a safer and more broadly applicable therapy. This would mean more people (military and general public) could benefit from this therapy, resulting in a significant improvement in quality of life for both the affected individuals and their families. Knowledge accrued in this project will also be transferable to other diseases that affect Service Members where similar methods to control immunity could be applied (i.e. end stage organ failure and autoimmunity).

Benefit to Reconstructive Transplant Research: Our work will establish a framework for the continuous optimization of CAR-Treg use (by the scientific community) and define potential for synergy with other established and innovative adjunct therapies. Moreover, our studies will identify whether unique interactions between VCA and Tregs need to be accounted for.

Proposal Title:	Engineering an Effective CAR Treg Combination Therapy to Control VCA Rejection
Log Number:	RT220003P1
Current PI Name:	Giorgio Raimondi
Award Number:	HT9425-23-1-0627
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	09-14-2023

Rationale: Vascularized composite allotransplantation (VCA), including face, limb, and penile transplantation, is used to restore the appearance and function of patients with severe tissue loss. A limitation of current VCA approaches is that immunosuppressive drugs are used to stop the patient's immune system from rejecting the graft. These drugs have side effects, such as increasing the risk of cardiovascular and kidney disease, and globally suppress immunity, putting patients at risk of infection and cancer. Moreover, immunosuppression often fails to prevent rejection: >50% of patients have transient or permanent graft rejection. There is an urgent need to develop more effective and less toxic treatments to control immunity in VCA.

Our team is focused on harnessing one of the natural ways that the immune system uses to regulate itself to improve VCA outcomes. Specifically, a special type of white blood cell known as T regulatory cells (or Tregs) has a unique ability to prevent inappropriate immune responses. We know this function of Tregs can be applied in transplantation to promote long-term graft acceptance. We recently made a significant improvement in how Tregs can be used in transplantation by applying a cell engineering strategy involving the creation of a custom- designed protein called a chimeric antigen receptor (CAR). When CAR are expressed on Tregs, they are more potent and specific. However, we also know that CAR-Tregs on their own are not strong enough to control rejection, so we are studying how to use them with other types of immunosuppression that are less toxic than traditional forms. This work revealed the two exciting approaches that are the focus of this proposal: to combine CAR-Tregs with co-stimulation blockade and/or with a microparticle technology to improve their survival. Our combined expertise in CAR-Tregs, VCA immunotherapy, and immunosuppression positions us to make rapid progress in this highly innovative, yet clinically-feasible, approach.

Objectives: Our hypothesis is that, in combination with non-toxic immunosuppression, CAR-Treg therapy will be an effective way to minimize VCA rejection. Our objectives are to use mouse models of VCA to test whether CAR-Treg therapy prevents VCA rejection and to optimize combination therapies for a maximal efficacy.

Focus Areas: “Develop novel approaches for immune tolerance and “reduce immunosuppression toxicity.”

Patient Impact: This work will help make VCA accessible to patients who would benefit from transplant of tissues such as a face, arm, leg, and/or penis. In the past 15 years, ~40% of combat injuries sustained by Service Members involved severe extremity and craniofacial trauma. There are also many civilians who have accidents causing similar injuries. Many of these patients would benefit from VCA.

Clinical Applications, Benefits, and Risks: CAR-Treg therapy (with or without a novel immunosuppressive protocol) would be applicable in any patient undergoing VCA. The most likely first application would be in upper extremity (e.g., hand) transplantation since this is the most commonly performed form of this procedure, and hence there is the largest patient population. The benefit would be the opportunity to reduce

conventional immunosuppressive therapy and its associated side effects, as well as the overall risk of rejection. The main risk of CAR-Treg therapy is that it may not work as expected and lead to unwanted episodes of rejection. If this were to happen, then conventional immunosuppression could be quickly restored and, if deemed necessary, CAR-Tregs could be eliminated by use of T cell depleting drugs.

Time to Patient-Related Outcome: First-in-human trials of CAR Treg therapy are in progress, so by the end of this project (2026), there will be safety and efficacy data from these studies which can be used to support application in VCA. For combination therapy, some of our approaches use clinical-grade drugs and so could be readily implemented; others will require scale-up and more preclinical validation. Evidence of efficacy in the proposed studies would allow us to seek further funding to support the latter efforts.

Benefit to Patients/Families with Traumatic Injury: Improving the outcomes of VCA will change its benefit /risk ratio and make it a safer and more broadly applicable therapy. This would mean more people (military and general public) could benefit from this therapy, resulting in a significant improvement in quality of life for both the affected individuals and their families. Knowledge accrued in this project will also be transferable to other diseases that affect Service Members where similar methods to control immunity could be applied (i.e. end stage organ failure and autoimmunity).

Benefit to Reconstructive Transplant Research: Our work will establish a framework for the continuous optimization of CAR-Treg use (by the scientific community) and define potential for synergy with other established and innovative adjunct therapies. Moreover, our studies will identify whether unique interactions between VCA and Tregs need to be accounted for.

Proposal Title:	Targeting the Natural Immunosuppressive Capabilities of Endogenous Beta-Adrenergic Neural Pathways to Prolong VCA Graft Survival and Reduce Toxicity Risks
Log Number:	RT220006
Current PI Name:	Elizabeth Repasky
Award Number:	HT9425-23-1-0529
Current Contracting Organization:	Health Research Inc., Roswell Park Division
Current Performing Organization:	Health Research Inc., Roswell Park Division
Web Approval Date:	09-15-2023

Today, thanks to advances in emergency care and surgical procedures, military personnel can survive catastrophic limb and facial injuries incurred during combat. Civilians incur similarly devastating losses as the result of accidents or medically necessary surgical procedures. But these individuals then face challenging physical disabilities and psychological disorders that take an incalculable toll not only personally but on families and caregivers as well. Prosthetic devices are useful, particularly for lower limb deficits, but many people find upper limb prostheses unwieldy and lacking in sensitivity. More recently, surgical procedures have been perfected to allow for successful transplantation of faces, upper limbs, and hands from cadaver donors. This type of specialized transplant contains several different tissues (skin, bone, blood vessels, connective tissue, muscles, and nerves) and the procedure is known as vascular composite allotransplantation (VCA). In the best-case scenarios, the blood vessels to these transplants can be surgically connected to the vascular system of the recipient and the nerves regenerate so that the person regains sensation and normal function in the transplant. This is truly life- altering. The problem is that the recipients' own immune system tries to reject the transplant, so patients must take very high doses of immunosuppressant drugs for the rest of their lives. These drugs have significant side-effects and include increasing susceptibility to life-threatening infections and cancers. To date, over 100 VCAs have been performed, and 85% of these patients experience one or more episode(s) of rejection which begins a vicious cycle in which immunosuppressive drugs must be intermittently discontinued which can lead to rejection of the graft. Since this surgery is performed solely to improve quality of life and is not "life-saving," there are ethical issues associated with these risks.

The primary research award focus area we are addressing in our proposal is to develop less-toxic regimens to improve VCA survival by dampening immune rejection at the graft site. This proposal leverages extensive work in our lab showing for the first time that the nervous system can be harnessed to modulate immune responses for clinical benefit in cancer and bone marrow transplantation. Our new idea is that we can manipulate the mechanisms by which the nervous system naturally regulates immune responses by repurposing drugs that are commonly prescribed for asthma (beta-agonists). These drugs target mediators that are part of our bodies' own natural relay system that allows the sympathetic nervous system to activate the "fight or flight" response and regulate immune responses. Our hypothesis is that we can develop a protocol that combines the immunosuppressive actions of beta-agonists and reduced doses of toxic immunosuppressant drugs currently used to maintain VCA. This is based on our recently published paper showing that we can safely prolong survival of VCA transplants just by adding a beta-agonist to a standard regimen of immunosuppressive drug (Tacrolimus) that, by itself, is not effective. In this proposal we will use our newly established and validated mouse models of VCA to (1) optimize the combination therapy, (2) study how beta-agonists suppress both immediate and long-term vascular damage and graft rejection driven by an over-active immune response, and (3) study details of how beta-agonists impact immune cells. These

studies will examine novel immunological endpoints to monitor the effectiveness of the new combination drug regimen that could be useful as new biomarkers in patients who could receive this new therapeutic strategy in the future.

Overall, our study addresses several points in the award focus areas, including studying why VCAs induce strong immune responses, improving immune tolerance, determining what special types of immunosuppression are needed to protect VCA, looking for a non-invasive diagnostic tool of rejection, and determining how our treatment regimen affects recovery of functionality of the VCA. The need for improved immunosuppressive regimens is clear. Because beta-agonists are already in use in the clinic for other medical problems, their safety profile is already well established. Thus, clinical trials testing the ideas emanating from our research are immediately feasible. The data from these experiments will provide a clear rationale that can be used to develop an effective clinical strategy that will benefit people given VCA by maintaining the grafts while reducing the dose (and toxic side effects) of currently used immunosuppressive drugs.

Proposal Title:	A Novel Point-of-Care Rejection and Immunosuppression Monitoring Assay (PRIMA) for Vascularized Composite Allotransplantation (VCA)
Log Number:	RT220010
Current PI Name:	Trong Nguyen
Award Number:	HT9425-23-1-0600
Current Contracting Organization:	Intelligent Optical Systems, Inc.
Current Performing Organization:	Intelligent Optical Systems, Inc.
Web Approval Date:	09-14-2023

Trauma is common in both military and civilian settings, and burns, amputation, and other non-salvageable tissue injuries can significantly impact form and function. Vascularized composite allotransplantation (VCA) involves transplants of multiple tissues such as muscle, bone, nerve, and skin as functional units). VCA can serve to replace lost or damaged tissue following a traumatic event and can have a significant impact on the restoration of form and function. VCA has gained increasing importance in clinical practice and may evolve into an important component of multidisciplinary approaches to reconstruction after severe injuries. To date, sources have reported as many as 150 upper extremity and 40 face transplants performed worldwide.

VCA such as hand or face transplants require life-long immunosuppressant drugs, so the transplant is not rejected. Tacrolimus is an FDA-approved drug used in majority of VCA protocols. Too much immunosuppression with tacrolimus can lead to kidney damage (requiring kidney transplantation), cancer, or infections, and too little immunosuppression can increase risks of transplant rejection. Timely diagnosis and treatment of acute and chronic rejection is also equally important to ensure graft survival. In order to improve life enhancing benefits of VCA in VCA, the risks of lifelong, high-dose or multi-drug systemic immunosuppression must be reduced but also rejection has to better managed.

It is thus critical to frequently monitor drug levels of tacrolimus, as well as levels of markers of kidney function in VCA patients, but also be able to predict when rejection episodes may occur or how severe they may be when they happen so that they receive the right dose of tacrolimus to help grafts to survive without the risk of adverse effects on the kidney. Current methods of laboratory monitoring of tacrolimus level and kidney markers require complicated or costly tests that are time-consuming and require patients to access special laboratories. This may cause missed laboratory tests/visits or even delayed reporting of results such as drug levels leading to improper management of patients with increased risk of toxic side effects.

The PRIMA technology will combine measurements of key inflammation biomarkers with immunosuppressant drug levels and early kidney function biomarkers in an integrated, easy-to-use, point- of-care device that uses a tiny amount of fingerstick sampling of blood, similar in format to a glucose meter. Our technology builds on our experience with other rapid assays in VCA for immunosuppressant drug levels that have confirmed their accuracy in prior studies. When combined with kidney damage and inflammation markers, this technology will help to both identify rejection episodes early and follow treatment to limit rejection and toxicity.

Our PRIMA technology will help to both individualize drug treatment as well as predict rejection risk to limit drug toxicity and immunologic attrition of the graft. The ease of use, low cost, and rapid analysis enabled by the proposed technology represents a significant advancement to better enable monitoring and optimization of immunosuppressant drug levels, protocol adherence, and treatment progression. Improving

adherence and optimizing existing immunosuppressant drug protocols is a near-term goal that we believe can be impactful as a means to reduce long term toxicity of immunosuppression and improve the safety and efficacy of protocols.

Proposal Title:	A Novel Point-of-Care Rejection and Immunosuppression Monitoring Assay (PRIMA) for Vascularized Composite Allotransplantation (VCA)
Log Number:	RT220010P1
Current PI Name:	Vijay Gorantla
Award Number:	HT9425-23-1-0601
Current Contracting Organization:	Wake Forest University Health Sciences
Current Performing Organization:	Wake Forest University Health Sciences
Web Approval Date:	09-14-2023

Trauma is common in both military and civilian settings, and burns, amputation, and other non-salvageable tissue injuries can significantly impact form and function. Vascularized composite allotransplantation (VCA) involves transplants of multiple tissues such as muscle, bone, nerve, and skin as functional units). VCA can serve to replace lost or damaged tissue following a traumatic event and can have a significant impact on the restoration of form and function. VCA has gained increasing importance in clinical practice and may evolve into an important component of multidisciplinary approaches to reconstruction after severe injuries. To date, sources have reported as many as 150 upper extremity and 40 face transplants performed worldwide.

VCA such as hand or face transplants require life-long immunosuppressant drugs, so the transplant is not rejected. Tacrolimus is an FDA-approved drug used in majority of VCA protocols. Too much immunosuppression with tacrolimus can lead to kidney damage (requiring kidney transplantation), cancer, or infections, and too little immunosuppression can increase risks of transplant rejection. Timely diagnosis and treatment of acute and chronic rejection is also equally important to ensure graft survival. In order to improve life enhancing benefits of VCA in VCA, the risks of lifelong, high-dose or multi-drug systemic immunosuppression must be reduced but also rejection has to better managed.

It is thus critical to frequently monitor drug levels of tacrolimus, as well as levels of markers of kidney function in VCA patients, but also be able to predict when rejection episodes may occur or how severe they may be when they happen so that they receive the right dose of tacrolimus to help grafts to survive without the risk of adverse effects on the kidney. Current methods of laboratory monitoring of tacrolimus level and kidney markers require complicated or costly tests that are time-consuming and require patients to access special laboratories. This may cause missed laboratory tests/visits or even delayed reporting of results such as drug levels leading to improper management of patients with increased risk of toxic side effects.

The PRIMA technology will combine measurements of key inflammation biomarkers with immunosuppressant drug levels and early kidney function biomarkers in an integrated, easy-to-use, point- of-care device that uses a tiny amount of fingerstick sampling of blood, similar in format to a glucose meter. Our technology builds on our experience with other rapid assays in VCA for immunosuppressant drug levels that have confirmed their accuracy in prior studies. When combined with kidney damage and inflammation markers, this technology will help to both identify rejection episodes early and follow treatment to limit rejection and toxicity.

Our PRIMA technology will help to both individualize drug treatment as well as predict rejection risk to limit drug toxicity and immunologic attrition of the graft. The ease of use, low cost, and rapid analysis enabled by the proposed technology represents a significant advancement to better enable monitoring and optimization of immunosuppressant drug levels, protocol adherence, and treatment progression. Improving

adherence and optimizing existing immunosuppressant drug protocols is a near-term goal that we believe can be impactful as a means to reduce long term toxicity of immunosuppression and improve the safety and efficacy of protocols.

Proposal Title:	A Multimodal Approach to Reduce VCA Immunogenicity and Improve Functional Outcome
Log Number:	RT220017
Current PI Name:	George Bittner
Award Number:	HT9425-23-1-0019
Current Contracting Organization:	Texas, University of, at Austin
Current Performing Organization:	Texas, University of, at Austin
Web Approval Date:	09-14-2023

Alignment with Focus Area: This application is directed toward the RTRP Multidisciplinary Investigator-Initiated Research Award for Vascularized Composite Allotransplantation (VCA), or limb transplant, face transplant, etc. Specifically, this is a research proposal to determine how prolonged denervation of VCAs influences VCA immunogenicity utilizing a novel polyethylene glycol (PEG- fusion) technology and to develop a novel localized immunosuppression (ISN) technology to maintain ISN by less-toxic regimens of maintenance ISN.

Problem: VCA is the biologic (non-prosthetic) restorative solution for military personnel and civilians with catastrophic injuries to their face, limbs, and other non-vital composite tissues. While similar ISN regimens are used for both solid organ transplantations (SOTs) and VCA, VCAs are more immune reactive, or immunogenic, and incur a higher rate of acute rejection than SOTs. Decreasing acute or chronic rejection and improving functional outcome would impact the number of amputated or excised VCA.

Solutions: Our interdisciplinary team composed of experienced VCA surgeons and academic scientists proposes a multi-modal project to reduce VCA immunogenicity and develop improved methods of ISN. We propose to address aspects of unique VCA immunogenicity due to prolonged lack of sensation and motor function produced by the slow and incomplete process of peripheral nerve regeneration (years for most VCA recipients). Lack of nerve function for prolonged periods of time cause at least three changes that increase immunogenicity: impaired blood and lymph flow due to poor vascular tone and muscle inactivity, atrophy of muscles, and increasing risk and severity of traumatic injury to the VCA, which can provoke episodes of acute rejection. Our team has developed a novel technique to repair severed axons (PEG-fusion) that will immediately reconnect (rather than slow regeneration by axonal outgrowth) severed nerves between the donor and host. This restores electrical activity to the nerves and produces a highly accelerated return of function and sensation. This rapid return of nerve function is expected to prevent atrophy, improve blood flow, and minimize traumatic injury to the VCA and optimize overall function.

Our team is also developing a novel technology to locally suppress the immune reaction to VCAs. Current clinical practice often uses systemic anti-inflammatories (corticosteroids, FK506) to control chronic demyelinating disease and other inflammation-linked pathologies. Clinically, however, systemic use of ISNs can have adverse effects on kidneys and immune response to pathogens for severely injured wounded Warriors and civilians. In contrast, in pilot studies, we have data showing that local immune suppression using methyl prednisolone (MP) can reduce the local immune response.

Action: Two clinical trials are underway for PEG-fusion repair, one of simple nerve lacerations by Neuraptive Therapeutics, Inc., and another trial led by members of our team to repair single lacerations and segmental loss injuries using autografts. There are no obstacles that would prevent direct translation of PEG-fusion for VCAs in human patients, and safety has been demonstrated. Localized ISN is an innovative, yet early-stage technology applicable to VCAs, in addition to many other conditions where localized ISN would be safer and less expensive than whole-body ISN.

Military Benefit: Recipients of VCAs currently use whole-body ISN that puts them at higher risk for pathogens and cancer. It is expensive and painful to clinically monitor. Recipients also currently must wait years to regain sensation and motor function, where consequences of prolonged denervation likely contribute to immunogenicity and increased episodes of rejection. The outcomes of our research most relevant for military Service Members with VCAs are that they would regain motor and sensation within week and months (rather than years) and have a therapy for localized ISN.

Proposal Title:	A Multimodal Approach to Reduce VCA Immunogenicity and Improve Functional Outcome
Log Number:	RT220017P1
Current PI Name:	Jaimie Shores
Award Number:	HT9425-23-2-0020
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	09-14-2023

Alignment with Focus Area: This application is directed toward the RTRP Multidisciplinary Investigator-Initiated Research Award for Vascularized Composite Allotransplantation (VCA), or limb transplant, face transplant, etc. Specifically, this is a research proposal to determine how prolonged denervation of VCAs influences VCA immunogenicity utilizing a novel polyethylene glycol (PEG- fusion) technology and to develop a novel localized immunosuppression (ISN) technology to maintain ISN by less-toxic regimens of maintenance ISN.

Problem: VCA is the biologic (non-prosthetic) restorative solution for military personnel and civilians with catastrophic injuries to their face, limbs, and other non-vital composite tissues. While similar ISN regimens are used for both solid organ transplantations (SOTs) and VCA, VCAs are more immune reactive, or immunogenic, and incur a higher rate of acute rejection than SOTs. Decreasing acute or chronic rejection and improving functional outcome would impact the number of amputated or excised VCA.

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Proposal Title:	A Multimodal Approach to Reduce VCA Immunogenicity and Improve Functional Outcome
Log Number:	RT220017P2
Current PI Name:	Casey Sabbag
Award Number:	HT9425-23-2-0021
Current Contracting Organization:	Metis Foundation
Current Performing Organization:	Texas, University of, Health Science Center at San Antonio
Web Approval Date:	09-14-2023

Alignment with Focus Area: This application is directed toward the RTRP Multidisciplinary Investigator-Initiated Research Award for Vascularized Composite Allotransplantation (VCA), or limb transplant, face transplant, etc. Specifically, this is a research proposal to determine how prolonged denervation of VCAs influences VCA immunogenicity utilizing a novel polyethylene glycol (PEG- fusion) technology and to develop a novel localized immunosuppression (ISN) technology to maintain ISN by less-toxic regimens of maintenance ISN.

Problem: VCA is the biologic (non-prosthetic) restorative solution for military personnel and civilians with catastrophic injuries to their face, limbs, and other non-vital composite tissues. While similar ISN regimens are used for both solid organ transplantations (SOTs) and VCA, VCAs are more immune reactive, or immunogenic, and incur a higher rate of acute rejection than SOTs. Decreasing acute or chronic rejection and improving functional outcome would impact the number of amputated or excised VCA.

Solutions: Our interdisciplinary team composed of experienced VCA surgeons and academic scientists proposes a multi-modal project to reduce VCA immunogenicity and develop improved methods of ISN. We propose to address aspects of unique VCA immunogenicity due to prolonged lack of sensation and motor function produced by the slow and incomplete process of peripheral nerve regeneration (years for most VCA recipients). Lack of nerve function for prolonged periods of time cause at least three changes that increase immunogenicity: impaired blood and lymph flow due to poor vascular tone and muscle inactivity, atrophy of muscles, and increasing risk and severity of traumatic injury to the VCA, which can provoke episodes of acute rejection. Our team has developed a novel technique to repair severed axons (PEG-fusion) that will immediately reconnect (rather than slow regeneration by axonal outgrowth) severed nerves between the donor and host. This restores electrical activity to the nerves and produces a highly accelerated return of function and sensation. This rapid return of nerve function is expected to prevent atrophy, improve blood flow, and minimize traumatic injury to the VCA and optimize overall function.

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Military Benefit: Recipients of VCAs currently use whole-body ISN that puts them at higher risk for pathogens and cancer. It is expensive and painful to clinically monitor. Recipients also currently must wait years to regain sensation and motor function, where consequences of prolonged denervation likely contribute to immunogenicity and increased episodes of rejection. The outcomes of our research most relevant for military Service Members with VCAs are that they would regain motor and sensation within week and months (rather than years) and have a therapy for localized ISN.

Proposal Title:	Localized and Inflammation-Responsive Immunotherapy of VCA Rejection
Log Number:	RT220021
Current PI Name:	Giorgio Raimondi
Award Number:	HT9425-23-1-0543
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	09-14-2023

Close to 40% of combat injuries sustained by Service Members in the last 15 years involved severe extremity and craniofacial trauma. Despite the best reconstructive efforts using native tissue, these injuries frequently result in need of amputations. Eighty percent of amputees are then permanently retired while experiencing amputation-related disabilities that include poor professional and social interactions. Vascularized Composite Allotransplantation (VCA) has become a viable approach for functional restoration, and it enables patients to return to a quality of life higher than that afforded by any currently available prosthesis. However, the toxic and debilitating side effects (e.g., infections, nephrotoxicity, cardiovascular disease, diabetes, and cancer) of the multi-drug immunosuppressive therapy necessary to preserve the transplanted limb from rejection counterbalances its benefits and prevents widespread use. In particular, the use of calcineurin inhibitors (e.g., tacrolimus), the current mainstay therapy in VCA, is associated with substantial morbidity and is relatively ineffective in preventing rejection long-term. Minimizing the need for immunosuppression or even altering the recipient's immune system to tolerate the transplant (by replacing calcineurin inhibitors with tolerance sparing/promoting drugs) will impact the life of a multitude of Service Members by pushing the benefit/risks ratio toward making VCA safer and more broadly applicable.

Biologic agents such as Belatacept (which reduces the activation of lymphocytes) have been developed to overcome this limitation. Despite a significant reduction in side effects, clinical studies have showed that Belatacept is not as effective as an immunosuppressant. Our own studies, however, indicate that the capacity of Belatacept to regulate the immune response against a transplant can be amplified by the co-administration of the JAK-inhibitor Tofacitinib. This combination limits the activation of the destructive arms of the immune system while promoting the function of the regulatory ones. The only limiting factors in the translation of this approach to a clinical application are the difficulties in maintaining the proper concentration of Tofacitininb in the body and the possible toxic effect associated with systemic exposure. To solve this problem, we propose to exploit cutting-edge advances in biomaterial design for drug delivery and improved understanding of graft rejection via a collaboration between three Principal Investigators with complementary expertise.

The goal of our study is to optimize and demonstrate the efficacy of a novel and clinically relevant drug delivery platform designed to suppress the rejection response in a localized and tunable fashion via a regimen that is permissive of immunomodulatory mechanisms. We are developing a novel dual-component therapeutic delivery platform that delivers JAK inhibitors from both microcrystalline drug deposits and from lipid nanoparticles encapsulated within a gel network. Inhibitors are released first from the microcrystalline phase locally to modulate infiltrating immune cells. Nanoparticles are released later in response to local enzymatic activity at the transplant site that occurs during rejection. Released particles traffic to the lymphoid tissues, the site of priming of the rejection response, to deliver their payload. This new material is easily syringe injected into or next to the transplant during surgery. Combining this material with the administration of Belatacept will render a regulated and localized synergism that our experimental data indicate is feasible and very effective in modulating the rejection response.

Thanks to the manufacturing scalability of the biomaterials investigated (capable to meet the demand of clinical use), our proposed studies in a small animal model of VCA will generate the necessary data to initiate a follow-up study in a preclinical large animal model and seek FDA approval. Independently from the outcome, our studies will be vastly informative for the nascent field of bioengineering for the manipulation of transplant rejection. If successful, this strategy will be transformative for VCA patients (service members and civilians), as it will change how immunosuppression is delivered and actuated and will avoid deleterious side effects, while also minimizing the risk of long-term graft loss. The knowledge accrued through this study would improve our understanding of how to optimize drug delivery to maximize the regulation of a transplant rejection response (beyond VCA). It will also impact a broader patient population, where targeted and controlled immunomodulation is needed, such as vaccination or cancer treatment. The proposed strategy addresses Focus Area: Reduce the risks of VCA-associated immunosuppression.

Subtopic: Develop novel approaches for achieving VCA immune tolerance / Identify unique immunotherapy requirements for VCA compared to solid organ transplants.

Proposal Title:	Localized and Inflammation-Responsive Immunotherapy of VCA Rejection
Log Number:	RT220021P1
Current PI Name:	Joel Schneider
Award Number:	HT9425-23-1-0544
Current Contracting Organization:	The Geneva Foundation
Current Performing Organization:	National Cancer Institute
Web Approval Date:	09-14-2023

Close to 40% of combat injuries sustained by Service Members in the last 15 years involved severe extremity and craniofacial trauma. Despite the best reconstructive efforts using native tissue, these injuries frequently result in need of amputations. Eighty percent of amputees are then permanently retired while experiencing amputation-related disabilities that include poor professional and social interactions. Vascularized Composite Allotransplantation (VCA) has become a viable approach for functional restoration, and it enables patients to return to a quality of life higher than that afforded by any currently available prosthesis. However, the toxic and debilitating side effects (e.g., infections, nephrotoxicity, cardiovascular disease, diabetes, and cancer) of the multi-drug immunosuppressive therapy necessary to preserve the transplanted limb from rejection counterbalances its benefits and prevents widespread use. In particular, the use of calcineurin inhibitors (e.g., tacrolimus), the current mainstay therapy in VCA, is associated with substantial morbidity and is relatively ineffective in preventing rejection long-term. Minimizing the need for immunosuppression or even altering the recipient's immune system to tolerate the transplant (by replacing calcineurin inhibitors with tolerance sparing/promoting drugs) will impact the life of a multitude of Service Members by pushing the benefit/risks ratio toward making VCA safer and more broadly applicable.

Biologic agents such as Belatacept (which reduces the activation of lymphocytes) have been developed to overcome this limitation. Despite a significant reduction in side effects, clinical studies have showed that Belatacept is not as effective as an immunosuppressant. Our own studies, however, indicate that the capacity of Belatacept to regulate the immune response against a transplant can be amplified by the co-administration of the JAK-inhibitor Tofacitinib. This combination limits the activation of the destructive arms of the immune system while promoting the function of the regulatory ones. The only limiting factors in the translation of this approach to a clinical application are the difficulties in maintaining the proper concentration of Tofacitininb in the body and the possible toxic effect associated with systemic exposure. To solve this problem, we propose to exploit cutting-edge advances in biomaterial design for drug delivery and improved understanding of graft rejection via a collaboration between three Principal Investigators with complementary expertise.

The goal of our study is to optimize and demonstrate the efficacy of a novel and clinically relevant drug delivery platform designed to suppress the rejection response in a localized and tunable fashion via a regimen that is permissive of immunomodulatory mechanisms. We are developing a novel dual-component therapeutic delivery platform that delivers JAK inhibitors from both microcrystalline drug deposits and from lipid nanoparticles encapsulated within a gel network. Inhibitors are released first from the microcrystalline phase locally to modulate infiltrating immune cells. Nanoparticles are released later in response to local enzymatic activity at the transplant site that occurs during rejection. Released particles traffic to the lymphoid tissues, the site of priming of the rejection response, to deliver their payload. This new material is easily syringe injected into or next to the transplant during surgery. Combining this material with the administration of Belatacept will render a regulated and localized synergism that our experimental data indicate is feasible and very effective in modulating the rejection response.

Thanks to the manufacturing scalability of the biomaterials investigated (capable to meet the demand of clinical use), our proposed studies in a small animal model of VCA will generate the necessary data to initiate a follow-up study in a preclinical large animal model and seek FDA approval. Independently from the outcome, our studies will be vastly informative for the nascent field of bioengineering for the manipulation of transplant rejection. If successful, this strategy will be transformative for VCA patients (service members and civilians), as it will change how immunosuppression is delivered and actuated and will avoid deleterious side effects, while also minimizing the risk of long-term graft loss. The knowledge accrued through this study would improve our understanding of how to optimize drug delivery to maximize the regulation of a transplant rejection response (beyond VCA). It will also impact a broader patient population, where targeted and controlled immunomodulation is needed, such as vaccination or cancer treatment. The proposed strategy addresses Focus Area: Reduce the risks of VCA-associated immunosuppression.

Subtopic: Develop novel approaches for achieving VCA immune tolerance / Identify unique immunotherapy requirements for VCA compared to solid organ transplants.

Proposal Title:	Localized and Inflammation-Responsive Immunotherapy of VCA Rejection
Log Number:	RT220021P2
Current PI Name:	Julia Patrone
Award Number:	HT9425-23-1-0545
Current Contracting Organization:	Johns Hopkins University, Applied Physics Laboratory
Current Performing Organization:	Johns Hopkins University, Applied Physics Laboratory
Web Approval Date:	09-14-2023

Close to 40% of combat injuries sustained by Service Members in the last 15 years involved severe extremity and craniofacial trauma. Despite the best reconstructive efforts using native tissue, these injuries frequently result in need of amputations. Eighty percent of amputees are then permanently retired while experiencing amputation-related disabilities that include poor professional and social interactions. Vascularized Composite Allotransplantation (VCA) has become a viable approach for functional restoration, and it enables patients to return to a quality of life higher than that afforded by any currently available prosthesis. However, the toxic and debilitating side effects (e.g., infections, nephrotoxicity, cardiovascular disease, diabetes, and cancer) of the multi-drug immunosuppressive therapy necessary to preserve the transplanted limb from rejection counterbalances its benefits and prevents widespread use. In particular, the use of calcineurin inhibitors (e.g., tacrolimus), the current mainstay therapy in VCA, is associated with substantial morbidity and is relatively ineffective in preventing rejection long-term. Minimizing the need for immunosuppression or even altering the recipient's immune system to tolerate the transplant (by replacing calcineurin inhibitors with tolerance sparing/promoting drugs) will impact the life of a multitude of Service Members by pushing the benefit/risks ratio toward making VCA safer and more broadly applicable.

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Subtopic: Develop novel approaches for achieving VCA immune tolerance / Identify unique immunotherapy requirements for VCA compared to solid organ transplants.

Proposal Title:	Immunogenicity in Subzero Stored VCA
Log Number:	RT220045
Current PI Name:	Byoung Chol Oh
Award Number:	HT9425-23-1-0593
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	Pending

No lay abstract provided

Proposal Title:	Local Delivery of Bioengineered Mesenchymal Stem Cell-Derived Extracellular Vesicles for Targeted Immunomodulation in Vascularized Composite Allotransplantation
Log Number:	RT220050
Current PI Name:	Wensheng Zhang
Award Number:	HT9425-23-1-0555
Current Contracting Organization:	Metis Foundation
Current Performing Organization:	Texas, University of, Health Science Center at San Antonio
Web Approval Date:	Pending

No lay abstract provided

Proposal Title:	Applying Enhanced Electroceutical Treatments and Identifying Electrical Biomarkers for SCI
Log Number:	SC220001
Current PI Name:	Darren Svirskis
Award Number:	HT9425-23-1-0492
Current Contracting Organization:	Auckland, University of
Current Performing Organization:	Auckland, University of
Web Approval Date:	06-12-2023

Service Members on the front lines of battle can suffer traumatic spinal cord injuries (SCIs) leaving them to contend with a compromised quality of life as a result of paralysis. Service Members experience a higher rate of SCIs compared to civilians, with 429 spinal injuries per million people per year, compared to 54 per million per year in the civilian population. There are very few treatment options available for people who have suffered a SCI and the body's natural healing mechanisms are generally not enough to provide meaningful recovery.

Paralysis in SCI is caused by damage to the spinal nerves that connect the brain to the rest of the body. One emerging therapy that has shown potential is the regeneration of spinal nerves using electrical stimulation. We have developed an ultra-thin implant that can be placed directly over the injured area of the spinal cord to encourage healing. This implant contains electrodes that can both record the natural electrical activity in the spinal cord and create an electric field through the damaged tissue to stimulate nerve regeneration. By regenerating damaged spinal nerves, we aim to reestablish the connection between the brain and the body and restore movement to patients with paralysis.

In this project we have two main objectives. First, we will demonstrate electrical stimulation can reliably promote healing by testing our implant in a preclinical animal model of SCI. We will use cutting-edge materials that can deliver safe and strong electrical stimulation directly to the spinal cord. Second, we will use our implant to record electrical activity from the spinal cord and the injury site, allowing us to understand how signaling occurs in a healthy cord, and how it changes after injury and during treatment and recovery.

Successful completion of this research will demonstrate that electrical stimulation treatments can regenerate damaged spinal nerves, resulting in functional improvements in a preclinical model of SCI. At the completion of this grant, we will require a translational phase to prepare our approach for patients, and subsequently, expect to achieve person-related outcomes in an estimated 8 years from our current position.

Proposal Title:	An Effective Strategy to Promote Axon Regeneration and Functional Recovery After Spinal Cord Injury
Log Number:	SC220048
Current PI Name:	Shuxin Li
Award Number:	HT9425-23-1-0611
Current Contracting Organization:	Temple University-Of The Commonwealth System of Higher Education
Current Performing Organization:	Temple University-Of The Commonwealth System of Higher Education
Web Approval Date:	08-17-2023

Spinal cord injuries (SCI) cause devastating paralysis, loss of sensation below the level of the injury, and deficits of various autonomic functions, including bowel, bladder, and sexual functions. People who suffer from SCI usually lose the ability to walk and other essential abilities to live independently. Loss of bladder function greatly increases the risks of urinary tract infections, and SCI frequently causes other major long-term health risks, such as deep vein thrombosis, bone fractures, pressure sores, and autonomic dysreflexia, all of which are life-threatening. Currently, treatments to reverse paralysis and return other lost functions are not available, and people who have suffered SCI are permanently disabled.

It has long been recognized that the greatest hope for functional recovery is to regenerate nerve fibers and restore their connections that are damaged by SCI. It is extremely challenging to achieve robust regeneration in the central nervous system (CNS) and to regain lost functions in adult mammals. However, in the past decades, researchers have made numerous breakthrough discoveries to regenerate damaged nerve fibers by targeting different genes and signaling pathways, including phosphatase and tensin homolog and some transcription factors for controlling neuronal growth. However, none of these approaches have been translated to clinics yet, and there is a persistent need to identify better targets and improved therapeutic methods. The best gene targets are probably those with the potential to impact multiple genes simultaneously. Among them, the transcriptional factor ZNF362 appears very important for controlling age-dependent decline in nerve regeneration in mammals.

This project targets ZNF362, a critical signaling regulator downstream of the let-7 miRNA. We have recently shown that Let-7-associated genes are critical for regulating neural cell growth in adult mammals. Among them, ZNF362 plays an important role in controlling the growth failure of mature nerve cells after CNS injury. We plan to study the potential essential role of ZNF362 in controlling the age-dependent loss of nerve growth capacity in adult CNS and stimulate robust nerve regeneration and functional recovery after SCI by inhibiting ZNF362. We designed the small sequence-targeting peptide drugs that block ZNF362 function selectively. Our pilot studies demonstrated that treatments with our novel peptides against ZNF362 promoted robust regrowth of motor and sensory CNS nerves in adult rodents. We thus propose to evaluate the efficacy of our novel peptide drugs in promoting nerve regeneration and functional recovery after SCI using adult rat models.

Aim 1: We will determine whether four ZNF362 antagonist peptides promote in vivo nerve regrowth and functional recovery in adult rats with transection SCI, aiming to select the top two optimal peptides. We have verified the high effectiveness of these four peptides in promoting nerve growth in vitro.

Aim 2: We will validate the efficiency of the top two optimal ZNF362 peptides in promoting robust regrowth of motor nerve tracts and locomotor recovery in adult rats with contusion SCI, a translational model mimicking the lesions of most SCI patients.

We have shown that transgenic ZNF362 deletion promotes robust CNS nerve regeneration in adult mice and that our ZNF362 peptides are highly effective for promoting nerve regrowth in vitro and in vivo. We anticipate that our novel peptide drugs will significantly advance our ability to treat SCI in adult mammals.

Impact: Our proposed studies using contusion SCI and delayed drug delivery have the potential to lead to important new treatments for SCI patients. The results from our initial preclinical experiments with acute and subacute injuries should apply to the great numbers of individuals who suffer significant SCI every year. If our peptides are successful with contusion SCI, we plan to move this work to a peptide safety assessment and more translational studies in clinically relevant models, including cervical and more severe SCI. Our future studies will direct to chronic SCI treatments. The practical advantages of systemically deliverable drugs over the transgenic approaches and highly invasive therapies employed before should facilitate translation to clinical trials with SCI patients. Therefore, the outcome is likely to advance to an investigational new drug application, and the success would significantly contribute to both improving patient quality of life and advancing the field of SCI research.

Relevance to Military Health: This proposal seeks to develop noninvasive and highly successful regenerative-based therapies for SCI patients in both the military and general American public. The success of this project may improve the quality of life of many people who have suffered SCI and reduce the SCI-associated health and financial burdens to patients, families, military, and public.

Proposal Title:	A Novel Therapy to Improve Reproductive Potential in Men with Spinal Cord Injury (SCI)
Log Number:	SC220060
Current PI Name:	Emad Ibrahim
Award Number:	HT9425-23-1-0720
Current Contracting Organization:	Miami, University of, Coral Gables
Current Performing Organization:	Miami, University of, Coral Gables
Web Approval Date:	10-03-2023

The majority of men with a spinal cord injury are in the age range where raising a family is a major life goal. Ninety percent of these men will be infertile in large part because the spinal cord injury has caused a unique set of problems. (1) Most men with spinal cord injury cannot ejaculate with sexual activity so they are not able to get their wives pregnant in the usual manner; however, there are techniques available to easily obtain sperm in these cases. (2) Once sperm are obtained, 90% of the time, the quality of the sperm is not normal, with the majority of the sperm not moving or dead, and the sperm that are moving are moving poorly. To date, improvements to the sperm quality have been seen in the test tube in the laboratory. Recently, a pilot study was conducted on volunteer men with spinal cord injury which showed that there may be a type of medication which may be given orally to improve the infertile condition. This pilot study was the first to report an improvement in sperm motility after administration of an oral medication in men with a spinal cord injury. The improvement was seen within 1 month of taking the medicine.

This research study will test the effect of a medicine, taken orally, that blocks a particular chemical pathway in the body that is linked to a cause of infertility (poor sperm motility) in men with a spinal cord injury. More specifically, it will determine if the infertility (sperm motility) is improved and if the medicine is well-tolerated by the subjects who are taking it. The safety of the medicine will be tested by examining the genetic material (DNA) the sperm carries inside of it. The study will also examine different dosages of the medicine.

If the medicine is shown to be safe, the risks of giving an oral medication to improve fertility will be limited to the potential side effects of the medicine (so far minimal) and the benefits will be wide ranging. The benefits will impact social and quality of life issues for the man with a spinal cord injury and his family. There will also be benefits to the health care delivery system, and the field of medical research. Most men with spinal cord injury must depend on some form of medical assistance for semen retrieval. The choice of an assisted reproductive technique to achieve a pregnancy offered to any couple (with or without a male partner with spinal cord injury) generally is determined by the number of motile (strong swimming) sperm available. For example: The availability of 5 million motile sperm in the ejaculate separates the need for in vitro fertilization from intrauterine insemination (tremendously less costly and less complicated); and the presence of 15 million motile sperm in the ejaculate can allow many couples the chance for intravaginal insemination which can be taught to be carried out at home in many instances. Improvement in the number of motile sperm available for a couple's use results in the ability to individualize the care and assistance they need as well as in an improvement in medical resource utilization.

In the field of medical research, the first remedy for a problem, whether surgical or medical, usually leads to other research looking for improvements over the original description. The medicine to be used in this study was chosen because it is already in use for other illnesses. The demonstration of its effectiveness will lead to further medical research such as whether the same mechanism for causing infertility is active in noninjured

men (15% of the general population). Additionally, other medicines could be investigated which may be more effective. Also, different points in the chain of biochemical events may be targeted either alone, or in combination with the pathway we have concentrated on, that may produce a better overall result.

We anticipate that this study will lead to improved treatment of infertility in men with spinal cord injury and allow for simpler, less expensive options for starting a family.

Proposal Title:	Intrarectal Mechanoreceptor Sensitization to Induce Defecation After Spinal Injury
Log Number:	SC220103
Current PI Name:	Lesley Marson
Award Number:	HT9425-23-1-0403
Current Contracting Organization:	Dignify Therapeutics
Current Performing Organization:	Inotiv
Web Approval Date:	05-16-2023

This project aims to address an area of great unmet need by restoring voluntary control over the time and place of bowel emptying to people with spinal cord injury (SCI) using a prescription drug. Most SCI individuals must employ manual stimulation of the rectum to initiate defecation, which can be undignified, burdensome, and time consuming. Regular and efficient emptying of the bowel is necessary to prevent complications of constipation, fecal impaction, and incontinence. The unpredictable timing and incomplete bowel emptying produced by reflex bowel movements in people with SCI is socially stigmatizing and the ensuing medical, financial, social, and psychological sequelae impair quality of life. Improved bowel control is ranked among the highest priorities of people with SCI.

Most people with SCI frequently supplement their bowel programs with oral laxatives, suppositories, or enemas. The timing of their effects is unpredictable, and they may themselves cause incontinence since diarrhea is a common side-effect. According to the directions for use provided on their packaging, no laxative should be used for more than 7 consecutive days. This precaution reflects the risk of gastrointestinal damage, colorectal cancer, and loss of intestinal muscle tone reported after chronic use of these products. Despite this warning, many people with SCI are forced to use laxatives every day because no alternatives are available.

We propose to address this unmet medical need by replacing time-consuming bowel programs with a fast-acting, short-lasting prescription drug to induce defecation “on demand.” The drug will induce rapid and efficient bowel emptying at a time and place chosen by the person with SCI. Any excess drug will be rapidly eliminated from the body with the feces, so that its effects will be short-lasting and predictable. Our objective is to enable people with SCI to complete a daily bowel program in around 15 minutes and then be free to go about their daily tasks without worrying about fecal incontinence, and in doing so will improve the quality of life for SCI individuals.

Dignify has completed experiments with both spinally injured and naive rats that confirm the ability of our drug to induced rapid onset, short-lasting defecation when it is applied directly into the rectum. We are seeking funding from Spinal Cord Injury Research Program to conduct the necessary preclinical work to enable us to examine the effects of our drug in healthy able-bodied volunteers and people with SCI. This involves identifying the most suitable formulation of our drug that produces rapid defecation with minimal absorption into the bloodstream. In accordance with regulatory requirements, the safety of our optimized formulation will be tested in preclinical studies to ensure that it does not cause damage to the lining of the rectum after repeated daily administration. On completion of these studies, Dignify will prepare and submit an Investigational New Drug application to the Food and Drug Administration for initiation of clinical trials. It is hoped that the initiation of the phase 1 clinical trials could commence within 1 year of completion of the proposed studies.

Proposal Title:	Understanding Interpersonal Violence Against People with SCI and Its Psychosocial Impacts
Log Number:	SC220139
Current PI Name:	Susan Robinson-Whelen
Award Number:	HT9425-23-1-0396
Current Contracting Organization:	MEMORIAL HERMANN HEALTH SYSTEM
Current Performing Organization:	MEMORIAL HERMANN HEALTH SYSTEM
Web Approval Date:	05-04-2023

Objectives and Rationale

This study will identify negative psychosocial effects of interpersonal violence (IPV) experienced by people with spinal cord injury (SCI). Psychosocial refers to psychological and social factors. IPV is the use of force or power by one person against another person. It includes physical, sexual, psychological, and financial abuse as well as disability-related abuse, such as neglecting basic care needs or withholding assistive devices. IPV can have serious negative effects on psychological and social health. People with SCI are at high risk for IPV. They also are at high risk for poor psychosocial health including depression, anxiety, post-traumatic stress, loneliness, and social isolation. It is therefore concerning that there is very little research on IPV and its harmful effects on people with SCI, including Veterans. In fact, the few available studies focusing on people with SCI only ask about sexual IPV or about IPV experienced over a person's lifetime, making it unclear if the IPV was experienced before or following injury. The proposed project is the first known national study of the negative psychosocial impacts of postinjury IPV on people with SCI.

The objective of the proposed project is to increase the understanding of postinjury IPV and its negative effects on psychosocial health among people with SCI, including Veterans. The specific aims are to identify (1) types of postinjury IPV experienced by people with SCI, relationships to perpetrators, what helps or prevents seeking help, and the negative psychosocial effects of IPV; (2) risk factors for postinjury IPV, such as race, education and other demographics, level of injury, and other SCI characteristics, and experience with IPV before SCI; and (3) the relation between postinjury IPV and psychosocial health: depression, anxiety, PTSD, loneliness, and social isolation. To address these aims, we will conduct two studies. In Study 1, we will remotely interview up to 30 people with SCI who have experienced IPV after their injury. We will ask about their IPV experiences, types of abusers, help-seeking experiences, and perceptions of the negative psychosocial effects of IPV. Study 2 will draw on the interview findings to develop a national survey administered to 350 people with SCI. The survey will provide new information related to the nature, risk factors, and the psychosocial effects of IPV in people with SCI.

Applicability and Impact: The proposed project will make a significant contribution toward advancing SCI research while directly addressing the Fiscal Year 2022 Spinal Cord Injury Research Program Investigator-Initiated Research Award "Psychosocial Issues" Focus Area. This project will systematically respond to a critical issue that has been largely neglected by both the SCI and the IPV literature. Given that people with disabilities are at high risk for severe IPV, unique types of IPV, and IPV by more abusers, this project will be applicable to all persons with SCI, including Veterans. We anticipate our findings will provide a wealth of information that can be used to increase awareness of IPV and its consequences. We will share our project findings broadly through diverse organizations and stakeholder groups.

Dissemination materials, including factsheets, infographics, and animated videos, will be tailored for specific audiences, focusing on education, prevention, and supports to promote psychosocial health and recovery. We will distribute these products widely through our institutions and rich networks of organizations committed

to serving the disability community. We will initiate the dissemination throughout the funding period and thus, anticipate them to have an immediate impact. Materials developed for healthcare providers will focus on providing trauma-informed care, understanding risk factors, eliminating barriers to help seeking, recognizing and screening for IPV, responding to IPV, and making appropriate referrals to address the psychosocial impacts of IPV.

Our longer-term goal is to use the findings from this study to set the stage for future development and evaluation of education and intervention programs to address IPV and its psychosocial impacts among people with SCI, including Veterans. First, we plan to adapt our group safety awareness and abuse prevention program designed for women with mobility impairments to tailor it for men and women with SCI. Given the potential long-term psychosocial consequences of IPV, we also plan to work with our community advisors to develop a group self-care and healing intervention program for IPV survivors with SCI by expanding our existing psychological health promotion program for women with SCI. The findings of the proposed project will advance the policy and practice of advocates, researchers, and clinicians in the fields of violence and disability as well as other providers of services to people with SCI, particularly regarding the experience of SCI as a risk factor for abuse. Ultimately, our findings will lead to increased safety, improved psychosocial health, and enhanced quality of life of the people in the at-risk and marginalized spinal cord injured population.

Proposal Title:	Synergistically Enhanced Neuroprotection via Dexmedetomidine-Induced Early Hypothermia and ERK Activation After Spinal Cord Injury
Log Number:	SC220152
Current PI Name:	Lingxiao Deng
Award Number:	HT9425-23-1-0700
Current Contracting Organization:	Indiana University
Current Performing Organization:	Indiana University
Web Approval Date:	08-30-2023

Objectives and Rationale: Spinal cord injury (SCI) results in many devastating physical problems that have long-lasting impact on an individual's life and the quality of their life. These include changes/dysfunction in movement, feelings and sensations, and urine discharge. SCI is initially caused by the impact of an external force. Changes within the spinal cord following this impact such as swelling and reduced blood supply in the injured site (called "secondary injury") increases the severity of the injury. It is, therefore, very important to find an effective treatment that can delay the progression of these changes, protect further tissue damage and limit the functional impairment. Treatment that lowers the body temperature can protect the spinal cord tissue and limit the secondary injury. This treatment is most effective if done as soon as possible after injury. Unfortunately, the beneficial effect of this cooling therapy remains significantly limited in clinical practice because of the time needed to transport and medically stabilize the patient and determine potential other injuries. Our goal is to provide patients with SCI access to low body temperature treatment as soon as possible, within the "golden time window (less than 4 hours after injury)" that maximizes the protective effects. In our pilot study, mice who sustained SCI received a one-time injection of dexmedetomidine (Dex), an Food and Drug Administration (FDA)-approved drug, within 1 hour of injury, which cooled the whole body for up to 16 hours without the need for any special cooling equipment. This early onset and long-lasting effect of lowering body temperature greatly improved physical movement and urinary function after SCI. Moreover, Dex has been shown to activate a molecule that leads to a series of chemical changes in cells that also protects the spinal cord. This further protection may lessen some of the secondary injuries following SCI and subsequent disability from the injury. Our idea is that Dex can provide a unique and powerful protective effect after SCI by rapidly inducing low body temperature and reducing secondary injuries. Furthermore, refining the procedure of using this drug will extend the time for the patients to access low temperature therapy. It may also reduce the time needed to maintain low body temperature, and allow this therapy to be more accessible to a larger number of patients who may avoid or reduce the secondary injuries that lead to substantial disability.

Relevance to Military Health: SCIs account for nearly 11% of Service Member deaths and affects thousands of military Service Members serving overseas, with significant disability for the remainder of their life. The poor medical conditions and delayed transportation from the battlefield, make it very difficult for injured soldiers to have access to effective neuroprotective treatment, such as low body temperature, within the "golden" therapeutic time window. Our proposal will explore a new treatment approach using Dex that conveniently, rapidly, and effectively induces lower body temperature without the need for auxiliary cooling equipment and can provide a powerful new treatment option for use on the battlefield.

Impact in the Field of SCI Research: There is urgent need within the area of SCI research to develop effective therapies that can initiate strong neuroprotection as early as possible after injury. To the best of our knowledge, this study will be the first to confirm the novel great protection of the FDA-approved drug Dex and determine how it protects spinal cord cells following SCI. Our study will evaluate the effect of Dex-induced low body temperature therapy on urinary function and explore if there are gender differences in recovery following treatment with low body temperature. Taken together, these findings will refine the

protocol for using Dex-induced hypothermia in order to maximize the therapeutic effects and improve patient outcome and quality of life. This will have great impact on soldiers in combat but also for non-military people with SCI. This could be later implemented in rural communities world-wide without easy access to hospital care.

Proposal Title:	Early Detrusor Chemodenervation to Preserve Bladder Compliance and Longevity After Spinal Cord Injury
Log Number:	SC220183
Current PI Name:	Zin Khaing
Award Number:	HT9425-23-1-0522
Current Contracting Organization:	Washington, University of
Current Performing Organization:	Washington, University of
Web Approval Date:	06-15-2023

Rationale: Spinal cord injury (SCI) is among the most devastating traumatic injuries, and there are approximately 300,000 Americans living with SCI. In addition to the loss of upper and lower extremity function, more than 80% of all patients with SCI suffer from neurogenic bladder, a condition caused by nerve impairment to the bladder and its control muscles (sphincters). Neurogenic bladder leads to increased lifetime health risks for persons with SCI, including urinary tract infection and loss of kidney function, and these complications negatively impact quality of life (QoL). Therefore, new and innovative ways to manage neurogenic bladder and promote bladder health are crucial to the long-term well-being of persons with SCI.

Injecting the bladder muscle with botulinum toxin A (BoNT-A) is an approved treatment for urinary urgency, frequency, and incontinence due to neurogenic bladder. This treatment prevents the overactivity and increases bladder compliance (the ability of the bladder to store urine). However, bladder BoNT-A injections, also called chemodenervation, are currently only offered to patients after other treatment options have failed. By that time, often years after the initial injury, permanent bladder muscle changes, such as thickening and scarring, have already occurred. Here, we propose an innovative use of an already approved treatment to examine whether chemodenervation performed early in the recovery process can increase bladder compliance and longevity of bladder health, and reduce muscle thickening, scar formation after SCI.

Objectives: In this proposal, we will investigate whether early chemodenervation mitigates deleterious bladder wall changes and preserves bladder storage function following SCI. A rat model of acute SCI will be used to test our hypothesis, as well as answer fundamental questions regarding (1) the optimal therapeutic window for early treatment, (2) how to preserve bladder function over time, and (3) the mechanism of nerve changes that underlie neurogenic bladder development. We hypothesize that chemodenervation will prevent or delay the progressive loss of bladder compliance, thereby preserving bladder health in the chronic stage of SCI. We will also perform a pilot clinical trial and examine the feasibility of treating patients early (

Research Impact, Benefits, and Risks: This proposal directly addresses the needs of and treatments for persons with SCI and neurogenic bladder. Bladder function is one of the top health priorities of persons with SCI. Given that almost of all SCI patients suffer from bladder dysfunction (>80%), limiting or stopping the development of changes in bladder tissue will likely have a huge impact on the treatment and care of neurogenic bladder-related complications. By helping to preserve bladder compliance, this intervention will decrease incontinence (leaky bladder), the risk of urinary tract infections, and help to preserve kidney function over the lifetime of a patient with SCI. Moreover, early treatment will likely also increase the longevity of bladder health. These outcomes will go a long way to improving the well-being and QoL for large numbers of persons with SCI.

The pilot clinical trial in this proposal will be a feasibility study, to confirm that the effects seen in the rat model will be identifiable in human tissue and in human bladder function. If the results from the pilot

clinical trial are positive, we will move to a larger scale multisite randomized clinical trial. We anticipate that the results from such a trial would be available in 5-6 years, indicating whether prevention of neurogenic bladder changes is possible.

Proposal Title:	Baroprosthesis to Reduce Secondary Damage of the Traumatic Spinal Cord Injury
Log Number:	SC220199
Current PI Name:	Gregoire Courtine
Award Number:	HT9425-23-1-0547
Current Contracting Organization:	Ecole Polytechnique Federale de Lausanne
Current Performing Organization:	Ecole Polytechnique Federale de Lausanne
Web Approval Date:	07-27-2023

Each year, tens of thousands of people around the world are paralyzed after suffering a spinal cord injury (SCI). Despite decades of research, doctors caring for patients who have just suffered an SCI have few treatments that might improve their ability to move and feel again. One of the only treatments that can potentially improve recovery during the period immediately after a SCI is to control patients' blood pressure at optimal targets. However, controlling blood pressure after SCI within a narrow target range has been very hard. Moreover, the drugs that are used to control blood pressure can actually cause harm. This challenge puts doctors in a difficult situation, since trying to help a patient by controlling their blood pressure can actually worsen their injury.

We recently developed a new treatment that uses electricity to modulate the cells of the spinal cord that control blood pressure. We call this treatment the neuroprosthetic baroreflex. We showed that the neuroprosthetic baroreflex can control blood pressure precisely in rodents, monkeys, and humans with SCI. Importantly, the neuroprosthetic baroreflex does not seem to carry the same risk for harm as existing treatments to control the blood pressure. The neuroprosthetic baroreflex has now entered clinical trials to manage blood pressure during the months and years that followed an SCI.

The neuroprosthetic baroreflex could also help doctors care for patients immediately after they have suffered an SCI. However, before this can happen, we need to answer two important questions about managing blood pressure immediately after SCI. First, the protocols that doctors use to manage blood pressure are based on the imperative need to avoid causing harm with potentially dangerous drugs. Therefore, we need to identify the best protocols for doctors to manage blood pressure without the risks of current treatments. Second, doctors do not currently know why the control of blood pressure allows patients to recover better movement and feeling after SCI. Understanding exactly why the control of blood pressure is so beneficial could allow us to identify the most important time window after injury and optimal settings to control a patient's blood pressure.

This research could help nearly everyone who suffers an SCI. What we learn could help reduce the risk people with SCI face during their initial management in the hospital. This would be of great benefit to patients, since it would give the doctors taking care of them more and better options to treat them. Ultimately, better treatment immediately after injury would likely improve patients' ability to move and feel in the months and years afterwards.

This research is also one of our most immediately achievable ways to make a difference in the treatment of patients with SCI. Controlling blood pressure is already a key treatment that doctors use immediately after an SCI. If we could discover better guidelines and improved treatments to control blood pressure, doctors would be able to use these discoveries to treat patients with SCI much faster than, say, a newly discovered drug. More specifically, we expect that the neuroprosthetic baroreflex could help doctors manage blood pressure in patients who have just suffered an SCI within the next 3-5 years.

To identify new protocols for blood pressure management, we will perform experiments in mice treated with the neuroprosthetic baroreflex. Specifically, we will maintain the mice at different levels of blood pressure during the first week after SCI, and will study how a given blood pressure level impacts the evolution of spinal cord damage and functional recovery. We will use cutting-edge molecular methods to study the complex molecular changes that occur within the spinal cord in the days and weeks after SCI. Therefore, there are no risks to patients as part of this research.

Overall, this proposal could lead to a new treatment to manage blood pressure very precisely in the immediate period after someone suffers an SCI. Ultimately, this treatment could help improve their ability to move and feel in the months and years after injury. It would also give doctors a powerful new treatment that carries less risk of harming their patients.

Proposal Title:	Baroprosthesis to Reduce Secondary Damage of the Traumatic Spinal Cord Injury
Log Number:	SC220199P1
Current PI Name:	Aaron Phillips
Award Number:	HT9425-23-1-0548
Current Contracting Organization:	Calgary, University of
Current Performing Organization:	Calgary, University of
Web Approval Date:	07-27-2023

Each year, tens of thousands of people around the world are paralyzed after suffering a spinal cord injury (SCI). Despite decades of research, doctors caring for patients who have just suffered an SCI have few treatments that might improve their ability to move and feel again. One of the only treatments that can potentially improve recovery during the period immediately after a SCI is to control patients' blood pressure at optimal targets. However, controlling blood pressure after SCI within a narrow target range has been very hard. Moreover, the drugs that are used to control blood pressure can actually cause harm. This challenge puts doctors in a difficult situation, since trying to help a patient by controlling their blood pressure can actually worsen their injury.

We recently developed a new treatment that uses electricity to modulate the cells of the spinal cord that control blood pressure. We call this treatment the neuroprosthetic baroreflex. We showed that the neuroprosthetic baroreflex can control blood pressure precisely in rodents, monkeys, and humans with SCI. Importantly, the neuroprosthetic baroreflex does not seem to carry the same risk for harm as existing treatments to control the blood pressure. The neuroprosthetic baroreflex has now entered clinical trials to manage blood pressure during the months and years that followed an SCI.

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This research could help nearly everyone who suffers an SCI. What we learn could help reduce the risk people with SCI face during their initial management in the hospital. This would be of great benefit to patients, since it would give the doctors taking care of them more and better options to treat them. Ultimately, better treatment immediately after injury would likely improve patients' ability to move and feel in the months and years afterwards.

This research is also one of our most immediately achievable ways to make a difference in the treatment of patients with SCI. Controlling blood pressure is already a key treatment that doctors use immediately after an SCI. If we could discover better guidelines and improved treatments to control blood pressure, doctors would be able to use these discoveries to treat patients with SCI much faster than, say, a newly discovered drug. More specifically, we expect that the neuroprosthetic baroreflex could help doctors manage blood pressure in patients who have just suffered an SCI within the next 3-5 years.

To identify new protocols for blood pressure management, we will perform experiments in mice treated with the neuroprosthetic baroreflex. Specifically, we will maintain the mice at different levels of blood pressure during the first week after SCI, and will study how a given blood pressure level impacts the evolution of spinal cord damage and functional recovery. We will use cutting-edge molecular methods to study the complex molecular changes that occur within the spinal cord in the days and weeks after SCI. Therefore, there are no risks to patients as part of this research.

Overall, this proposal could lead to a new treatment to manage blood pressure very precisely in the immediate period after someone suffers an SCI. Ultimately, this treatment could help improve their ability to move and feel in the months and years after injury. It would also give doctors a powerful new treatment that carries less risk of harming their patients.

Proposal Title:	Targeting PARP Signaling as a Novel Treatment for Urologic Complications of Spinal Cord Injury
Log Number:	SC220218
Current PI Name:	Rosalyn Adam
Award Number:	HT9425-23-1-0446
Current Contracting Organization:	Children's Hospital, Boston
Current Performing Organization:	Children's Hospital, Boston
Web Approval Date:	05-26-2023

Although survival rates have increased steadily over the past decades, spinal cord injury (SCI) still has a devastating impact on injured individuals, their caregivers, and their families. Active-duty military personnel are at significantly higher risk of SCI than individuals in the general population due to the risks associated with military training and service. The most visible effect of SCI is limb paralysis that may affect both lower and upper limbs to differing extents. Less obvious is the loss of bladder and bowel control, which can lead to psychological distress and a significantly diminished quality of life as a result of incontinence, increased likelihood of urinary tract infection, and elevated risk of damage to the kidneys. SCI is extremely costly to individuals, both financially and psychologically, and in spite of significant advancements in care of individuals with SCI, there is currently no cure. The relatively young age at which military personnel are likely to incur their spinal injury, as well as the increased likelihood of surviving their injuries means that such individuals and their families bear a lifelong health and financial burden from their condition.

A significant proportion of the clinical expenditures for individuals with SCI is directly related to management of the bladder, and while current medications to improve bladder control provide some symptomatic relief, they are often associated with undesirable side effects and variable long-term efficacy. To explore new avenues for treatment of the urinary complications of SCI, our group has performed an in-depth analysis of the molecular signals that drive changes in the bladder in animal model of SCI. This analysis has identified a protein in cells called PARP-1 that is activated by SCI, in parallel with increases in deposition of collagen, a substance that increases stiffness of the bladder wall making it less flexible. Treatment of animals with SCI with inosine, a molecule known to inhibit PARP-1, led to reduced collagen deposition. Previous studies from our group showed that inosine could decrease overactivity of the bladder in animals with SCI. Together, these results suggest that PARP-1 may be responsible for signaling that leads to damaging complications of SCI. Based on our new data, we will investigate how PARP-1 leads to structural and functional changes in the bladder following SCI by determining how treatment of SCI animals with additional PARP inhibitors leads to changes in cells responsible for collagen production, and how they impact bladder function.

Although the proposed studies will use an animal model of SCI, we believe the results of this research will be relevant to humans with spinal injury, since the mechanisms that regulate collagen production in the bladder are similar in both species. If successful, we anticipate results from our studies could lead to the use of PARP inhibitors in humans with SCI to diminish complications, since PARP inhibitors are already approved for use in diseases such as cancer. Studies such as ours that investigate the basic mechanisms that underlie the complications of SCI have the potential to uncover new strategies for treatment and to impact the health and quality of life of paralyzed military personnel and their families.

Proposal Title:	Targeting Serotonergic Receptors to Treat Spinal Cord Injury
Log Number:	SC220219
Current PI Name:	Yu-Shang Lee
Award Number:	HT9425-23-1-0412
Current Contracting Organization:	Cleveland Clinic Foundation
Current Performing Organization:	Cleveland Clinic Foundation
Web Approval Date:	05-10-2023

Significant efforts have been made in the development of new treatments for spinal cord injury (SCI), from basic science research through clinical application. However, the far more challenging goal of restoring bladder function to improve the quality of life among SCI patients has still been largely underachieved. The spinal cord segments below the injured level undergo remodeling to adapt to changes in spinal circuits that control lower urinary tract (LUT) function (bladder and external urethral sphincter). Particularly, the 5-HT (a neurotransmitter released form the nerve fibers) and 5-HT receptors have been shown to play important roles in regulating LUT function in both normal and SCI conditions. To develop a clinically applicable therapeutic to improve LUT dysfunctions caused by SCI, we first will use comprehensive approaches with pharmacological interventions to activate 5-HT receptors to determine their efficacy on improving LUT function in a clinically-relevant contusive SCI model. Importantly, our recent study showed that long-term activation of 5-HT receptors (a subtype of 5-HT receptors) by a promising compound called NLX-112 can improve LUT function after contusive SCI. NLX- 112 is a drug that is currently in a phase 2 clinical trial to treat Parkinson’s disorders. We will further compare the pharmacological efficacy on improving LUT function between NLX-112 and the current clinically available compounds, and study the potential mechanisms underlying NLX-112 improvement of LUT function after SCI. Therefore, success in this project will move NLX-112 forward toward clinical application in SCI patients to address LUT dysfunctions.

Proposal Title:	Critical Time Window for Rehabilitation After Incomplete Spinal Cord Injury: Early vs Late Locomotor Training
Log Number:	SC220241
Current PI Name:	Milapjit Sandhu
Award Number:	HT9425-23-1-0418
Current Contracting Organization:	Shirley Ryan AbilityLab
Current Performing Organization:	Shirley Ryan AbilityLab
Web Approval Date:	06-12-2023

Background/Rationale: Most patients who sustain a spinal cord injury (SCI) lose the ability to walk and many never recover this ability even after prolonged rehabilitation. This loss of mobility imposes severe constraints on quality of life and the ability to return to community living. The average age of SCI onset is 27 years for military Service Members; thus, individuals with SCI have strong incentives to resume active community participation and employment – each depending in large part on restoration of locomotor function. While there are many innovative therapies under development, we rely heavily on retraining of motor function via massed practice to promote recovery. We know that intensive motor training promotes recovery by helping the nervous system to restructure neural circuits and to reprogram their functions. It is thought that the neural trauma associated with SCI initiates many of these corrective functions and that high-intensity training helps to remodel this plasticity. What remains unknown is the optimal time window during which the injured spinal circuitry is most responsive to training.

It is our hypothesis that the most effective time to initiate such training (i.e., the time-sensitive window of plasticity) arises within the first few weeks after injury. This hypothesis is derived from retrospective studies and from our pilot data, which suggest that increased time from injury to initiation of rehabilitation is associated with poorer walking performance. Studies in animal models of SCI and parallel work in other neurological deficits, such as stroke, also support the notion that the capacity of the injured nervous system to respond to various training protocols may change radically according to time elapsed since injury. Accordingly, the overall objective of our study is to determine if there is a critical period of plasticity post-SCI in which training maximally improves function. To do this, we plan to compare the effects of high-intensity treadmill training, an intervention designed to restore locomotor function, administered in addition to routine care during the acute in-patient rehabilitation phase, and compare that with delayed high-intensity training provided at 3 or 6 months after the initial spinal trauma. Our specific aims are the following.

Aim 1: To determine the critical time window for plasticity post-SCI by evaluating the effectiveness of 20 hours of additional high-intensity locomotor training provided in the early, subacute, or chronic stage following SCI.

Aim 2: To determine the relationship between functional improvement achieved over the course of training and post-intervention walking activity, including community ambulation, using wearable sensors for up to 1 year post-SCI.

Study Design: We will employ a randomized, multi-site, placebo-controlled, and repeated measures clinical trial study design. We will enroll 108 individuals with acute traumatic SCI at cervical or thoracic level (C5–T12) upon their admission to inpatient rehabilitation at our two clinical study sites: Shirley Ryan AbilityLab, Chicago, and Baylor Scott White Institute for Rehabilitation, Dallas, Texas. In Aim 1, we will deliver 20 additional hours of high-intensity body weight supported treadmill training (target 65%-80% maximum heart rate), in addition to their standard rehabilitation. This will be administered over a period of 4-6 weeks and will be initiated at an early (

Impact: In alignment with the Department of Defense's mission to provide the best care and outcomes for military Service Members, Veterans, and civilians, this study addresses the FY22 SCIRP Focus Area of Rehabilitation and Regeneration. Establishing precision neurorehabilitation requires identifying the time window of fruitful adaptive plasticity. This study will inform us whether a therapeutic window exists after acute SCI that could be engaged using high-intensity locomotor training. This information may help patients and clinicians appreciate the importance of therapy timing after SCI and update the standard-of-care clinical practice guidelines. Our findings may also be of value to the Centers for Medicare & Medicaid Services or commercial third-party payers of healthcare services who make decisions regarding reimbursement. Finally, our project may open avenues to expand the science of post-SCI motor recovery. A clinically important future direction would be to identify novel techniques, including pharmacological and cellular therapies, noninvasive electrical stimulation, or acute intermittent hypoxia, that may be capable of prolonging or re-opening a period of enhanced plasticity after SCI.

Proposal Title:	Preservation of Spinal Cord Tissue Resulting from Spinal Cord Injury Ischemia: Testing of a Novel Oxygen Release Therapeutic
Log Number:	SC220248
Current PI Name:	Prodip Bose
Award Number:	HT9425-23-1-0562
Current Contracting Organization:	Florida, University of
Current Performing Organization:	Florida, University of
Web Approval Date:	07-12-2023

Cervical spinal cord injury (C-SCI) has been a major component of SCI with lingering quality of life due to eroding disabilities with locomotion (gait) and increased muscle tone (spasticity) along with other infirmities that produce a substantial health care responsibility. There are rapidly growing concerns that injury-induced ischemia (e.g., caused by the stretch and avulses post-capillary venules as well as sulcal arterioles, resulting in hemorrhage, reduced spinal cord vascular perfusion, vasospasm, and hypoperfusion) is a significant risk factor for lingering SCI-disabilities. Accordingly, there is an urgent need to test the safety and efficacy of therapeutic measures that target the ischemic zone by utilizing a novel experimental O2 therapeutic (e.g., NanO2), which has excellent potential for rapid translation. When the spinal cord is stretched, contused, partially severed, or compressed by bleeding, loss of nerve function distal to the injury site occurs. Other injuries, including hemorrhage and brain injury often happen. The global incidence is 10.5/100,000 per year. In combat, spinal cord injury accounts for over 11% of casualties. The severity and anatomy of the injury contribute to the outcome, rehabilitation, and complications. Neck injury is the most common and creates the most disability. The worse cases, para/quadriplegia, are permanent, impacting employment or military service. Victims require long-term care with large expenditures from society and reduced quality of life. Secondary infections, decubitus ulcers, muscular contractions, and physiotherapy could be lessened by immediate nerve salvaging therapy. Today, the speed of evacuation to medical care is the best ever.

There is no pharmaceutical agent to salvage spinal cord neurons. When injured, the spinal cord blood flow is compromised leading to swelling, decreased oxygen delivery, and extension of injury to cells not killed by the primary event. Cord swelling reduces blood flow causing more neuron death. NanO2 is a perfluorocarbon emulsion comprised of nanoparticles of fluorinated oils that dissolve large quantities of oxygen. They are 1/100-1/1000th the size of red blood cells. Intravenous NanO2 allows areas of decreased blood flow to receive dissolved oxygen when the red cells do not flow. NaO2 is in human stroke trials to deliver needed O2 to prevent acute ischemia while allowing time for clot-busting drugs to be delivered. In traumatic brain injury, NanO2 is capable of salvaging brain tissue. In SCI, there is no definitive therapy wherein giving a short-acting drug would allow a patient to get to therapy. The use of NanO2 will primarily salvage neurons that otherwise would die. Our proposed preclinical animal studies will utilize the NanO2 (NuVox, Tucson, Arizona) in dosages being utilized in humans (plus1/2 and about 2x that dose). Our study will use intravenous NanO2 because that is something that a first responder could perform. The studies will be conducted over 3 years in rats to define the best dose and delay from injury to treatment (20 minutes, 1 hour, 2 hours, and 4 hours). This treatment could quickly transition to human phase 2 trials. These studies will have a standard moderate neck SCI (previously well-defined as a model) with a focus on a major outcome variable (gait movement/functional preservation). It is vitally important that therapy should demonstrate an improvement in nerve-muscle function. Markers of anatomic injury will be assessed with magnetic resonance imaging (MRI) and by microscope inspection of the spinal cord at the end of the experimentation. Certain biomarkers will be assessed to discern the treatment effect. The applicability of this research is to create an intravenous fluid that could be given to a victim within the first few minutes of his /her injury. For the military, this would mean that a combat medic or first echelon hospital could give NanO2.

Furthermore, these studies will potentiate the effectiveness of other existing ways to reduce tissue swelling to improve rehabilitation. Every nerve saved will contribute to the functional movement of the leg or arm. This research will have applications for civilian and military injury victims. Often the initial minor injuries do not manifest themselves until secondary tissue swelling begins. The NanO2 emulsion being tested is approved for use in Europe as an echocardiogram contrast agent. It does not have toxicities attributed to other older generations of perfluorocarbons. This one is unique with a short dwell time in the body. It can be given in multiple dosages, just as used in stroke. The data from these studies will be reported to the scientific community, the Department of Defense, NuVox Inc., and there will be an application pending with the Food and Drug Administration to do human studies in acute moderate to severe spinal cord injury. It is believed that within five years of beginning this work military and civilian medicine could have a new drug for usage in spinal injury.

Proposal Title:	Antiphospholipid Antibodies for the Diagnosis of Lyme Disease
Log Number:	TB220023
Current PI Name:	Linden Hu
Award Number:	HT9425-23-1-0526
Current Contracting Organization:	Tufts University School of Medicine
Current Performing Organization:	Tufts University School of Medicine
Web Approval Date:	02-09-2023

This proposal addresses the Fiscal Year 2021 Tick-Borne Disease Research Program Focus Area of Diagnosis and specifically seeks to develop a test capable of distinguishing active infection and previous exposure, and/or monitoring of response to treatment.

Currently, the diagnosis of Lyme disease relies on the detection of the host immune response to the causative organism, Borrelia burgdorferi. This type of testing, called serologic testing, has several limitations. First, the host immune response typically takes some time to develop, resulting in a lag between the time someone is infected and the time serologic testing becomes positive. This can cause delays in initiating treatment that are associated with more severe disease and a higher risk of post-treatment Lyme disease symptoms. Another major issue with current serologic testing is once the test is positive, it stays positive for long periods of time, making it useless for tracking the success of treatment or for detecting reinfection.

In the course of fundamental research on the metabolism of the Lyme disease bacteria, our lab has identified a series of antibodies that may arise earlier in infection and decline faster after treatment. These antibodies do not recognize bacterial proteins detected by currently available tests but are instead raised against host molecules (phospholipids) that B. burgdorferi co-opts for its own purposes. Normally, the host immune response suppresses the production of antibodies to its own molecules, but in the context of the bacterial infection, it now can recognize these targets. Also, because these targets have likely been seen by the immune system before, but were suppressed, antibodies may be ramped up and produced more quickly when they are recognized in conjunction with B. burgdorferi. In addition, because the host tries to limit antibodies that have the potential to damage itself, once the infection is cleared and the stimulus is removed, the immune system quickly shuts down further production of antibodies to these molecules.

As such, testing for these anti-phospholipid antibodies to host molecules could address multiple key failings of the current diagnostic tests. Our preliminary studies show that anti-phospholipid antibody levels decline steadily in most patients after antibiotic treatment and return to baseline levels at time-points when antibodies used in traditional Lyme diagnostics remain very elevated. In addition, it appears that patients who remain symptomatic after antibiotic therapy have higher levels of these anti-phospholipid antibodies.

This suggests that these types of autoantibodies may have a role in following the response of patients to antibiotic treatment and in differentiating symptoms that result from Lyme disease from other infections (e. g., long COVID).

As part of this project, we will first broaden the search for different types of antiphospholipid autoantibodies to include a larger number of different lipids/phospholipids to find the combination of antigens with the best characteristics for a monitoring test. We will also determine if specific antibody classes (IgM, IgG subclasses, IgA) may be the most sensitive/specific for following resolution of disease. The experiments proposed here will examine the levels of each of the candidate antibodies throughout the multiple stages of Lyme disease using a sample from the extensive collections of Dr. Adriana Marques (National Institutes of Health) and Dr. John Aucott (Johns Hopkins University). We will test for specificity by studying samples from patients with other types of infection or autoimmune diseases. Using this information, we will design a diagnostic panel of antibodies optimized for a return to baseline levels after successful treatment and where

elevation is most closely linked to persistent symptoms. Finally, we will assess the final test configuration using an independent validation panel of samples.

The lack of a good test for cure has greatly hampered clinicians in the care of patients who have persistent symptoms after Lyme disease. It is likely that patients with symptoms after Lyme disease are a heterogenous group with different reasons for persistent symptoms. Our hope is that the antiphospholipid auto-antibody tests will identify a subset of patients that may respond best to additional therapy. At the conclusion of this project, we will have identified the best diagnostic targets to achieve this goal and will begin work to design a testing kit for which we could seek regulatory approval within 3-5 years.

Proposal Title:	Direct Detection of Tick-Borne Agents in a Single Point-of-Care Test
Log Number:	TB220033
Current PI Name:	Rafal Tokarz
Award Number:	HT9425-23-1-0830
Current Contracting Organization:	Columbia University Medical Center
Current Performing Organization:	Columbia University Medical Center
Web Approval Date:	02-09-2023

Low accuracy and the inability to simultaneously target the wide range of potential causative pathogens are the primary limitations of direct tests for tick-borne diseases. Currently, optimal laboratory diagnosis requires a wide range of individual, costly tests that may fail to detect the causative pathogen. The lack of accurate early diagnosis can then lead to improper therapy and to persistent and long-lasting symptoms. Thus, accurate early detection is critical, and requires a test that provides superior simultaneous detection of a wide spectrum of tick- borne agents. Just as important, this test needs to be based on methods and equipment that are already widely available and can be adapted in any diagnostic laboratory.

To overcome current testing limitations, we have developed a diagnostic test called TBDCapSeq that is based on a technique called Next Generation Sequencing (NGS). This technique has been progressively used for over 15 years and has rapidly improved all facets of clinical research. NGS tests do not focus on a single pathogen, but instead provide specific information about all microbes present within a tested sample. The primary limitation for using NGS-based diagnosis were the very high costs of the tests and the complexity of the machines and the analysis process. However, new small, cheaper and portable machines have offset these limitations. Based on the speed of current technological innovation, we anticipate that in the next decade portable NGS tests will begin to replace currently used PCR tests as the primary point of care diagnostic platform. Portable NGS also provides a unique immediate opportunity for improvement of tick-borne disease testing. In our previous published work, we have demonstrated the superior accuracy of our TBDCapSeq assay and its ability for simultaneous detection of multiple agents. Our efforts in this project will be focused on adapting our existing assay to these new cheaper and portable sequencers. Concurrently, we will approach the Food and Drug Administration (FDA) and follow their guidelines for thorough assay validation necessary to obtain regulatory approvals for diagnostic use. These experiments will be completed within a 2-year frame.

With this work we will address multiple components of the Diagnosis Focus Area of the Fiscal Year 2022 Tick-Borne Disease Research Program. The availability of TBDCapSeq would fulfill the following crucial needs: (i) Rapid direct detection of any tick-borne agent in a single assay; (ii) The ability to detect all tick-borne co-infections; (iii) Uncover the emergence of new pathogen variants; and (iv) Reduce costs associated with direct testing. Following the validation experiments and FDA approval, TBDCapSeq could be adapted in any clinical laboratory. We would first pursue implementation of our assay for tick-borne disease testing within the department of Pathology at Columbia University. Concurrently, we would seek out commercial partners in order to facilitate commercial availability to the diagnostic community. Our assay would considerably impact patient care of military and civilian patients by improving early diagnosis. In addition, patients would now be tested for a full range of tick-borne agents, increasing the likelihood of detecting co-infections. And by greatly reducing the costs associated with testing, TBDCapSeq would provide affordable testing for the military Service Members as well as the civilian population.

Proposal Title:	Development of a Subunit Vaccine Against Babesiosis Caused by Babesia microti
Log Number:	TB220063
Current PI Name:	Edouard Vannier
Award Number:	HT9425-23-1-0677
Current Contracting Organization:	Tufts Medical Center
Current Performing Organization:	Tufts Medical Center
Web Approval Date:	02-09-2023

In the United States, most cases of human babesiosis are caused by Babesia microti and are acquired from the bite of an infected deer tick. Babesia microti is a small parasite that invades, multiplies inside, and eventually bursts red blood cells open, causing anemia. As the infection progresses, patients typically experience fever, chills, sweats, headache, myalgia, and anorexia. In individuals older than 50 years, babesiosis can be so severe that hospital admission is warranted. Despite antimicrobial therapy, lung inflammation and/or loss of kidney function can develop and be fatal. The number of cases reported each year to health authorities has increased 10-fold in the past two decades. Current measures to prevent babesiosis aim at reducing tick exposure and removing ticks embedded in the skin. The proposed research aims at developing a subunit vaccine against babesiosis caused by B. microti. As such, it responds to a Fiscal Year 2022 Tick-Borne Disease Research Program Focus Area outlined for “Prevention.”

Our vaccine strategy is to trigger an antibody response that will interrupt the lifecycle of Babesia microti in the vaccinated host. To identify Babesia microti proteins against which an infected host develops a strong antibody response, we screened the entire set of proteins produced by the parasite. Using a strain of laboratory mice that relies on antibodies to resolve Babesia microti infection, we discovered that these antibodies recognize a small set of parasite proteins. We probed for functional evidence that neutralization of those proteins drives resolution of infection. In doing so, we identified three parasite proteins that are well suited for use in a subunit vaccine. We propose to identify vaccine compositions that confer full protection to young and old mice from B. microti.

We will produce the three prioritized proteins and verify that these proteins are recognized by antibodies made during the course of B. microti infection in mice and in humans. Each vaccine composition will comprise one of the three proteins mixed with one of two adjuvants, compounds that promote antibody production. One adjuvant is used in seasonal flu vaccines for the elderly. The other adjuvant strengthens vaccine-induced immunity against a parasite that causes malaria. We will immunize mice of a strain in which old age exacerbates the burden of B. microti but will initiate our studies by seeking to reveal protection in young age. Anticipating that protection conferred by immunization with a single protein will be partial, we will combine two or three of the prioritized proteins as long as they each confer partial protection and will seek to identify a combination that confers full protection to young mice. We will assess whether this combination confers equal protection to old mice. If not, we will vary the adjuvant and/or the dose or frequency of protein. We will assess whether vaccine compositions that confer protection from the life stage of B. microti that causes disease in the host (the vaccine target) also confer protection from the life stage of B. microti that is delivered in the skin by an embedded tick and quickly progresses to the life stage that causes disease.

Aside from being life-threatening, severe babesiosis can be difficult and costly to treat. Veterans who are beyond 50 years of age will benefit the most from a vaccine against babesiosis because they are at highest risk of severe illness. Active-duty Service Members, despite a low risk for severe babesiosis due to their young age, will benefit from a vaccine against babesiosis because they will escape symptoms. By reducing

the duration of B. microti infection in asymptomatic carriers who may donate blood, a vaccine will protect the blood supply, thereby benefiting any military Service Member in need of a blood transfusion. These considerations extend to military beneficiaries as well as the general population.

The proposed research will generate proof-of-concept data to support the development of a subunit vaccine for babesiosis. Once the project is completed, a vaccine that uses parasite proteins or fragments thereof could reach the market in 5 years or less. The success of mRNA vaccines for COVID-19 has spurred many efforts to apply this approach to other infectious diseases, including malaria. If using the mRNA technology to express the validated Babesia microti proteins in humans, a vaccine for babesiosis could reach the market in record time.

The proposed research will yield tangible outcomes before a vaccine reaches the market. In the clinic, when a patient with weakened immunity fails to clear Babesia microti, one could guide the duration of antimicrobial therapy by monitoring the appearance, in blood, of IgGs specific for B. microti antigens that we identify as protective. The proposed research will also advance our knowledge of Babesia microti, particularly of its entry and replication in red blood cells. This knowledge could be applied to other parasites of red blood cells.

Proposal Title:	Impact of Alpha Gal Syndrome on Early Bioprosthetic Heart Valve Degradation
Log Number:	TB220066
Current PI Name:	Joseph W. Turek
Award Number:	HT9425-23-1-0881
Current Contracting Organization:	Duke University
Current Performing Organization:	Duke University
Web Approval Date:	07-10-2023

Alpha-gal is a carbohydrate that is expressed in all animals except for humans. It is found in red meat, dairy, and other animal-derived products including surgical implants and drugs. The human immune system uniformly tolerates this foreign, non-human, carbohydrate, although some patients develop Alpha-gal Syndrome (AGS), or an allergy to it. This allergy is caused by a bite from the Lone Star tick. Symptoms are delayed and exist on a continuum, ranging from vague abdominal pain to life-threatening anaphylaxis following exposure to alpha-gal. AGS continues to be a chronic, underreported and often unrecognized allergic condition, effecting an estimated 20% of people in regions endemic to the Lone Star tick, like the Southeastern United States.

Alpha-gal is present on tissue valves used in heart surgery. Tissue valves are artificial heart valves made from pig or cow tissue. They are popular because they can be placed through a minimally invasive approach and do not require patients to be on blood thinners after surgery. Historically though, younger patients were precluded from receiving tissue valves because younger patients have stronger immune responses, which cause more damage when the foreign valve is initially implanted, shortening the expected lifetime of the valve from 15 years to 5. Our team (Drs. Joseph Turek, study Principal Investigator and Scott Commins, study Consultant/Contractor) have shown that patients with AGS, or an allergy to alpha-gal, have a stronger immune response to tissue valves than their peers. Alpha-gal is removed from animal-derived surgical implants by the immune system during the first year after implantation. We believe the stronger immune response associated with AGS, a tick-borne disease, contributes to increased valvular damage within the first year, predisposing patients to early valve failure following tissue-based heart valve replacement.

In this study, we will (1) measure how an alpha-gal allergy (AGS) effects valve function in patients undergoing tissue-based valve replacement, (2) use a novel animal model to demonstrate the isolate effect of AGS on valve function following tissue-based valve replacement , and (3) attempt to prevent AGS related valvular damage in the animal model using a commercially available allergy medication. In Aim 1, we will be collecting blood from patients undergoing aortic valve replacement at Duke University, once preoperatively and once at patients' 1-year follow-up. Patients testing positive for an alpha-gal allergy at either time will be considered to have AGS. Valve function based on routine ultrasound and the need for redo valve replacement will be compared between these two study groups (those with and without AGS). In Aim 2, we will use special pigs that lack alpha-gal (like humans), give them Lone Star tick toxin (simulating a tick bite), and create an AGS model. By implanting aortic valves from other pigs with and without alpha-gal into these AGS pigs, we can isolate and measure the true impact AGS has on valve structure and function. In Aim 3, we will utilize the same approach from Aim 2, but selectively treat pigs with Omalizumab, a Food and Drug Administration-approved allergy medication that has been shown to treat refractory AGS patients with severe symptoms.

Heart disease is the leading cause of death in the United States, with annual costs over $350 billion. Valvular heart disease accounts for $23 billion of this total cost, affecting 11% of Americans. Tissue-based heart valves represent the majority of these repairs and will continue to outpace mechanical valve replacement.

Early valve damage following tissue-based heart valve replacement affects approximately 20% of patients and is associated with premature valve failure and redo valve replacement. The number of adults 65 and older is expected to double over the next 30 years, making AGS-related valve damage an unmet public health concern worth studying, particularly in the Southeastern United States where AGS is reported to effect up to 20% of the population. By understanding and addressing the clinical implications of AGS in valvular heart surgery (short-term), we have the opportunity to extend the functional life of replacement heart valves and improve the value of care for military members and their families (long-term).

Proposal Title:	Immune System Disturbances After Borrelia burgdorferi Infection
Log Number:	TB220074
Current PI Name:	Nicole Baumgarth
Award Number:	HT9425-23-1-0701
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	02-09-2023

Infections with B. burgdorferi (Bb) cause persistent infections in many mammalian species, yet these species have an intact immune system. It can be concluded that Bb has ways to evade or even suppress the immune system in order to achieve persistence. Indeed, our previous work showed that Bb infection suppresses the development of fully functional antibody responses both to Bb, and also to other unrelated antigens. Yet, antibody responses are critically important for regulating interaction with the many bacteria and other microorganisms that surround us.

This proposal seeks to understand whether the suppression of effective antibody responses by Bb can change the function of the gastrointestinal tract and cause the gut to become “leaky” and let some of the microbes from the lumen of the gut enter the blood stream. Such a breach in the normal barriers can cause a variety of ongoing symptoms that might persist, even after the Bb infection is treated with antibiotics, potentially explaining some of the ongoing symptoms experienced by patients suffering from Post-Treatment Lyme Disease Syndrome (PTLDS).

Thus, this proposal seeks to address multiple areas research directly relevant for the Department of Defense Fiscal Year 2022 Tick-Borne Disease Research Program: (a) Bb immune evasion, (b) pathogenesis of persistent clinical manifestations associated with Lyme disease, and (c) the effects of tick-borne diseases on local and systemic immune responses. Expected outcomes of our studies would help identify new therapeutic therapies aimed at treating symptoms associated with PTLDS.

Proposal Title:	ANAM Performance in Evaluating Injury and Psychological Health Risk: Establishing the Connection Between Cognition, Health, and Readiness
Log Number:	TP210693
Current PI Name:	Vy Nguyen
Award Number:	HT9425-23-2-0001
Current Contracting Organization:	Henry M. Jackson Foundation
Current Performing Organization:	U.S. Army Research Institute of Environmental Medicine (USARIEM)
Web Approval Date:	10-12-2022

Traumatic brain injury, musculoskeletal injury, and psychological health pose a significant health and medical readiness concern for the military. The cognitive health of Service Members is also of considerable concern because their cognitive abilities are often affected after a traumatic brain injury. Previous studies show that cognitive function may be related to injury, return to duty after an injury, and psychological health conditions such as posttraumatic stress disorder and depression. Changes in cognitive function that may persist and decline throughout the lifespan of Service can also be affected by traumatic brain injury and psychological health.

Given the findings from these previous studies, the proposed project seeks to apply the Automated Neuropsychological Assessment Metrics (ANAM) battery. The ANAM battery is a library of neurocognitive tests administered to Service Members before deployment so that there is a baseline measure of cognitive function to compare to if they experience a traumatic brain injury. Service Members are also administered the ANAM battery after a traumatic brain injury. These neurocognitive data are collected from all Service Members who deploy because of a mandated clinical testing policy and are maintained in a one-of-a-kind data repository that also incorporates medical and personnel data. The proposed project will evaluate whether the performance on ANAM battery can be used to assess the risk of traumatic brain injury, musculoskeletal injury, and psychological health conditions and to assess the time to return to duty after an injury. The proposed project will also evaluate whether certain factors such as traumatic brain injury and psychological health affect changes in ANAM performance over time. Findings will address gaps identified under the program's Focus Area "Understand" sub-area (a) by evaluating neurocognitive tests to improve understanding of the pre-exposure risk factors contributing to an individual's recovery and long-term outcomes following a brain injury.

The proposed research will be conducted among all deploying active-duty Service Members using historical data from as far back as 2007 in a data repository. Much of the project effort will be on data analysis because existing data will be used to carry out the goals of this 4-year project, and results are expected to be start being disseminated in the second year of the project. Results will provide a better understanding of the relationships between neurocognitive function and risk of traumatic brain injury, musculoskeletal injury, and psychological health outcomes that directly impact their health and well-being. Making use of tests that are already being collected, this important research will help identify neurocognitive risk factors for health conditions that are common and debilitating for Service Members with the goal of reducing the likelihood of them occurring even years later. Based on findings from this project, the ANAM battery can potentially be used in interventions to reduce the risk of these conditions in Service Members and to inform clinical guidelines in terms of decisions as to when to return an injured Service Member back to duty.

Proposal Title:	Targeted Plasticity Therapy for the Treatment of Post-Traumatic Stress Disorder
Log Number:	TP220002
Current PI Name:	Seth Hays
Award Number:	HT9425-23-1-0818
Current Contracting Organization:	Texas, University of, at Dallas
Current Performing Organization:	Texas, University of, at Dallas
Web Approval Date:	09-12-2023

Post-traumatic stress disorder (PTSD) is a common source of service-connected disability. Although current treatments can help some people, many others do not benefit or experience intolerable side effects. Providing consistent, effective relief for individuals with PTSD is of clear importance to our Service Members and Veterans.

This project builds on our initial success toward developing a novel therapy to treat chronic PTSD. Our approach uses electrical stimulation of a nerve in the neck to promote rewiring of specific cells in the brain and to activate the calming rest-and-digest response. Combining this nerve stimulation with prolonged exposure (PE), a common type of behavioral therapy, serves to potentiate the benefits of PE and thereby reduce the symptoms of PTSD.

Our research in animal models shows the potential of this nerve stimulation therapy. Building on these studies, we have begun a first-in-human trial to test this intervention in individuals with chronic PTSD. Preliminary findings show that the therapy is safe, tolerable, and reduces core symptoms in individuals with treatment-resistant PTSD. The remaining steps to bring this therapy to clinical practice are completion of successful phase 2 and 3 studies.

Here, we propose an expeditious effort to accomplish the first of these goals and lay the foundation to complete the second. In this project, we will perform a rigorous phase 2 clinical study to investigate whether the therapy improves recovery in individuals with chronic PTSD. Additionally, we will determine the most effective way to deliver therapy in order to maximize benefits. Finally, we will begin the steps needed to smoothly transition to a subsequent phase 3 study once the proposed project is completed. We will leverage the expertise of our community- based lived experience consultant throughout the study to gain perspective on recruitment, implementation, and future application of our therapy.

Our group is seriously committed to developing new and improved treatments for neurological disorders. We have successfully converted a similar approach into the first FDA-approved treatment for chronic stroke. The project proposed here will lead directly to a Phase 3 pivotal study for chronic treatment-resistant PTSD, which is a necessary step toward eventual clinical use. Ultimately, these efforts are focused on translating this innovative therapy to yield real, tangible benefits for Service Members, Veterans, and their families.

Proposal Title:	Design Microbiome-Based Therapies to Prevent and Ameliorate Post-Traumatic Stress Disorder
Log Number:	TP220055
Current PI Name:	Yang-Yu Liu
Award Number:	HT9425-23-1-0880
Current Contracting Organization:	Brigham and Women's Hospital, Inc.
Current Performing Organization:	Brigham and Women's Hospital, Inc.
Web Approval Date:	09-13-2023

An estimated 2 million women Veterans live in the US today. Exposure to trauma is highly prevalent in U.S. women Veterans due to prolonged deployments with war zone exposure, combat-related injuries, and military sexual trauma. Post-traumatic stress disorder (PTSD) is a debilitating mental disorder that occurs in some persons after exposure to a traumatic event such as physical or sexual violence, combat, or a serious accident. At least 1 in 9 American women will meet lifetime criteria for a PTSD diagnosis, and women's risk of PTSD is twice that of men. The prevalence of PTSD is significantly higher among women Veterans than women civilians and men Veterans. An estimated 20% of women Veterans who served in Iraq and Afghanistan have been diagnosed with PTSD. Women are the fastest growing group of Veterans, making up 9.4% of the Veteran population in 2014, which is estimated to increase to 16% by 2040. Among women, PTSD is also more likely to be chronic and is associated with worse functional impairment and distinct neurobiological profiles. Moreover, PTSD is a major risk factor for chronic diseases such as diabetes, coronary heart disease, stroke, and cardiometabolic disease in women. Understanding why some women develop PTSD and others do not is critical to inform prevention and intervention development. Although a substantial body of research has greatly improved our knowledge regarding prevalence, clinical symptoms, and consequences of PTSD over the last decades, much remains to be learned about why some persons are more vulnerable to developing PTSD after trauma, why some persons are resilient, and the mechanisms driving the relation between PTSD and chronic diseases. The potential role of the gut microbiome in mental health and a range of chronic diseases is increasingly recognized, although its role in PTSD specifically has yet to be understood. To date, only four studies have examined the PTSD–gut microbiome relationship. Moreover, these studies have several methodological limitations.

The overarching challenge is that we do not fully understand the PTSD-gut microbiome relationship and hence lack rational design of microbiome-based therapeutics for the prevention or amelioration of PTSD. Our central hypothesis is that microbiome-based interventions can prevent and ameliorate PTSD. Our overall objective is to initiate a unique project to evaluate the PTSD-gut microbiome relationship and develop synbiotics (a combination of prebiotics and probiotics) to prevent or ameliorate PTSD, leveraging a population-based cohort and an etiologically relevant mouse model.

To accomplish the overall objective, we have assembled a multidisciplinary team with expertise in human microbiome, trauma/PTSD epidemiology, resilience/positive health, molecular neurobiology, animal models of stress, and clinical microbiology. This team will pursue four research projects:

Project 1. Differentiate the gut microbiome for women who develop PTSD following trauma versus those who are resilient and resistant in a population-based cohort.

Project 2. Reveal the causation between PTSD versus resilience to trauma and gut microbiome using a novel computational method.

Project 3. Quantify the impact of trauma exposure on the gut microbiome and neuronal activity using a mouse model.

Project 4. Test whether microbiome-based interventions will reverse a CSDS-induced susceptibility phenotype in mice.

Our proposed research is significant because it will provide foundational evidence to inform future studies on microbiome-targeted interventions for preventing or mitigating PTSD and its adverse consequences for physical health. This is directly relevant to the sub-area (3.a) of the three FY22 TBIPHRP FPA Focus Areas. The proposed research will offer a paradigm for the design of microbiome-based therapies to prevent and ameliorate PTSD.

Posttraumatic Stress Disorder (PTSD) is a psychiatric condition that may occur in people who have experienced or witnessed traumatic or horrifying events. PTSD has been linked to an increased risk of various chronic diseases and eating disorders. The gut microbiome plays a critical role in modulating the immune, metabolic, psychological, and cognitive activities of the host. Understanding the association of PTSD with the gut microbiome may improve the physical and mental health of people with PTSD. Yet, this association remains understudied. To date only four observational human studies have examined the PTSD-gut microbiome association and no human studies have examined the microbiome in persons resilient following trauma. Those studies have several methodological limitations that have constrained progress in the field thus far. In this project, we employ the strongest methods available to address those methodological limitations.

Our objective is to differentiate the gut microbiome for women who develop PTSD following trauma versus those who are resilient and resistant in a population-based cohort. First, we will perform community-level analyses to interrogate overall microbiome differences across four groups of women (n=2,605) in a population-based cohort. Second, we will perform differential abundance, classification, and multivariate analyses to identify microbial species/pathways associated with PTSD, resilience and resistance considering a wide range of host factors (e.g., age, body mass index, diet, childhood trauma, medications) captured in this cohort.

The human gut microbiome, comprised of communities of microorganisms and their genes, is increasingly recognized as an essential component of normal physiology, with important roles in health and disease. There is a growing body of basic science that indicates the presence of a “gut-microbiota-brain axis” linking gut microbiota and mental health. The gut microbiome may be especially important for PTSD. In a recent study, the authors followed a unique trauma-exposed cohort for 15 years, from early childhood to adolescence, repeatedly assessing post-traumatic symptomatology. They transplanted the fecal samples from trauma-exposed adolescents (PTSD vs. No PTSD) into germ-free mice, finding that those mice transplanted with PTSD microbiomes compared with those receiving resilient microbiomes exhibited anxious behavior. This result implies that the microbial trauma profile is at least partially responsible for the trauma-related phenotype. So far there are only two interventional studies examined the effect of microbiome-targeted supplementation on PTSD in combat Veterans. One study focused on FSWW08 (a fermented soy formulation), while the other focused on Lactobacillus reuteri DSM 17938 (a commercially available probiotic). None of the interventions was designed based on any established causal relationship between PTSD and the gut microbiome.

The objective of Project-2 is to reveal the causation between PTSD versus resilience to trauma and gut microbiome using a novel computational method—the generalized microbe-phenotype triangulation (GMPT) method. The central hypothesis of Project-2 is that some microbial species can prevent the host to develop PTSD. We will first analyze the WMS sequencing data of 2,605 women in NHSII to identify putative PTSD-causal species and pathways using the GMPT method. We will then design candidate synbiotics based on the putative PTSD-causal species/pathways.

Animal models have been extensively used to study the role of gut microbiome in brain disorders, e.g., autism, Parkinson's Disease, and Alzheimer's Disease. Although PTSD is a complex heterogeneous phenotype that is difficult to model in animals, animal studies are pivotal to advances in understanding PTSD and the neurobiology of stress. In fact, animal models are useful complements to human studies to investigate causal relations where such human studies are neither ethical nor feasible. We simply cannot randomly assign humans to trauma exposure and assess their gut microbiome before and after. Among others, chronic social defeat stress (CSDS) has been suggested to be an etiologically relevant animal model for PTSD-related phenotypes. In our pilot studies, we have successfully implemented the CSDS paradigm in male and female mice along with a comprehensive characterization of behavioral outcomes including supervised machine learning approaches and established whole-brain c-Fos activity mapping following CSDS. Importantly, we have demonstrated that the CSDS paradigm is able to alter gut microbiome composition in mice.

The objective of Project-3 is to study the impact of CSDS on the mouse gut microbiome. Project 3 consists of the following two specific aims: Aim 3.1: Test whether chronic social defeat stress will lead to susceptible- vs. resilient- specific changes in the gut microbiome of male and female mice. Our hypothesis is that CSDS-induced changes in gut microbiome will predict stress susceptible and stress resilient mice. Aim 3.2: Test whether CSDS-induced gut microbiome changes are associated with neuronal activities and transcriptional alterations in the brain. Our hypothesis is that CSDS-induced changes in gut microbiome are associated with neuronal activities and transcriptional alterations in the brain.

Clinical studies suggest a key role of the gut microbiome in stress responsiveness and affective disorders, including anxiety and depression. In addition, human studies demonstrate that microbiota-targeting diets and bacterial strains can have a positive impact on mood, anxiety, cognition, stress, and HPA axis functionality. Thus, microbiome-based therapeutics hold great promise in treating mental disorders. In this project, we will systematically test whether the administration of resilient-specific fecal transplants as well as PTSD-based candidate synbiotics (designed in Project 2) can reverse a stress-induced susceptibility phenotype and that these changes are associated with transcriptional alterations in the brain. Being able to reproduce a resilient-specific microbiome in patients, for example with PTSD, has the potential to induce (behavioral and transcriptional) resilience and therefore provides a basis for novel treatments.

The objective of Project-4 is to test whether microbiome-based interventions will reverse a CSDS-induced susceptibility phenotype in mice. Project 4 consists of the following two specific aims:

Aim 4.1. Test whether the administration of resilient-specific fecal microbiota can reverse a CSDS-induced susceptibility phenotype in mice. Our hypothesis is that resilient-specific fecal microbiota transfer will promote stress resilience in mice following CSDS.

Aim 4.2. Test whether the administration of candidate synbiotics (designed in Project 2) can reverse a CSDS-induced susceptibility phenotype in mice. Our hypothesis is that candidate synbiotics designed in Aim 2.2 will promote stress resilience in mice following CSDS.

Proposal Title:	Measuring Autonomic Nervous System in Warfighters Following Exertion and Resting Stressors (Measuring ANSWERS)
Log Number:	TP220063
Current PI Name:	Wesley Cole
Award Number:	HT9425-23-1-0642
Current Contracting Organization:	North Carolina at Chapel Hill, University of
Current Performing Organization:	North Carolina at Chapel Hill, University of
Web Approval Date:	09-13-2023

Measuring Autonomic Nervous System in Warfighters following Exertion and Resting Stressors (Measuring ANSWERS) seeks to build on a promising area of traumatic brain injury research. Specifically, there is emerging evidence that the autonomic nervous system (ANS), our “fight or flight” control system, is disrupted after mild traumatic brain injury (mTBI). This disruption to ANS functioning potentially contributes to common post-mTBI symptoms, such as slowed thinking, balance issues, cognitive disruptions, sleep problems, and mood disturbances. However, research has not yet clearly linked measures of ANS functioning and those symptom or functional measures commonly used in mTBI clinical care. Such links would have important implications for post-mTBI care. Measures of ANS functioning, such as heart rate variability and pupillary light reflex, are objective and cannot be exaggerated or minimized by patients. Currently, many clinical recommendations for returning someone to activity after mTBI rely on self-reported symptoms which are prone to misrepresentations. Additionally, ANS functioning would serve as a marker of physiologic recovery, which may be a more accurate indicator than currently used assessments of whether and when an individual is ready to return to unrestricted activity.

There are two key Aims in Measuring ANSWERS. First, we will connect measures of ANS functioning (heart rate variability and pupillary light reflex) to common post-mTBI clinical and symptom measures, including cognition (i.e., reaction time), balance, ocular functioning, physical symptoms (e.g., headaches, sleep), and mood. Our test battery is adapted from other studies with Warfighters we are conducting, as our goal is to adapt findings to best serve military populations. A group of 50 active-duty military Service Members and 100 student sports club level athletes will be enrolled. Within these groups, individuals with a history of mTBI will be compared to those with no history of mTBI. For Aim 2, we seek to understand the trajectory of ANS functioning from immediately after mTBI through recovery. We will assess 50 sports club athletes who sustained a recent mTBI 3 times: within 3 days of injury, at self-reported symptom recovery, and within a week of being medically cleared to return to unrestricted activity. We will use the same battery of tests from Aim 1.

Age- and sex-matched controls will also be assessed at similar time points to serve as a comparison. For both Aims, participants will also engage in a virtual reality-based assessment that mimics real-world activities. This will allow us to determine if recovery on ANS measures and current mTBI measures translates to successful real-world performance.

Measuring ANSWERS synergizes our collective expertise and leverages our military and sports mild traumatic brain injury (mTBI) experience to execute a project that will expand our understanding of the biological underpinnings of mTBI and how our current assessments and clinical decision-making tools can be improved for the benefit of the Warfighter. The Measuring ANSWERS project will occur at The Matthew Gfeller Center on the campus of UNC-Chapel Hill. The Matthew Gfeller Center has a long history of collaboration with partners who have lived experience and insights into the dynamic and complex issues surrounding civilian and military mTBI. We have been committed to engaging military community partners and stakeholders in our work as a result. This extends beyond community members serving in an advisory

capacity as we have created true partnerships between our scientific clinical researchers and military community members. These relationships guide and shape our research goals and study design, and our projects have the ultimate goal of translating research findings to benefit and better serve military populations.

Findings from Measuring ANSWERS will further our understanding of the role of the ANS in mTBI symptoms and recovery. This is especially relevant for service members who are often returned to dynamic and challenging environments with increased risk for further injury. Having a more objective indicator of recovery from mTBI that has been linked to successful return to “real-world” performance would increase the confidence that Service Members are ready to be safely returned to duty. Additionally, further clarifying how disrupted ANS functioning contributes to mTBI symptoms could have implications for treatment, particularly in those service members and Veterans with persistent symptoms after mTBI. We plan to complete this study over a 3- year funding period with rapid dissemination of study findings in order to positively impact clinical practice as soon as possible.

Proposal Title:	The REACH Intervention for Caregivers of Veterans and Service Members with TBI: Efficacy and Implementation Planning Across the VA Polytrauma System of Care
Log Number:	TP220091
Current PI Name:	Paul Perrin
Award Number:	HT9425-23-1-0563
Current Contracting Organization:	Virginia, University of
Current Performing Organization:	Virginia, University of
Web Approval Date:	09-13-2023

Objectives/Rationale: Family caregivers are the primary supports for Veterans and Service Members (V /SMs) with traumatic brain injury (TBI). Many TBI caregivers provide over 60 hours a week of care and experience negative effects of their role, including health problems, strain, depression, anxiety, and even suicidal thoughts. Unfortunately, there is no structured, research-backed, and widely used clinical intervention to bring much- needed services to caregivers of V/SMs with TBI. Therefore, this study will adapt, rigorously test, and develop concrete plans to implement nationally across the U.S. Department of Veterans Affairs (VA) Polytrauma System of Care (PSC) a previously developed six-session telehealth caregiver intervention, “Resources for Enhancing All Caregivers’ Health” (REACH), for the unique needs of caregivers of V/SMs with TBI.

Community-Based Participatory Research (CBPR) Approach: Capturing and integrating CBPR input is central to all three aims of the proposed study: (a) engagement and adaptation (b) randomized waitlist-control trial, and (c) implementation planning. CBPR subject matter experts (SMEs) will be core members of our research team; four will be caregivers of V/SMs with TBI, 8-10 will be TBI clinicians, three will be PSC clinical researchers, and two will be PSC administrators. The input from caregivers who share their lived experience will not only guide the development of the intervention, but they and other SMEs will be key to decision-making as we refine the content; this process will depend upon their modifications and concurrence.

Given the goal of delivering an effective and meaningful caregiver intervention throughout the entire VA PSC, our approach to implementation planning will depend upon the engagement of clinicians who will ultimately champion the uptake of REACH-TBI. Further, we need administrator buy-in for true systems change.

Applicability and TBIPHRP Focus Areas: This proposal aligns with three “Treat” focus areas: (3a) using telehealth to promote sustained recovery during the chronic phase of injury and overcome gaps in care delivery to rural or resource-limited environments; (3b) using proven family- and caregiver-level methods for reducing barriers to care for TBI challenges; and (3c) using services and implementation research to increase VA provider access to and adoption of a research-backed intervention for caregivers of V/SMs with TBI. Population Helped: Caregivers of V/SMs with TBI will be assigned to either the REACH-TBI (55) or a waitlist-control (55) group for 3 months, after which the initial assignments will be switched for the subsequent 3 months. At 3- and 6-month follow ups, we expect that caregivers receiving REACH-TBI will show improved strain, depression, anxiety, self-efficacy, and military health care frustration compared to the waitlist-control.

Clinical Applications, Benefits, and Risks: While we do not anticipate any more than minimal risks (e.g., temporary sadness when talking about caregiving challenges), if effective, REACH-TBI will have powerful clinical applications as the first research-backed intervention for caregivers of V/SMs with TBI that can be fully implemented across one of the largest health systems serving V/SMs with TBI, thereby filling a critical gap in clinical services. The VA PSC and the VA Caregiver Support Program's National Caregiver Center are poised to support a full-scale implementation of REACH-TBI, and this study will help make that strategy concrete.

Timeline: In the first 6 months, we will adapt and standardize REACH-TBI based on the TBI research literature and interviews with TBI caregiver and clinician SMEs. In months 7-36, we will evaluate the effectiveness of REACH-TBI in improving the hypothesized outcomes, as well as work with our SMEs to interpret the results of open-ended interviews that will empower participants to be the experts regarding how we can best modify the delivery of the intervention for maximum benefit to TBI caregivers throughout the PSC. In months 37-48, we will work with our SMEs to develop an actionable implementation plan for a subsequent rollout of REACH- TBI across the full VA PSC. Therefore, we will have a REACH-TBI intervention by 7 months, evidence of its effectiveness for caregiver outcomes at 36 months, and a full PSC-wide implementation plan at 48 months.

Relevance to Military Health: The proposed study—for the first time in the VA's history—will link the over 100 clinics in the PSC and the National Caregiver Center to adapt, test, and develop a plan to implement at a national level a proven intervention for caregivers of V/SMs with TBI. REACH-TBI has potential to serve the caregivers currently supporting the needs of nearly half a million V/SMs with TBI, remediating the adverse strain and mental health effects of caregiving, as well as improving self-efficacy and military health care frustration. This study will also provide a model for working from a true CBPR framework to integrate perspectives of caregivers of V/SMs with TBI, TBI clinicians and clinical researchers, and PSC administrators in all phases of TBI intervention development and implementation, thereby affecting systems change and empowering families to access resources critical to the care and wellbeing of V/SMs with TBI.

Proposal Title:	Turning Training into Action: Translating Training of Behavioral Health Providers into Evidence-Based Practices
Log Number:	TP220099
Current PI Name:	Shelley MacDermid Wadsworth
Award Number:	HT9425-23-1-0479
Current Contracting Organization:	Purdue University
Current Performing Organization:	Purdue University
Web Approval Date:	09-12-2023

This project addresses the “Treatment” focus area of the TBIPHRP, specifically, “increasing provider adoption and availability of evidence-based treatments.” Two persistent and thorny challenges that interfere with the quality of behavioral health care for military families are: (a) ensuring that providers can effectively deliver evidence-based therapies (EBTs) and (b) that individuals in need can find and connect with well-prepared providers. These challenges are particularly acute for the reserve component personnel (Tanielian et al., 2014, 2018), who systematically receive less and lower-quality care for their mental health problems than personnel serving in the active component (Hummer et al., 2021).

The proposed project builds on the Star Behavioral Health Providers (SBPH) program, which trains community-based therapists to serve military members and their families. Our objective is to strengthen the implementation of SBHP by testing the effectiveness of methods for providing follow-on support after training and for promoting and facilitating provider engagement with RC personnel and their families. We will focus on Cognitive Behavioral Therapy for Insomnia and Cognitive Processing Therapy for PTSD, two well-validated evidence-based therapies that are recommended as front-line treatments for their respective conditions by both the Department of Defense and the Department of Veterans Affairs. These conditions are among the most common for military personnel, especially those who have experienced wartime deployments (Schvey et al., 2021).

This study will identify strategies that can be used to promote competent application of evidence-based practices with both military and civilian populations, and to strengthen connections between community-based providers and military-connected clients, which would offer wide benefits inside and outside the military. The study carries no direct risks to military families, but it is possible we will learn more about ineffective than effective strategies, which will limit contributions to the field. The study will be conducted in two phases lasting a total of 45 months.

This project directly targets civilian mental health providers in the SBHP program in order to increase community-based EBT care for military-connected patients, particularly Reserve Component personnel and their families. Planned interventions are also predicted to increase the use of gold-standard therapies, helping to improve the quality of services that these Service Members and their family members receive when seeking care in the community. Further, it is expected that the findings of the proposed study will generalize to other clinical settings and populations of interest including active-duty personnel, Veterans, and their families thereby facilitating the delivery of EBT care to these populations.

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Proposal Title:	The Epichaperome: A Novel Therapeutic Target for Blast Traumatic Brain Injury
Log Number:	TP220102
Current PI Name:	Clifton Dixon
Award Number:	HT9425-23-1-0400
Current Contracting Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Current Performing Organization:	University of Pittsburgh-of the Commonwealth System of Higher Ed.
Web Approval Date:	06-09-2023

Exposure to blast waves during training or deployment can affect the brain. Blast traumatic brain injury of all severities can result in chronic disturbances of cognitive, behavioral, emotional, and physical functioning. Normal complex cognitive function requires optimal functioning of neuronal networks. It has been well documented that, when the brain undergoes stress (e.g., stroke, neurodegeneration, trauma), heat shock proteins are induced to assist in protein folding, degradation and other functions. Also called chaperones, they function to maintain cellular function by aiding in protein folding, activation, degradation, or disaggregation in normal cells. Chaperomes are composed of chaperones, co-chaperones, and other factors and function to modulate the structural and functional architecture of protein networks. Under conditions of cellular stress, the chaperomes become biochemically “rewired” to form a network of stable, high-molecular-weight complexes, recently called epichaperomes. It has recently been demonstrated that the epichaperomes are responsible for the disturbance of protein-protein interaction networks which ultimately become dysfunctional and can drive brain pathology. These epichaperome mediated protein network changes have been identified and therapeutically targeted. Inhibition of epichaperomes result in the degradation of disease-causing proteins and normalization of affected cellular regulatory pathways. A member of a new class of drugs called epichaperome inhibitors, PU-AD is an oral, brain-permeable inhibitor of heat shock 90 in epichaperomes. PU-AD has been shown to reduce the epichaperome in models of Alzheimer’s disease and Parkinson’s disease. We have preliminary data that PU- AD treatment can attenuate cognitive deficits after experimental traumatic brain injury. It is currently unknown whether blast TBI results in the formation of epichaperome responses. The overall goal of the project is to provide proof of principle that blast TBI produces an epichaperome response and that the anti-epichaperome drug PU-AD reverses epichaperome levels and improves learning and memory. The project will first define the time course of epichaperome formation after experimental blast. Next, we will test the efficacy of PU-AD therapy on cognitive function after experimental blast. If epichaperomes are determined to result from blast TBI, a novel therapeutic target will be identified that could benefit survivors of blast traumatic brain injury. The project focus area of Treat and the sub-focus area of “Treatments that promote functional recovery, including interventions administered acutely, during the post-acute phase, or during the chronic phase of injury.” The epichaperome is a novel mechanism for aberrant brain function after blast traumatic brain injury. Therapies, such as PU-AD are already being evaluated clinically for other brain disorders and could rapidly be translated to treat military personnel with brain injuries resulting from blast exposure.

Proposal Title:	Identifying Circuit Mechanisms of MDMA and Methylone to Develop Plasticity-Based PTSD Treatment
Log Number:	TP220107
Current PI Name:	Alfred Kaye
Award Number:	HT9425-23-1-0458
Current Contracting Organization:	Yale University
Current Performing Organization:	Yale University
Web Approval Date:	06-09-2023

The objectives of the proposed study in Dr. Alfred Kaye's and Dr. Christopher Pittenger's laboratories at Yale University are to determine the mechanisms by which MDMA and methylone may induce therapeutic plasticity in PTSD using cutting-edge neural circuits methods. MDMA is expected to be approved as a transformative treatment for PTSD, while similar compounds with distinct effects like methylone are not well understood. Methylone has been administered in two clinical studies in healthy volunteers, is well-tolerated (Poyatos, et al., 2021; Poyatos, et al., 2021b), and recently, Transcend Therapeutics, with headquarters in New York NY received authorization to proceed with an open-label PTSD study in the UK to study TSND-201.

A rapid-acting (single treatment), long-lasting (durable), non-hallucinogenic treatment for psychological health condition(s) would support the DOD and/or VA goals of a rapid treatment for psychological health conditions including ASR and PTSD (Focus Area Treat 3a). To develop TSND-201 to its fullest potential and optimize a dosing regimen, additional preclinical research must be conducted to understand methylone's mechanism of action and impact on neuroplasticity.

The need for improved pharmacological treatments for PTSD has been recognized as a crisis by the VA PTSD Pharmacology Working Group (Krystal, et al 2017). While selective serotonin reuptake inhibitors are FDA-approved and show efficacy, 40% of patients may be treatment-refractory (Stein et al., 2002; Zohar et al., 2002). Recently, a phase 3 trial of MDMA augmentation of psychotherapy showed a remarkable effect (d=0.91 on CAPS-5 rating scale, Mitchell et al 2021). MDMA-assisted psychotherapy is based on its effects in enhancing the plasticity, although the precise mechanisms by which this occurs are not well understood. The broader class of potential PTSD treatments, MDMA-like compounds called entactogens, were identified based on similar biochemical effects and because they seemed to produce an enhancement of therapies for dealing with difficult memories. The entactogen methylone (TSND-201) would provide a novel therapeutic with no hallucinogenic treatment affects that would provide another therapeutic tool.

A rapid-acting (single treatment), long-lasting (durable), non-hallucinogenic treatment for psychological health condition(s) would support the DOD and/or VA goals of a rapid treatment for psychological health conditions including ASR and PTSD.

Proposal Title:	Identifying Circuit Mechanisms of MDMA and Methylone to Develop Plasticity-Based PTSD Treatment
Log Number:	TP220107P1
Current PI Name:	Christopher Pittenger
Award Number:	HT9425-23-1-0459
Current Contracting Organization:	Yale University
Current Performing Organization:	Yale University
Web Approval Date:	06-09-2023

The objectives of the proposed study in Dr. Alfred Kaye's and Dr. Christopher Pittenger's laboratories at Yale University are to determine the mechanisms by which MDMA and methylone may induce therapeutic plasticity in PTSD using cutting-edge neural circuits methods. MDMA is expected to be approved as a transformative treatment for PTSD, while similar compounds with distinct effects like methylone are not well understood. Methylone has been administered in two clinical studies in healthy volunteers, is well-tolerated (Poyatos, et al., 2021; Poyatos, et al., 2021b), and recently, Transcend Therapeutics, with headquarters in New York NY received authorization to proceed with an open-label PTSD study in the UK to study TSND-201.

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A rapid-acting (single treatment), long-lasting (durable), non-hallucinogenic treatment for psychological health condition(s) would support the DOD and/or VA goals of a rapid treatment for psychological health conditions including ASR and PTSD.

Proposal Title:	Law Enforcement Officers and Traumatic Brain Injuries
Log Number:	TP220111
Current PI Name:	Jaclyn Caccese
Award Number:	HT9425-23-1-0520
Current Contracting Organization:	Ohio State University, The
Current Performing Organization:	Ohio State University, The
Web Approval Date:	08-01-2023

Rationale/Objective: Our long-term goal is to raise awareness, monitor, and optimize civilian law enforcement officer (LEO) health and wellness following traumatic brain injury (TBI), with the primary focus on preserving quality of life and careers of those who protect and serve our community. In the process of protecting and serving our community daily, LEOs are often called to respond to stressful, life-threatening, and potentially traumatic situations, often putting their own lives in danger. This work puts them at risk for experiencing TBIs, musculoskeletal injuries, and psychological health conditions (e.g., post-traumatic stress disorder [PTSD]). Our LEOs currently lack the health care, educational resources, and support they need, potentially causing long-term health consequences. Therefore, we propose the following three Aims. Aim 1 will determine how many LEOs have a history of TBI and psychological health conditions; we will also ask questions about how they sustained the TBIs (e.g., sports, military service, on duty). Aim 2 will use sensors in mouthguards to measure head impact exposures during combative training and will use sensors on headgear and vests to measure blast exposures. Aim 3 will use a series of performance assessments before and after head impact and blast exposures; testing will include cognitive, reaction time, jump performance, heart rate, balance, gait and sleep assessments.

CBPR Approach: Our community-based partners include Executive Director of the National Association of State Head Injury Administrators, Rebeccah Wolfkiel, and Franklin County Sheriff's Office Deputies, Scott Paur and Joshua Walters. Our community-based partners have been involved in the design of the proposed project and will be involved in every stage of the research process, from recruitment and implementation to data dissemination.

Impact: This proposal will target FY22 TBIPHRP IIRA Focus Area 1, "Understand by determining how many LEOs have a history of TBIs and psychological health conditions," (Aim 1) and Focus Area 2, "Prevent and assess by measuring head impacts and blasts exposures in LEOs during training activities," (Aim 2) and determining the effects of these head impacts and blast exposures on human performance (Aim 3). Ultimately, this proposal will improve our understanding of TBIs and psychological health conditions among LEOs.

Beneficiaries: Our findings will allow us to draw actionable insights to inform training and operational decision-making and to increase awareness of the potentially negative effects of head impacts and blast exposures within the law enforcement community; we will disseminate findings to relevant stakeholders who can provide health care and educational resources to LEOs.

Clinical Applications: Findings will help us develop a framework for monitoring that may be used in the future for LEOs or U.S. Service Members and will provide risk mitigation strategies, such as calculating the minimum safe distance for breachers in training exercises. Risk mitigation strategies not only include monitoring and reducing the magnitude of blunt head impacts and low-level blast exposures, but also wellness initiatives within law enforcement organizations (e.g., rehabilitation programs, peer-mentoring for Veterans to address transition to civilian life, behavioral screening tools). Importantly, these data are needed as a first step in developing injury thresholds and exposure standards.

Projected Timeline to Achieve the Expected Outcomes: With the help of our community-based partners, we will disseminate findings to relevant stakeholders, including local, state, and federal agencies and organizations by the end of year 3. We have already started educational initiatives focused on TBIs and psychological health conditions in LEOs by presenting at the 2022 Ohio Public Safety Symposium. Finally, we will present study findings each year at the Military Health System Research Symposium.

Relevance to Service Members, Veterans, and/or Military Beneficiaries: An estimated 35-40% of new LEO recruits are Operation Enduring Freedom/Operation Iraqi Freedom Veterans. Both men and women who decide to pursue post-military careers in law enforcement may have undiagnosed TBIs and/or psychological health conditions. Therefore, occupational health care providers for LEOs must become familiar with the potential needs of veterans within their practice. In addition, due to similarities in occupational hazards between LEOs and U.S. Service Members, we can use LEOs to develop a framework for monitoring that informs critical decision making about health and wellness for both LEOs and U.S. Service Members.

Proposal Title:	Law Enforcement Officers and Traumatic Brain Injuries
Log Number:	TP220111P1
Current PI Name:	James Onate
Award Number:	HT9425-23-1-0521
Current Contracting Organization:	Ohio State University, The
Current Performing Organization:	Ohio State University, The
Web Approval Date:	08-01-2023

Rationale/Objective: Our long-term goal is to raise awareness, monitor, and optimize civilian law enforcement officer (LEO) health and wellness following traumatic brain injury (TBI), with the primary focus on preserving quality of life and careers of those who protect and serve our community. In the process of protecting and serving our community daily, LEOs are often called to respond to stressful, life-threatening, and potentially traumatic situations, often putting their own lives in danger. This work puts them at risk for experiencing TBIs, musculoskeletal injuries, and psychological health conditions (e.g., post-traumatic stress disorder [PTSD]). Our LEOs currently lack the health care, educational resources, and support they need, potentially causing long-term health consequences. Therefore, we propose the following three Aims. Aim 1 will determine how many LEOs have a history of TBI and psychological health conditions; we will also ask questions about how they sustained the TBIs (e.g., sports, military service, on duty). Aim 2 will use sensors in mouthguards to measure head impact exposures during combative training and will use sensors on headgear and vests to measure blast exposures. Aim 3 will use a series of performance assessments before and after head impact and blast exposures; testing will include cognitive, reaction time, jump performance, heart rate, balance, gait and sleep assessments.

CBPR Approach: Our community-based partners include Executive Director of the National Association of State Head Injury Administrators, Rebeccah Wolfkiel, and Franklin County Sheriff's Office Deputies, Scott Paur and Joshua Walters. Our community-based partners have been involved in the design of the proposed project and will be involved in every stage of the research process, from recruitment and implementation to data dissemination.

Impact: This proposal will target FY22 TBIPHRP IIRA Focus Area 1, "Understand by determining how many LEOs have a history of TBIs and psychological health conditions," (Aim 1) and Focus Area 2, "Prevent and assess by measuring head impacts and blasts exposures in LEOs during training activities," (Aim 2) and determining the effects of these head impacts and blast exposures on human performance (Aim 3). Ultimately, this proposal will improve our understanding of TBIs and psychological health conditions among LEOs.

Beneficiaries: Our findings will allow us to draw actionable insights to inform training and operational decision-making and to increase awareness of the potentially negative effects of head impacts and blast exposures within the law enforcement community; we will disseminate findings to relevant stakeholders who can provide health care and educational resources to LEOs.

Clinical Applications: Findings will help us develop a framework for monitoring that may be used in the future for LEOs or U.S. Service Members and will provide risk mitigation strategies, such as calculating the minimum safe distance for breachers in training exercises. Risk mitigation strategies not only include monitoring and reducing the magnitude of blunt head impacts and low-level blast exposures, but also wellness initiatives within law enforcement organizations (e.g., rehabilitation programs, peer-mentoring for Veterans to address transition to civilian life, behavioral screening tools). Importantly, these data are needed as a first step in developing injury thresholds and exposure standards.

Projected Timeline to Achieve the Expected Outcomes: With the help of our community-based partners, we will disseminate findings to relevant stakeholders, including local, state, and federal agencies and organizations by the end of year 3. We have already started educational initiatives focused on TBIs and psychological health conditions in LEOs by presenting at the 2022 Ohio Public Safety Symposium. Finally, we will present study findings each year at the Military Health System Research Symposium.

Relevance to Service Members, Veterans, and/or Military Beneficiaries: An estimated 35-40% of new LEO recruits are Operation Enduring Freedom/Operation Iraqi Freedom Veterans. Both men and women who decide to pursue post-military careers in law enforcement may have undiagnosed TBIs and/or psychological health conditions. Therefore, occupational health care providers for LEOs must become familiar with the potential needs of veterans within their practice. In addition, due to similarities in occupational hazards between LEOs and U.S. Service Members, we can use LEOs to develop a framework for monitoring that informs critical decision making about health and wellness for both LEOs and U.S. Service Members.

Proposal Title:	A Randomized Clinical Trial of Quetiapine to Reduce Post-Concussive Syndrome Polypharmacy
Log Number:	TP220114
Current PI Name:	Muhammad Baig
Award Number:	HT9425-23-1-0829
Current Contracting Organization:	Foundation for Advancing Veterans' Health Research
Current Performing Organization:	Foundation for Advancing Veterans' Health Research
Web Approval Date:	09-13-2023

An unprecedentedly high number of mild traumatic brain injuries (mTBIs) were survived by combat Veterans who deployed in support of conflicts following 9/11 and thus became known as one of the "signature injuries" of modern combat. Combat-related mTBI is frequently associated with a concurrent psychological comorbidity, including post-traumatic stress disorder (PTSD) and high levels of disability and health care service utilization as long-term physical, psychological, and cognitive consequences in Veterans. Unfortunately, there are no FDA-approved or otherwise established medications or strong recommendations in the DOD/VA clinical practice guidelines for pharmacologic treatment of post-concussive symptoms. This lack of evidence to guide care often results in the use of multiple central nervous system-acting medications in the attempt to provide symptomatic relief for post-concussive symptoms and comorbid psychological conditions – resulting in polypharmacy practices which lead to adverse outcomes. Unfortunately, even this polypharmacy practice often fails to provide adequate symptom relief for either post-concussive symptoms or psychological comorbidity and adds undesired side effects to the burden of Veterans. Therefore, a huge need in the field of mTBI research is to find a single effective medication that reduces the burden of post-concussive symptoms without adding to the burden of Veterans through polypharmacy.

Quetiapine, an atypical antipsychotic approved by the FDA, has a broad spectrum of actions at several receptors. Quetiapine's ability to reduce irritability and anxiety and improve sleep without impairing sleep architecture is a distinct benefit over other medications and theoretically may benefit patients with post-concussive symptoms. Though quetiapine does have risks and side effects as an atypical antipsychotic, none of these prevent its effective use in short-term treatment for mTBI rehabilitation. Compared to other anti-psychotics, quetiapine has less risk of extra-pyramidal symptoms. Also, the risks for diabetes and heart disease are dose related effects on metabolic dysregulation that are associated with the high doses used to treat psychotic disorders. However, thorough medical screening for metabolic risk factors coupled with low-moderate quetiapine doses use can prevent any of these complications. Furthermore, starting a low dose scheduled at bedtime and slowly titrating up to a desired level of sedation may regularize the circadian rhythm and greatly improve sleep without interfering with the sleep. Both the psychopharmacological rationale, available safety data, and preliminary findings from our group, strongly argue for the need of a larger-scale phase 3 randomized clinical trial to determine the effectiveness of quetiapine monotherapy. We propose the need for a randomized pragmatic trial evaluating the efficacy of quetiapine in Veterans with co-morbid mTBI and PTSD specifically as an adjunct to comprehensive rehabilitation services delivered as a standard of care.

Herein, we propose a randomized, pragmatic, open-label trial evaluating the efficacy of quetiapine monotherapy compared to TAU polypharmacy, as an adjunct to standard of care rehabilitation services in Veterans with mTBI and comorbid posttraumatic stress symptoms.

The proposed project addresses the Treat Focus Area for a Level 2 CTA under the TBIPHRP BAA. Veterans (N=146) from South Texas Veterans Healthcare System and New Mexico VA Healthcare System. with PTS symptoms following an mTBI who are not currently using quetiapine will be enrolled in a phase 3, two-site,

16-week, randomized open-label pragmatic trial of quetiapine monotherapy vs. TAU polypharmacy as an adjunct to standard of care outpatient rehabilitation treatment. TAU Participants will continue their standard care prescribed medication adjusted to maximize benefits and minimize risk. Participants in the quetiapine group will be cross-tapered off other psychotropic medications while adding a flexible dose regimen of quetiapine based upon tolerability and clinical response to maximize engagement in rehabilitation treatment.

An evidence-based monotherapy could greatly improve the outcomes of Veterans suffering with post-concussive symptoms following an mTBI and who have PTS symptoms making them especially vulnerable to the adverse effects of CNS-acting medications commonly used. Results of this study will inform the joint VA/DOD CPGs. Positive results with quetiapine in this trial could minimize side effects and increase the utilization rate and outcome success of using psychopharmacological monotherapy within rehabilitation programs in the VA system and could lead to new guidelines for clinical practice. The study is significant by showing that certain medications can effectively augment rehabilitation services to improve recovery from mTBI and PTSD within the VA standards of care programs and minimize polypharmacy practices. It will benefit the VA by reducing the ongoing demands of an unremitted chronic disease in Veterans.

Proposal Title:	Evaluating Reductions in Hippocampal Volume Related to Blast Exposure and Their Effect on Memory Function
Log Number:	TP220120
Current PI Name:	Sarah Martindale
Award Number:	HT9425-23-1-0789
Current Contracting Organization:	Salisbury Foundation for Research and Education, Inc.
Current Performing Organization:	Salisbury Foundation for Research and Education, Inc.
Web Approval Date:	09-12-2023

FY21 TBIPHRP IIRA Focus Area to be Addressed: Understanding injury (blast) and biological factors (hippocampal size) contributing to long-term outcomes (memory) following brain injury or traumatic events.

Objectives and Rationale: The overarching purpose of this proposal is to determine whether, when, and under what circumstances exposure to blast affects brain health long term, beyond what is expected for normal aging. The specific focus of this proposal will determine if reductions in hippocampal size as a result of blast exposure continue to change over time and how these changes relate to memory function. This focus builds upon our novel finding that increasing severity of blast exposure is related to lower hippocampal size in combat Veterans. The relationship between blast exposure and hippocampal size was strong, even after accounting for other conditions, including post-traumatic stress disorder and traumatic brain injury. This project will advance our research by identifying how size differences and/or size changes related to blast exposure affect memory performance, which relies on the hippocampus.

Previous research has shown a strong relationship between blast exposure and smaller hippocampal size in both animals and humans. The relationship between hippocampal size and memory performance is also well established. Previous research has demonstrated that exposure to a blast at close range is associated with poorer memory. However, we do not know whether blast exposure leads to a process of hippocampal degeneration that continues over time, which may also explain memory complaints among service members when a clear cause cannot be identified. Importantly, our preliminary finding demonstrates that changes in hippocampal size seen shortly after blast exposure may endure for many years. It also leads to the troubling possibility that the size of the hippocampus may continue to decline more than expected over time due to blast exposure.

The objective of this study is responsive to the Understand focus area in two ways. First, the proposed study will determine whether reduction in hippocampal size related to blast exposure is an ongoing process of neurodegeneration. We will identify potential characteristics of blast exposure that may be responsible for ongoing changes in hippocampal size. Identifying a specific characteristic of blast exposure, such as the severity of blast pressure experienced, would be highly informative to the development of in-theater protocols (e.g., rest following specific exposure) and treatment or intervention guidelines. Second, the proposed study will determine if hippocampal size explains the relationship between blast exposure and difficulties with learning and memory (i.e., neurocognitive disorders, commonly referred to as dementia).

In addition to the hippocampus, we will also evaluate other brain regions of interest to determine whether blast accelerates change beyond normal aging. If this is indicated, blast may be a contributing risk factor for negative outcomes, such as dementia. This is responsive to the DoD Warfighter Brain Health Initiative that has identified blast as an exposure of interest that might affect brain health. Overall, this study will increase what we know about the nature of problems following blast exposure and the blast event characteristics

associated with those outcomes. Knowledge gained from this research will support future studies in evaluation of potential strategies to mitigate or alleviate these negative outcomes in an informed and effective manner.

Outcomes: This research represents a necessary step along the translational science continuum towards clinical applications. Too little is currently known about the relationship between blast exposure, brain structure, and associated functional outcomes for effective interventions to be developed. Further, we know very little about the relationship between blast exposure and clinical outcomes in humans, which does not support application to person-related outcomes at this time. The current project will create new knowledge about how blast exposure affects clinical outcomes. When combined with the results of similar projects, this knowledge will form the basis for the development of meaningful interventions to lessen the negative effects of blast exposure. Once we know what blast-related factors are related to negative outcomes, then treatments and interventions to improve outcomes can be effectively developed.

Contributions to Advancing Understanding of Psychological Health Conditions: What we learn from this research will ultimately improve our understanding of outcomes following blast exposure in both biological and functional domains. An improved understanding of the factors related to these outcomes will provide the necessary foundation to support the development of interventions that lead to better, more effective treatments for Service Members.

Proposal Title:	SMART-CPT for PTSD and History of Concussion: A Pragmatic Implementation Trial
Log Number:	TP220123
Current PI Name:	Amy Jak
Award Number:	HT9425-23-1-0528
Current Contracting Organization:	Veterans Medical Research Foundation of San Diego
Current Performing Organization:	Veterans Medical Research Foundation of San Diego
Web Approval Date:	09-12-2023

The proposed work targets the third FY22 TBIPHRP PCRA Focus Area: “Treat,” by testing a behavioral (psychotherapy) intervention for helping Veterans with a history of mild traumatic brain injury (mTBI), post-traumatic stress disorder (PTSD), and cognitive complaints (such as memory or attention). These conditions occur together in a large proportion of post-9/11-era Veterans seeking treatment for persistent symptoms following mTBI. This common combination of conditions often complicates recovery from each condition and leads to poorer day-to-day functioning than would each of the conditions occurring in isolation since cognitive, emotional, and neurobehavioral symptoms are strongly intertwined. Typically, these conditions are treated separately from each other, despite overlap in symptoms and treatment strategies. Given the interconnectedness of these conditions, integrating treatments to fully target the overlapping symptoms simultaneously will lead to better outcomes for Veterans and reduce time and cost burdens. We previously conducted a preliminary study at a single site of an integrated intervention, SMART-CPT, which combines Cognitive Processing therapy (CPT) for PTSD (a gold standard treatment for PTSD) with cognitive symptom management and rehabilitation training (CogSMART) for treatment of persistent post-concussive symptoms and thinking difficulties associated with TBI.

We found that, in addition to reducing PTSD and post-concussive symptoms, SMART-CPT (as compared to standard CPT) led to significant improvements in thinking abilities in several areas, including attention, memory, and problem solving. However, additional data are needed to confirm the broad effectiveness and feasibility of SMART-CPT when delivered at different sites by different treatment providers. Particularly, we need to understand the implementation of the therapy to be sure it can successfully be carried out by VA, DOD, and/or community partner sites. We need to further study the acceptability of the treatment by Veterans and identify any barriers and/or facilitators of implementing SMART-CPT. Therefore, we propose to examine SMART-CPT in a multi-site hybrid implementation trial. Consistent with community based participatory research principles, the trial will be informed at all stages by a Veteran lived-experience consultant. We hypothesize those Veterans who complete SMART-CPT will have greater improvements in objective cognitive functioning, quality of life, and day-to-day functioning than those receiving standard CPT. We also hypothesize that SMART-CPT can be successfully implemented across both VA/DOD and community partner sites.

The proposed study targets Veterans with PTSD, cognitive complaints, and history of mTBI; half will be randomly assigned to receive SMART-CPT and half to receive standard CPT. Both interventions will last twelve weeks and will be delivered in an individual format. Veteran patients will also undergo assessments of mental health, quality of life, and cognitive functioning prior to therapy, at the end of therapy, and three months after the end of treatment. Expected near-term benefits include direct, immediate, and practical applications to treatment of Veterans. The intervention takes three months to complete and in this time we anticipate that individuals will see a notable reduction in post-concussive and PTSD symptoms, increases in cognitive functioning, and improvements in quality of life. We also anticipate ease of implementation of SMART-CPT by mental health clinicians in the near term, and optimization of implementation for expanded adoption of the treatment in the in the months after the completion of the trial. Both treatment groups are

receiving all the standard elements of CPT; therefore, both groups are receiving the standard of care for PTSD and risks to participating would be minimal and similar to participating in any psychotherapy. Because there are currently no empirically-supported treatments designed specifically to treat comorbid PTSD and TBI in aggregate, this study has the potential to fill a large treatment void, particularly in military and Veteran populations. Validating the SMART-CPT intervention to better treat this large proportion of the Veteran and military population with complex TBI would significantly improve and streamline treatment and reduce distress in individual service members and Veterans. Healthcare costs of those with complex TBI are 4-6x higher than those without complex TBI, and SMART-CPT can be delivered in a third less time than current treatments; this effectiveness paired with efficiency leads not only to better resolution of the distress experienced by those with complex TBI but the long-term impact of the proposed work would be reduced overall healthcare costs. The proposed trials particular focus on implementation will also lead to increased provider adoption and availability of evidence-based treatments, as well as treatment engagement, and identification and evaluation of methods for successful dissemination of SMART-CPT.

Proposal Title:	Development and Pilot Testing of eHealth Problem-Solving Training (ePST) for Adults with Traumatic Brain Injury
Log Number:	TP220141
Current PI Name:	Shannon Juengst
Award Number:	HT9425-23-1-0567
Current Contracting Organization:	TIRR Memorial Hermann
Current Performing Organization:	TIRR Memorial Hermann
Web Approval Date:	09-12-2023

Who Will This Help and How It Will Help Them? Traumatic brain injury (TBI) is a condition of growing concern in the United States, particularly among Veterans/Service members. TBI often leads to physical, cognitive, emotional, and behavioral symptoms that can affect reintegration into civilian life, social functioning and health, and quality of life. Cognitive and emotional changes after TBI are especially concerning, as they contribute to high rates of suicide, substance abuse, and social problems among Service Members with this condition. Therefore, treating or preventing these symptoms is critically important and could save lives.

Problem-Solving Training (PST) teaches a standard, step-by-step, problem-solving strategy [A=Assess; B=Brainstorm; C=Consider and Choose; D=Develop and Do; E=Evaluate; F=Flex] that can be used to solve problems or achieve goals. This strategy could be applied to whatever everyday problems and goals a person chose, making it flexible and adaptable to every individual. By supporting goal achievement and a person's ability to solve daily problems, we could prevent and improve function, health, and overall quality of life of Veterans/Service Members and civilians with TBI and their care partners.

Objectives and Rationale for the Proposed Project: Though PST is already delivered remotely over the phone or video call, it still requires time from a trained therapist and coordinating times for a therapist and person with TBI to meet. With more people having access to and comfort using mobile devices, more people could benefit from PST if we could adapt it to an electronic format. Therefore, to provide a more widely available, accessible, and effective support for persons living with the chronic psychological health consequences of TBI, we propose to co-design, co-develop, and pilot test an electronic and mobile version of PST (ePST) for adults with TBI in collaboration with our Community Advisory Board.

CBPR Approach and Implementation: We have established a Community Advisory Board (CAB) made up of diverse persons with lived TBI experience (survivors and care partners), community partners, and subject matter experts. We will engage with our Community Advisory Board through quarterly meetings, large group email communications, and smaller group (e.g., workgroup) emails and meetings. To foster equitable participation and collaborative decision-making, we established a plan following the Multi-Stakeholder Research Journey Engagement Roadmap. Our CAB will co-design and co-develop our ePST intervention, inform pilot testing and iterative adaptations, and actively participate in disseminating and implementing ePST at the end of the project.

Applicability and How This Project addresses the FY22 TBIPHRP PCRA Focus Areas: Our project directly addresses the Psychological Health and Traumatic Brain Injury Research program (PH/TBIRP) mission to optimize prevention, assessment, and treatment of psychological health issues and TBI in two focus areas: treat psychological health conditions via evidence-based interventions and prevent psychological health conditions by developing and optimizing interventions addressing upstream factors (i.e., problem-solving).

Potential Clinical Applications, Benefits, and Risks: ePST will be a low-risk, high-reward, cost-effective, time-efficient, and Service Member-informed intervention for managing and improving psychological health symptoms common after TBI. The telehealth platform will maximize efficiency and capacity to include a broader and more geographically remote population.

Projected Timeline to Achieve a Patient-Related Outcome: We propose to complete this project in 3 years. The first 18 months will be dedicated to designing and developing ePST, in partnership with our CAB. During months 18-30, we will pilot test ePST (vs traditional PST and a Hybrid ePST/PST) among 40 persons with TBI, during which time we will also determine changes to ePST based on their experiences and feedback. The final 6 months will be devoted to making these adaptations, to publishing our results, and to co-developing plans with our CAB to share ePST more broadly through our community partners to reach those who need it.

Impact on Health and Well-Being of Service Members, Veterans, and/or Military Beneficiaries: Improving effective problem-solving could prevent and improve poor psychological health among Veterans/Service Members with TBI. Therefore, PST/ePST could have an immediate impact on Veterans'/Service members' access to healthcare, even if they don't otherwise have access to psychological health interventions.

Proposal Title:	Targeting Inflammatory Astrocyte Reactivity in Mild Traumatic Brain Injury
Log Number:	TP220183
Current PI Name:	Jian Luo
Award Number:	HT9425-23-1-0879
Current Contracting Organization:	Palo Alto Veterans Institute for Research
Current Performing Organization:	Palo Alto Veterans Institute for Research
Web Approval Date:	09-12-2023

Traumatic brain injury (TBI) is a leading cause of mortality and disability in military and civilian populations. For Service Members and Veterans, TBI has become the “signature wound” and a significant health issue during times of both peace and war. Mild traumatic brain injury (mTBI, also referred to as concussion) accounts for 80% of TBI cases. mTBI is often recurrent (termed repetitive mTBI) for individuals engaged in military operations and in contact sports. Repetitive mTBI is associated with more severe and protracted cognitive, motor, and behavioral complications. Among them, cognitive impairment (when a person has trouble remembering, learning new things, concentrating, or making decisions) is one of the most common, most disabling, and longest-lasting symptoms, because it is a substantial source of disability that destroys a Veteran's productivity and quality of life. Currently there is no effective treatment available to improve cognitive function, largely due to the fact that our understanding of the injury mechanisms is still incomplete. Animal models play crucial roles in understanding the injury mechanisms and developing treatments of human TBI.

This application targets this urgent need to develop a unique treatment for mTBI recovery and cognitive impairment. The goal of this proposal is to develop a mechanism-based therapy targeting astrocytes, the most abundant and star-like cell type in the brain. Astrocytes are multifunctional cells that react to brain insults and are a key determinant of TBI outcome. Astrocytes’ reaction can have both beneficial and harmful effects on TBI outcome, and reducing their harmful effects will promote TBI recovery and improve brain function. Astrocytes represent one of the most intriguing opportunities to tackle cognitive impairment.

In our recent work, we identified a novel gene that promotes the formation of harmful and toxic astrocytes, providing a unique avenue for therapeutic intervention. Accordingly, we show that targeting this gene with genetic or pharmacological approaches greatly improves cognitive function in an established, highly reproducible mouse model of repetitive mTBI. This gene has not been studied in astrocytes and its function in promoting formation of harmful astrocytes in completely novel. In this application, we propose to first identify the mechanism of action for the gene activity in astrocytes. We will employ the genetic approach to target this gene and investigate how its activation contributes to brain injury and cognitive deficits. We will then use a pharmacological inhibitor to assess its effect on functional recovery in mice with brain injury. While the genetic targeting approach has an advantage of being cell type-specific and can provide us unique insight into the mechanistic basis of astrocyte action in TBI, pharmacological agents have the potential for translation to clinical testing.

In summary, this application aims to tackle cognitive impairment, a common and disabling symptom that TBI survivors often experience. When completed, results from the proposed research will inform us how astrocytes contribute to brain injury and cognitive impairment. They will also provide strong evidence of developing therapeutic strategies targeting this gene to support TBI recovery and cognitive improvement in humans. Together, our results will provide a strong foundation to translate our findings to clinical trials by developing similar or identical agents to the one tested in the animal subjects.

Proposal Title:	Clinical Effectiveness and Implementation of Massed Prolonged Exposure for PTSD Among Veterans in Intensive Outpatient Substance Use Treatment
Log Number:	TP220196
Current PI Name:	Sonya Norman
Award Number:	HT9425-23-1-0669
Current Contracting Organization:	Veterans Medical Research Foundation of San Diego
Current Performing Organization:	Veterans Medical Research Foundation of San Diego
Web Approval Date:	09-12-2023

Post-traumatic stress disorder (PTSD) and substance use disorder (SUD) often co-occur. PTSD+SUD comorbidity is associated with more severe PTSD, worse treatment outcomes for substance use, greater suicide risk and worse functioning than having one of these disorders. First-line treatments for PTSD, particularly Prolonged Exposure Therapy (PE), are effective in treating PTSD among those with an SUD, and delivering these treatments concurrent to SUD programming is recommended by the VA/DoD Clinical Practice Guidelines. While PE is one of the most effective treatment options for PTSD among those with PTSD+SUD, effects are smaller and dropout is higher than among people with PTSD without an SUD. A promising way to enhance outcomes is to offer PE in a massed format (M-PE; i.e., multiple sessions per week instead of once weekly). M-PE has been shown to be effective in improving PTSD symptoms and substantially reducing dropout in military and Veteran populations. Preliminary findings suggest M-PE delivered concurrent to intensive SUD programming is a promising strategy that warrants further study. Evaluating the effectiveness of M- PE delivery in of SUD intensive outpatient programming (IOP) in improving PTSD and other mental health outcomes and reducing dropout as compared to weekly PE delivery (W-PE) is the necessary next step in this critical research.

The primary goal of this project is to determine whether a promising way to treat PTSD among those with SUD, M-PE, will help Veterans with their PTSD symptoms and lead to better treatment completion rates more than PE delivered weekly and whether the massed format will reduce substance use comparably to weekly PE among those in intensive SUD treatment. We will also evaluate whether M-PE helps Veterans function better and feel less depressed. M-PE is a one-on-one talk therapy that is delivered over twelve sessions several times a week. The therapy is brief because of the massed format so that it can be delivered at times when lengthy interventions may not be realistic, such as military mental health clinics on bases where military personnel may be getting ready to redeploy. Our preliminary work with Veterans in intensive SUD treatment showed M-PE to lead to improvements in PTSD and depression symptoms with no dropout, making this larger evaluation of M-PE compared to PE delivered in the traditional longer (weekly) format a critical next step. We will accomplish this by randomly assigning participants who are in intensive SUD treatment to receive either M-PE or W-PE. We propose to have 200 Veterans who served post 9/11 go through this study, and the entire study is expected to take 4 years to complete. We will run the study across four VAs (San Diego, Tampa, Chicago, and Atlanta).

We will use a research design that will let us concurrently compare M-PE and W-PE while also learning about what strategies are helpful to implement M-PE into DOD and VA healthcare settings and what barriers may come up to make implementation more challenging. This design will prepare us to successfully implement M- PE into intensive substance use treatment if we find it to work well. We will include a panel

of Veterans that will help guide us on making decisions in how we conduct the study so that it is most relevant to Veterans. We will interview participants, therapists, and administrators to learn about the benefits and challenges of offering M-PE so that we can use that information to guide us in the future.

The proposed study holds promise for identifying a better method by which to treat Service Members and Veterans with PTSD and SUD, with better PTSD outcomes and lower dropout. If found to be effective, M-PE can be immediately delivered in VA and military healthcare settings since providers are already trained in it in both settings. The treatment is brief and thus would be minimally disruptive to an active-duty schedule or the busy life of a returning Veteran. The expected improvements in mental health problems will also positively impact Veterans' families and society more generally. Thus, the proposed study is highly responsive to the needs of the military.

The proposed project is relevant to two sub-areas within FY22 TBIPHRP CTA Focus Area 3 (Treat). We are evaluating an intervention for its ability to promote sustained functional recovery from PTSD which fits within sub-area a, and we are exploring implementation factors to increase provider adoption and availability of the treatment which fits within sub-area c.

Proposal Title:	Implementing and Evaluating a Patient-Centered PTSD Treatment Program for Military Personnel
Log Number:	TP220205
Current PI Name:	Alan Peterson
Award Number:	HT9425-23-2-0016
Current Contracting Organization:	Texas, University of, Health Science Center at San Antonio
Current Performing Organization:	Texas, University of, Health Science Center at San Antonio
Web Approval Date:	09-12-2023

Objectives and Rationale for the Proposed Project: Over the past 20 years, several large, randomized clinical trials (RCTs) by our own group on the treatment of post-traumatic stress disorder (PTSD) have established the efficacy of existing evidence-based cognitive-behavioral therapies for PTSD with military populations, though not to the same degree as with civilians. Numerous follow-on studies have added and evaluated treatment adaptations—including the type of therapy delivered, the frequency and total number of treatment sessions, and the modality of treatment delivery (e.g., in office, in home, via telehealth)—to improve efficacy with combat-related PTSD as well as the treatments' feasibility and acceptability in military settings. Despite trends toward continuously improving outcomes, however, there remains room for further improvement. Research also shows a seeming reluctance among many military personnel to first begin and then to complete evidence-based treatment (EBT), with low rates of those with a PTSD diagnosis initiating an EBT and high dropout rates among those who do. Reported reasons include patients not liking or feeling well suited for a particular treatment. Many also perceive a lack of control over their care, which can cause them to avoid treatment, drop out, or have poorer outcomes. The current study aims to overcome these barriers and potentially improve treatment engagement, retention, and outcomes by evaluating a Shared Decision-Making model of patient- centered healthcare that has been shown through research and our group' s own clinical experience to maximize patient engagement and satisfaction. Military participants will be offered and receive thorough descriptions of a menu of evidence-based cognitive-behavioral therapies to include prolonged exposure (PE), cognitive processing therapy (CPT), and written exposure therapy (WET). Options also will be provided on treatment frequency (condensed or spread out over several weeks), treatment modality (in the clinic or via telehealth), and length (number of sessions may vary by treatment type and/or patient response). This two-step, partially randomized preference trial will then follow a process that allows patients with a strong preference to select their particular treatment, and others to identify treatment arms to which they are willing to be randomized.

Investigators will examine which patient characteristics and preferences lead to selection of and improved outcomes with different treatment options, with the goal of guiding future efforts in personalized medicine. Investigators will also compare these outcomes to prior RCTs with military personnel. They hypothesize that those who engage in Shared Decision-Making will (1) show higher rates of treatment completion, and (2) have larger reductions in PTSD symptoms posttreatment.

Use of Community-Based Participatory Research (CBPR) in the Study: Feedback from a Lived Experience Consultation (LEC) who served as an Army Scout in Operation Iraqi Freedom has already been incorporated into the study design. Upon funding, we will continue to utilize the LEC and a Community Advisory Board.

Description of the Ultimate Applicability of the Research: If successful, study results will provide a framework that clinicians and patients can use to work together to identify personalized approaches to care that better enable patients to engage in and complete treatment with the greatest opportunity for recovery.

How the Project Addresses at Least one Sub-Area of the FY22 TBIPHRP PCRA Focus Areas: This project targets the Treat Focus Area with research that addresses immediate and long-term treatments for PTSD and improvements in systems of care. It will target the 3.a. and 3.b. Sub-Areas to evaluate interventions that promote sustained functional recovery with rapid treatments for PTSD.

Types of Patients Who Will Be Helped by the Research and How It Will Help Them: Shared Decision-Making could help improve PTSD care across the military healthcare system for military personnel with a PTSD diagnosis who avoid treatment or who drop out prematurely.

Potential Clinical Applications, Benefits, and Risks: The ultimate benefits are that more patients could be expected to start and continue treatment and have greater chances of recovery. Study risks to participants are minimal, potentially including a temporary increase in some stress-related symptoms that can occur during psychotherapy as they deal with traumatic memories. The benefit to participants is that all will receive an evidence-based PTSD therapy, and they even can designate the treatment or treatments that they prefer.

Projected Timeline to Achieve the Expected Patient-Related Outcomes: The timeline for the research project is 4 years. Findings can be disseminated and quickly incorporated into clinical practice.

How the Proposed Project Will Impact the Health and Well-Being of Service Members: The proposed project attempts to provide a means by which patients and clinicians can work together to overcome treatment barriers and engage Service Members in an EBT. Patients will have an opportunity for healing and recovery, improved or restored relationships, and maintenance or improvement in occupational and social functioning.

Proposal Title:	Implementing and Evaluating a Patient-Centered PTSD Treatment Program for Military Personnel
Log Number:	TP220205P1
Current PI Name:	Vanessa Jacoby
Award Number:	HT9425-23-2-0018
Current Contracting Organization:	Texas, University of, Health Science Center at San Antonio
Current Performing Organization:	Texas, University of, Health Science Center at San Antonio
Web Approval Date:	09-12-2023

Objectives and Rationale for the Proposed Project: Over the past 20 years, several large, randomized clinical trials (RCTs) by our own group on the treatment of post-traumatic stress disorder (PTSD) have established the efficacy of existing evidence-based cognitive-behavioral therapies for PTSD with military populations, though not to the same degree as with civilians. Numerous follow-on studies have added and evaluated treatment adaptations—including the type of therapy delivered, the frequency and total number of treatment sessions, and the modality of treatment delivery (e.g., in office, in home, via telehealth)—to improve efficacy with combat-related PTSD as well as the treatments' feasibility and acceptability in military settings. Despite trends toward continuously improving outcomes, however, there remains room for further improvement. Research also shows a seeming reluctance among many military personnel to first begin and then to complete evidence-based treatment (EBT), with low rates of those with a PTSD diagnosis initiating an EBT and high dropout rates among those who do. Reported reasons include patients not liking or feeling well suited for a particular treatment. Many also perceive a lack of control over their care, which can cause them to avoid treatment, drop out, or have poorer outcomes. The current study aims to overcome these barriers and potentially improve treatment engagement, retention, and outcomes by evaluating a Shared Decision-Making model of patient- centered healthcare that has been shown through research and our group' s own clinical experience to maximize patient engagement and satisfaction. Military participants will be offered and receive thorough descriptions of a menu of evidence-based cognitive-behavioral therapies to include prolonged exposure (PE), cognitive processing therapy (CPT), and written exposure therapy (WET). Options also will be provided on treatment frequency (condensed or spread out over several weeks), treatment modality (in the clinic or via telehealth), and length (number of sessions may vary by treatment type and/or patient response). This two-step, partially randomized preference trial will then follow a process that allows patients with a strong preference to select their particular treatment, and others to identify treatment arms to which they are willing to be randomized.

Investigators will examine which patient characteristics and preferences lead to selection of and improved outcomes with different treatment options, with the goal of guiding future efforts in personalized medicine. Investigators will also compare these outcomes to prior RCTs with military personnel. They hypothesize that those who engage in Shared Decision-Making will (1) show higher rates of treatment completion, and (2) have larger reductions in PTSD symptoms posttreatment.

Use of Community-Based Participatory Research (CBPR) in the Study: Feedback from a Lived Experience Consultation (LEC) who served as an Army Scout in Operation Iraqi Freedom has already been incorporated into the study design. Upon funding, we will continue to utilize the LEC and a Community Advisory Board.

Description of the Ultimate Applicability of the Research: If successful, study results will provide a framework that clinicians and patients can use to work together to identify personalized approaches to care that better enable patients to engage in and complete treatment with the greatest opportunity for recovery.

How the Project Addresses at Least one Sub-Area of the FY22 TBIPHRP PCRA Focus Areas: This project targets the Treat Focus Area with research that addresses immediate and long-term treatments for PTSD and improvements in systems of care. It will target the 3.a. and 3.b. Sub-Areas to evaluate interventions that promote sustained functional recovery with rapid treatments for PTSD.

Types of Patients Who Will Be Helped by the Research and How It Will Help Them: Shared Decision-Making could help improve PTSD care across the military healthcare system for military personnel with a PTSD diagnosis who avoid treatment or who drop out prematurely.

Potential Clinical Applications, Benefits, and Risks: The ultimate benefits are that more patients could be expected to start and continue treatment and have greater chances of recovery. Study risks to participants are minimal, potentially including a temporary increase in some stress-related symptoms that can occur during psychotherapy as they deal with traumatic memories. The benefit to participants is that all will receive an evidence-based PTSD therapy, and they even can designate the treatment or treatments that they prefer.

Projected Timeline to Achieve the Expected Patient-Related Outcomes: The timeline for the research project is 4 years. Findings can be disseminated and quickly incorporated into clinical practice.

How the Proposed Project Will Impact the Health and Well-Being of Service Members: The proposed project attempts to provide a means by which patients and clinicians can work together to overcome treatment barriers and engage Service Members in an EBT. Patients will have an opportunity for healing and recovery, improved or restored relationships, and maintenance or improvement in occupational and social functioning.

Proposal Title:	Point-of-Injury Intranasal ALM Drug Therapy to Reduce Secondary Injury and Improve Outcomes After TBI in Civilian and Military Resource-Limited Environments
Log Number:	TP220214
Current PI Name:	Geoffrey Dobson
Award Number:	HT9425-23-1-0917
Current Contracting Organization:	James Cook University
Current Performing Organization:	James Cook University
Web Approval Date:	09-13-2023

Background: Traumatic brain injury (TBI) is a global epidemic, affecting around 69 million people annually. TBI occurs when a sudden external force injures the brain potentially leading to death or disability. It can occur from a violent blow or a jolt to the head, or from an object penetrating the skull, such as a bullet or explosive fragments. The primary injury leads to secondary injury processes in both the brain and throughout the body including cell swelling and death; immune dysfunction; inflammation; bleeding and clotting derangements; and heart, lung and bowel dysfunction. No safe and effective drug therapy exists to treat mild to severe TBI. On the battlefield, TBI is estimated to be responsible for 20-25% of injuries. Since 2000, TBI has affected >450,000 U.S. military Service Members, including >51,000 moderate- to-severe injuries. These injuries cause difficulties with cognition, learning and sleep, as well as mental disorders such as depression, anxiety, and PTSD. TBI can also lead to later-life cardiovascular disease, as well as dementia and neurodegenerative diseases including Alzheimer's disease and Parkinson’s disease. Our study addresses major unmet needs in both military and civilian settings.

Objective and Rationale of the Project: Our objective is to provide the Department of Defense with a new frontline drug therapy to treat TBI in combat and other military settings immediately after the injury has occurred. The drug will prevent early death and further provide protection and stabilization of the casualty for up to 7 days in the field. The idea is to protect the brain and the whole body by reducing secondary injury progression and TBI-associated complications. If secondary injury progression can be reduced, many lives can be saved, military personnel will return to active duty faster, and TBI casualties will have reduced disability and neurodegenerative complications later in life. The aim of the proposed project is to evaluate the survival and protection effects of ALM, a novel neuroprotective drug therapy, in a military-relevant rat model of moderate TBI. We will examine injectable (IV) and nasal (spray) administration which will translate to simple use in austere locations or in civilian prehospital environments.

Focus Areas: The focus areas we address in our proposal are: (1) the acute treatment of TBI in rural or other resource-limited environments, and by peers, teams, or first responders/medics; (2) generation of evidence regarding the safety, efficacy, and utility of neuroprotective measures; and (3) interventions that promote sustained recovery and address neurodegenerative processes associated with TBI.

Applicability of the Research: Reduce early mortality and morbidity from moderate TBI.

Types of Patients: All military personnel, Veterans, their Families, and the civilian population.

Potential Clinical Applications, Benefits, and Risks: Early IV or intranasal treatment by first responders or combat medics after suspected TBI will reduce early mortality, secondary injury progression, and TBI-associated whole-body complications. Our small-volume drug therapy will also offer a key tactical

advantage because it will simplify treatment of combatants suspected of having TBI and other injuries in the field. In the civilian setting, ALM has broad applications from sport-related concussions, falls and car crashes, to mass casualty events including terrorist bombings. At this stage, we see no risks.

Timeline: We expect ALM use in clinical and field situations is feasible within 5-6 years if this award is successful. We would work with our civilian and military colleagues to expedite field use.

Impact on Well-Being of Service Members, Veterans, and Military Beneficiaries: This study has the potential to provide the first effective and safe EARLY treatment for TBI, which will improve recovery by reducing TBI-related morbidity such as cardiovascular dysfunction. In the long term, it may reduce development of psychological and neurodegenerative disorders, which will enhance well-being and quality of life for all Service Members and Veterans as well as other military beneficiaries.

Proposal Title:	CD98hc-Mediated Delivery of an Ultrapotent TrkB Agonist Antibody for Neuroprotection and Improved Cognitive Recovery After Traumatic Brain Injury
Log Number:	TP220230
Current PI Name:	Colin Greineder
Award Number:	HT9425-23-2-0010
Current Contracting Organization:	Michigan, University of
Current Performing Organization:	Michigan, University of
Web Approval Date:	08-01-2023

The Problem: Traumatic Brain Injury (TBI) has been called a “silent epidemic,” affecting more than 1.5 million people in the U.S. each year and contributing disproportionately to trauma-related death and disability in both military and civilian populations. Despite many attempts to identify effective treatments, there are no medications capable of saving brain cells or improving the cognitive or behavioral impairments that can occur after someone suffers from TBI.

One of the major reasons why research has been slow to identify effective treatments is that many promising drug candidates are not able to cross the so-called “blood-brain barrier,” a very tight layer of cells that prevents most molecules from crossing from the bloodstream into the brain tissue. One example of a promising drug candidate is a naturally occurring growth factor that we all have called brain-derived neurotrophic factor (BDNF). BDNF seems to be involved in the recovery from TBI. For instance, a genetic abnormality in the BDNF gene has been linked to worse outcomes in TBI and other forms of brain injury like stroke. Injecting BDNF directly into the brain (something that is feasible in animals but not possible in patients) reduces the death of brain cells and improves outcomes in animal models of stroke and neurodegenerative diseases like Alzheimer’s. Despite widespread consensus that BDNF has therapeutic potential, it is not considered a viable drug candidate and has not been investigated in clinical trials in TBI patients because of its inability to cross the blood-brain barrier.

Our Solution: Our team has developed a novel “brain shuttle” technology that hitchhikes on a transporter that normally brings essential amino acids across the blood brain barrier. We can use this brain shuttle to deliver antibodies into the brain, including one that mimics the natural function of BDNF by binding to and activating its receptor in the brain. We call this new drug the TrkB (pronounced “Track B”) antibody shuttle. Our preliminary work, done in healthy mice, shows that a single intravenous dose of TrkB antibody shuttle provides several days of BDNF-like protective effects in the brain.

Project Objective: Here, we will test the TrkB antibody shuttle in two mouse models of TBI – a mouse TBI model established at the University of Michigan and a rat model of blast-induced TBI developed at the Walter Reid Army Institute of Research. This blast-induced model has great relevance to the U.S. military because it simulates the injury that Warfighters suffer when an improvised explosive device detonates nearby. We expect the TrkB antibody shuttle will decrease the loss of brain tissue and improve cognitive and behavioral recovery in both models of TBI. This addresses one of the FY22 TBIPHRP focus areas (Treat) and sub-areas (Interventions that promote sustained functional recovery).

Who This Will Help: If the TrkB antibody improves outcomes in both models of TBI, this will represent a major breakthrough that could result in an innovative treatment for TBI patients. Those with severe TBI, who have the greatest risk of neurodegeneration and prolonged cognitive and behavioral impairments, will

be the most likely to benefit from this novel neuroprotective therapy. The TrkB antibody shuttle could also help millions of patients who suffer mild TBI, or concussion, who frequently experience cognitive symptoms that go unrecognized and untreated. The results will still be valuable even if the TrkB shuttle is not found to be efficacious because this work will increase our understanding of how the shuttle transports antibodies into the brain after a TBI, which will pave the way for delivering other therapeutic antibodies.

Timeline to Patient Impact: The timeline to clinical use is typically lengthy for new therapies. The TrkB antibody shuttle must be carefully tested in animal models before it can be tested in human clinical trials and commercialized. Unlike many other laboratory discoveries, however, our approach has a clear path to clinical use because we have already overcome many hurdles that typically delay the drug development process and the proposed experiments will overcome more. This will make future human clinical trials more likely to succeed.

Military Benefit and Impact: TBI is now the most common injury suffered by Warfighters. Therapeutics capable of protecting brain tissue and improving cognitive and behavioral outcomes could have a major impact on the lives of the thousands of service members affected by TBI each year. The experiments described in our proposal will provide a clear answer as to the therapeutic potential of the TrkB antibody shuttle in TBI and, if positive, will accelerate large animal testing, human clinical trials, and commercialization. Moreover, the work will provide proof-of-principle data for using our novel brain shuttle technology to test other therapies for TBI.

Proposal Title:	CD98hc-Mediated Delivery of an Ultrapotent TrkB Agonist Antibody for Neuroprotection and Improved Cognitive Recovery After Traumatic Brain Injury
Log Number:	TP220230P2
Current PI Name:	Peter Tessier
Award Number:	HT9425-23-2-0011
Current Contracting Organization:	Michigan, University of
Current Performing Organization:	Michigan, University of
Web Approval Date:	08-01-2023

The Problem: Traumatic Brain Injury (TBI) has been called a “silent epidemic,” affecting more than 1.5 million people in the U.S. each year and contributing disproportionately to trauma-related death and disability in both military and civilian populations. Despite many attempts to identify effective treatments, there are no medications capable of saving brain cells or improving the cognitive or behavioral impairments that can occur after someone suffers from TBI.

One of the major reasons why research has been slow to identify effective treatments is that many promising drug candidates are not able to cross the so-called “blood-brain barrier,” a very tight layer of cells that prevents most molecules from crossing from the bloodstream into the brain tissue. One example of a promising drug candidate is a naturally occurring growth factor that we all have called brain-derived neurotrophic factor (BDNF). BDNF seems to be involved in the recovery from TBI. For instance, a genetic abnormality in the BDNF gene has been linked to worse outcomes in TBI and other forms of brain injury like stroke. Injecting BDNF directly into the brain (something that is feasible in animals but not possible in patients) reduces the death of brain cells and improves outcomes in animal models of stroke and neurodegenerative diseases like Alzheimer’s. Despite widespread consensus that BDNF has therapeutic potential, it is not considered a viable drug candidate and has not been investigated in clinical trials in TBI patients because of its inability to cross the blood-brain barrier.

Our Solution: Our team has developed a novel “brain shuttle” technology that hitchhikes on a transporter that normally brings essential amino acids across the blood brain barrier. We can use this brain shuttle to deliver antibodies into the brain, including one that mimics the natural function of BDNF by binding to and activating its receptor in the brain. We call this new drug the TrkB (pronounced “Track B”) antibody shuttle. Our preliminary work, done in healthy mice, shows that a single intravenous dose of TrkB antibody shuttle provides several days of BDNF-like protective effects in the brain.

Project Objective: Here, we will test the TrkB antibody shuttle in two mouse models of TBI – a mouse TBI model established at the University of Michigan and a rat model of blast-induced TBI developed at the Walter Reid Army Institute of Research. This blast-induced model has great relevance to the U.S. military because it simulates the injury that Warfighters suffer when an improvised explosive device detonates nearby. We expect the TrkB antibody shuttle will decrease the loss of brain tissue and improve cognitive and behavioral recovery in both models of TBI. This addresses one of the FY22 TBIPHRP focus areas (Treat) and sub-areas (Interventions that promote sustained functional recovery).

Who This Will Help: If the TrkB antibody improves outcomes in both models of TBI, this will represent a major breakthrough that could result in an innovative treatment for TBI patients. Those with severe TBI, who have the greatest risk of neurodegeneration and prolonged cognitive and behavioral impairments, will

be the most likely to benefit from this novel neuroprotective therapy. The TrkB antibody shuttle could also help millions of patients who suffer mild TBI, or concussion, who frequently experience cognitive symptoms that go unrecognized and untreated. The results will still be valuable even if the TrkB shuttle is not found to be efficacious because this work will increase our understanding of how the shuttle transports antibodies into the brain after a TBI, which will pave the way for delivering other therapeutic antibodies.

Timeline to Patient Impact: The timeline to clinical use is typically lengthy for new therapies. The TrkB antibody shuttle must be carefully tested in animal models before it can be tested in human clinical trials and commercialized. Unlike many other laboratory discoveries, however, our approach has a clear path to clinical use because we have already overcome many hurdles that typically delay the drug development process and the proposed experiments will overcome more. This will make future human clinical trials more likely to succeed.

Military Benefit and Impact: TBI is now the most common injury suffered by Warfighters. Therapeutics capable of protecting brain tissue and improving cognitive and behavioral outcomes could have a major impact on the lives of the thousands of service members affected by TBI each year. The experiments described in our proposal will provide a clear answer as to the therapeutic potential of the TrkB antibody shuttle in TBI and, if positive, will accelerate large animal testing, human clinical trials, and commercialization. Moreover, the work will provide proof-of-principle data for using our novel brain shuttle technology to test other therapies for TBI.

Proposal Title:	Mitigating Traumatic Brain Injuries and Neurological Implications via the Immunometabolite Itaconate
Log Number:	TP220232
Current PI Name:	Pedro Cabrales
Award Number:	HT9425-23-1-0388
Current Contracting Organization:	California, University of, San Diego
Current Performing Organization:	California, University of, San Diego
Web Approval Date:	08-09-2023

The proposed work described herein is directly responsive to the topic areas (Understand and Treat) of the Traumatic Brain Injury and Psychological Health Research Program.

One of the major causes of death in the battlefield is traumatic brain injury (TBI), and this is worsened when the TBI is accompanied with severe hemorrhage; thus, one of the greatest opportunities to reduce mortality and morbidity in the battlefield scenario involves appropriate treatment of TBI concomitant to hemorrhage. These therapies need to provide fluid resuscitation and rapid restoration of the inadequate oxygen supply to the brain and the rest of the body. This proposal will test a novel target treatment based on the idea that the cellular energetic pathway is a key factor to better resuscitate from TBI with hemorrhage. The main goal of the present study is to find new ways to treat TBI in the presence of hemorrhage. To date, existing therapies for TBI in the presence of severe hemorrhage are severely limited and there is a great interest in therapeutically targeting the metabolism and the cellular response to reduce pressure, reduced perfusion, and limited oxygenation. Here, we aim to establish itaconate-induced alteration of metabolism as an important new, innovative, and untapped opportunity for therapeutic intervention to reduce cellular injury in TBI. Itaconate is easy to administer, water-soluble, and endogenously present in the human body. Taken together, adequate resuscitation with enhanced cellular energetic metabolism could be the key to increase survival from TBI in the presence of severe hemorrhage.

Proposal Title:	Prebiotic and Probiotic Interventions for Treatment of TBI-Induced Microbiome Dysfunction
Log Number:	TP220244
Current PI Name:	Kris Martens
Award Number:	HT9425-23-1-0538
Current Contracting Organization:	Ohio State University, The
Current Performing Organization:	Ohio State University, The
Web Approval Date:	09-12-2023

Traumatic brain injury (TBI) is a major health concern for the military and for society at large. In military populations, over 450,000 brain injury have been recorded by the DOD since 2000. These injuries have wide-ranging consequences that often last years beyond the initial injury event. For instance, military populations with TBI are at higher risk for several psychiatric conditions and have elevated rates of psychiatric impairments. A hallmark across many disorders is risky decision-making. Currently, there are no treatments for TBI or for the conditions that develop because of TBI.

The mechanisms by which TBI may cause such symptoms are complex. Moreover, these factors are often difficult to tease apart in patient populations for both practical and ethical reasons. To better study TBI mechanisms, we can use an animal model that mimics many of the conditions of human TBI. Our laboratory has established a rat model of TBI that demonstrates risky decision-making using a rodent gambling task. This assessment directly mimics a human neuropsychological assessment, the Iowa Gambling Task, which patients with TBI struggle with.

Measurements of the gut microbiome, the community of stomach and colon bacteria that help to process nutrients, show acute and chronic disruptions in patients with TBI. Our rat model mimics these findings and low gut bacterial diversity actually predict worse impairments on the rodent gambling task. Further, our pilot data shows that intervention can rescue some of these deficits. Thus, the goal of the current proposal is to investigate the gut microbiome as a potential mechanism and treatment target for TBI-induced psychiatric impairment.

The current research will address the TBI-PH Research Program areas of focus in the following fashion:

1a. Understand: Understanding of risk, protective, and biological factors contributing to an individual’s vulnerability to, response to, and long-term outcomes psychological health conditions and/or TBI.

3a Treat: Interventions that promote sustained functional recovery, including interventions administered acutely, during the post-acute phase, or during the chronic phase of injury.

To do this, we will undertake an approach in which we administer a pro- or pre-biotic diet before and after injury to promote gut health and prevent bacterial dysfunction. We will then reassess the rats on a battery of psychiatric measures, including our gambling task. We will also collect fecal samples to measure gut microbiome function, blood samples to explore how metabolism is affected, and ultimately brain samples to better understand pathological changes. In a second aim, we will take the fecal samples collected from TBI rats and transplant them into germ-free mice. This will allow us to isolate the gut microbiome and explore how it remodels the gut, brain, and behavior. These mice will have their gut microbiomes sampled and undergo metagenomic shotgun sequencing, a method to provide a much deeper understanding of the gut than is typically feasible in most studies.

We anticipate to conduct these studies over a 2-year period. From the findings of this, novel therapeutic interventions could be developed and integrated into military food supply programs (e.g., MREs, cafeterias). Ultimately, these studies will deepen our understanding of the mechanisms by which TBI causes impaired decision-making and may lead to therapeutic breakthroughs for a condition that has no treatments.

Proposal Title:	microRNA Biomarkers of Cumulative Blast-Mild Traumatic Brain Injury (Micro BCB)
Log Number:	TP220291
Current PI Name:	James Meabon
Award Number:	HT9425-23-1-0755
Current Contracting Organization:	Seattle Institute for Biomedical and Clinical Research
Current Performing Organization:	VA Puget Sound Health Care System
Web Approval Date:	10-03-2023

Slowly resolving disease processes can be initiated by mild traumatic brain injury (mTBI). There is increasing evidence that repetitive, subinjurious, head impact/blast exposures can provoke similar pathology. Training military units may sustain multiple injurious and subinjurious mTBI insults throughout their careers. For example, our published analyses estimate a typical Special Operations Forces (SOF) team experiences 54+ blasts per training week (>251 psi/week); equaling 648 subinjurious blasts/quarterly (>3, 000 psi/quarter/Soldier). Science is only now exploring these traumatic events' long-term effects, with definitive studies demonstrating repeated mTBI exposures increase the risk of Alzheimer's disease and related dementias.

During the chronic phase of the injury/recovery process, mTBI is largely diagnosed based on retrospectively recalling acute symptoms following the event, as well as behavioral and cognitive assessments. Similarly, diagnostics and prognostics for cumulative mTBI exposures are also lacking. Therefore, objective biomarkers enabling accurate diagnoses for identifying and managing individuals with probable mTBI would be useful. Although there has been progress in identifying acute mTBI biomarkers, few studies have identified candidate biomarkers for chronic mTBI resulting from significant cumulative blast exposure. Testing previously identified molecules for their diagnostic and prognostic performance in additional populations will help validate and establish their use in guiding medical care decisions, monitoring aggravation of previous mTBIs and will help support disability claims based on a history of TBI.

Our project aims to validate previously identified objective blood miRNA biomarker candidates. We will determine their performance for diagnosis, prognosis, or monitoring of chronic repetitive mTBI across the blast injury spectrum—ranging from repetitive low-intensity blast (LIB) training exposure to mild- intensity blast (MIB) typically encountered during combat. miRNAs are a class of small, stable ribonucleic acid molecules carried in the blood, allowing these biomarkers to be easily attained. The fact that they are easily measured by polymerase chain reaction (PCR)-based techniques additionally allows them to be inexpensive, highly accurate and deployable both within civilian and far forward operational settings.

miRNAs bind to cognate sequences in messenger RNA, preventing its expression as protein and thereby limiting the role of that protein in the function of the cell. miRNAs can therefore not only have profound effects on the trajectory of disease and injury recovery processes, but also knowing which miRNAs are differentially expressed along the injury and recovery process can indicate which molecular mechanisms may be beneficial to treat. We have previously identified a group of blood miRNAs with altered expression in Veterans with chronic repetitive blast-mTBI. Importantly, we identified altered disease pathways related to their expression levels reflecting the value of this powerful approach. Our recent work in support of this application has determined several of these miRNAs may serve as high performance diagnostics discriminating persons with and without blast-mTBI.

We have performed additional studies in mice identifying novel miRNAs that reflect cumulative LIB exposure, as well as additional miRNAs related to chronic MIB exposures. In both cases, our preliminary

work indicates the miRNAs relate to behavioral impairments and with diffusion tensor imaging (DTI) measures of hindbrain injuries in the superior cerebellar peduncle and medulla. Importantly, changes in DTI metrics may precede both neuronal loss and symptoms onset. Taken together, identifying blood biomarkers correlated with both symptom burden and neurodegenerative structural changes may help guide the neuroimaging-based monitoring of traumatic brain injury resolution as an essential part of personalized precision medicine. In Aim 2, we will test the hypothesis that serum miRNA biomarker concentrations predict the presence and extent of structural abnormalities in superior cerebellar peduncle and brainstem (measured via DTI methods). In Aim 3, we conduct a discovery-focused analysis of the combined sequencing data derived from Aim1 to identify promising new miRNAs for highly powered insights into the unknown mechanisms of blast injury.

Proposal Title:	The Combat Polytrauma Triad: Photosensitivity as a Link Between PTSD and Chronic Pain After TBI
Log Number:	TP220295
Current PI Name:	Mary Heinricher
Award Number:	HT9425-23-1-0703
Current Contracting Organization:	Oregon Health and Science University - Portland
Current Performing Organization:	Oregon Health and Science University - Portland
Web Approval Date:	09-12-2023

Veterans who have sustained a traumatic brain injury (TBI) are particularly likely to experience both chronic pain and posttraumatic stress disorder (PTSD), a combination referred to as the “polytrauma clinical triad.” Chronic pain and PTSD can occur together subsequent to even mild TBI and have significant, long-lasting (decades) impact on both psychological and physical function.

Effective treatments for PTSD and pain subsequent to TBI are limited, in part due to incomplete understanding of the brain mechanisms that tie these outcomes together after TBI. However, symptoms of PTSD and pain are mutually reinforcing, and one clue comes from the considerable overlap between brain circuits implicated in PTSD and those contributing to chronic pain. My laboratory has shown in animal studies that brain circuits contributing to chronic pain can be activated by psychological stress and by light, causing increased sensitivity to pain in both cases. We now have preliminary evidence from a largely Veteran population that elevated sensitivity to light is associated with chronic pain and pain-related disability, and that light is processed differently in individuals with high-impact chronic pain.

These individuals with high-impact chronic pain are more likely to report PTSD symptoms and to endorse elevated levels of depression, sleep-disturbances, and other disability. The goal of the proposed experiments is to determine whether photosensitivity could serve as a marker of PTSD and high-impact chronic pain after TBI. This marker would be quantitative and free from the stigma felt by individuals endorsing PTSD or pain.

Our objectives are to quantify sensitivity to light in Veterans with TBI with and without associated PTSD, and determine whether photosensitivity is associated with greater pain and pain-related disability, poor sleep, and poor functional outcomes in individuals living with PTSD and pain. We will also use functional brain imaging (“brain scans”) to determine whether a dim light stimulus can activate the pain-related brain regions in Veterans who have PTSD after TBI compared to those with TBI without PTSD. This would be strong evidence that pain pathways in the brain are changed in individuals who suffer from PTSD following a TBI.

The proposed project will help us better Understand PTSD and chronic pain after TBI. Pain in the absence of identifiable peripheral triggers is no longer dismissed as imaginary by most physicians, but underlying mechanisms are only now beginning to emerge with the concept of brain changes contributing to chronic pain. This work could thus provide insights into the mechanisms connecting pain and PTSD after TBI, and ultimately help identify individuals whose pain is caused by underlying brain changes.

This project also has relevance to Prevention of PTSD and pain after TBI by providing a quantitative measure that has the potential to link PTSD with chronic pain after TBI, identifying individuals with highest risk.

If we are correct, photosensitivity could be developed as a quantitative marker of brain changes contributing to PTSD and pain in Veterans with TBI, guiding their treatment and serving as a measure of their responses to different therapies. This work would also provide a better understanding of the brain mechanisms of pain

in individual Veterans with chronic pain or PTSD, giving us a simple, inexpensive test that could be applied in a standard clinical setting, and, importantly, validating these hard-to-explain symptoms. Because this test would provide us with a window into brain function, this could guide pharmacological treatments that act directly upon these brain mechanisms and reduce the need for unnecessary surgical procedures or chronic non-specific opioid use. Finally, these studies could pave the way for modifying the light environment as a way to improve pain, functional outcomes, and quality of life for these Veterans.

Proposal Title:	Silexan in the Treatment Of Post-Traumatic Stress Disorder (STOP) Trial: A Randomised, Placebo-Controlled, Double-Blind Trial
Log Number:	TP220299
Current PI Name:	Michael Berk
Award Number:	HT9425-23-1-0885
Current Contracting Organization:	Deakin University
Current Performing Organization:	Deakin University
Web Approval Date:	10-03-2023

Posttraumatic stress disorder (PTSD) is a common and debilitating mental illness. Current treatments for PTSD include psychotherapy and antidepressant medications. Many patients are unable to tolerate psychotherapy for PTSD and drop out of it. In addition, its effectiveness is limited. Up to 50% of patients who receive psychotherapy do not benefit from it. Antidepressant medications have only small benefits in PTSD. They also have unpleasant side effects that can make patients unwilling to take them. There is an urgent need to develop new treatments for PTSD that are effective and well- tolerated.

Silexan is a lavender oil derivative. It is taken orally in the form of capsules. It is currently available over-the-counter in Australia and the United States. Previous research has shown that it is an effective treatment for anxiety disorders, including Generalized Anxiety Disorder. It is also well-tolerated by patients. The only side effects that have been identified so far are mild gastrointestinal symptoms and these are uncommon. The results of a small pilot study suggest that Silexan may also be effective and well-tolerated in PTSD.

The STOP trial is a clinical trial that aims to investigate whether adding Silexan to treatment-as-usual improves PTSD symptoms in adults with PTSD. The trial will recruit 156 participants. Participants will take Silexan or a placebo daily in addition to their usual medications for 12 weeks. The severity of their PTSD symptoms will be assessed prior to and at the end of this 12-week period. The trial will begin in July 2023. It is expected to take 4 years for the data to be collected and analyzed and the results of the trial to be published.

The research team for the STOP trial includes an Australian Army Veteran with lived experience of PTSD. This Veteran served in the Australian Army for many years and was deployed during the Iraq War. He developed PTSD as a result of his experiences in Iraq. He has provided input into the design of the trial. He will chair a committee called the Community Advisory Board (CAB). The CAB will also include a second person with lived experience of PTSD and two psychiatrists. The role of the CAB will be to provide input and feedback to the research team from a lived experience perspective. It will meet regularly with the research team. It will also help the team to translate their findings into improved care for serving military members and Veterans with PTSD and other people suffering from PTSD.

The STOP trial addresses sub-area 3a of the FY22 TBIPHRP CTA Focus Areas because it is a clinical trial of an intervention for PTSD. The trial has the potential to obtain definitive evidence regarding the effectiveness of Silexan in PTSD. If Silexan is found to be an effective treatment for PTSD, the pool of patients who could potentially benefit from this treatment includes any adults with PTSD. Silexan is already available over the counter at a relatively low cost, so there will be few barriers to patients accessing this treatment.

Serving military members and Veterans are at high risk of developing PTSD. Nearly one-quarter of Iraq War Veterans suffer from PTSD. PTSD is an important contributing factor to the high rates of disability experienced by Veterans and their elevated risk of suicide. Existing treatments for PTSD have significant

limitations. Silexan has the potential to provide an important alternative treatment for serving members and Veterans with PTSD.

Proposal Title:	NOP Receptor Modulator Treatment Optimizes Cognitive, Locomotor, and Sensory Outcomes of Mild Concussive TBI with and Without PTSD
Log Number:	TP220330
Current PI Name:	Kelly Standifer
Award Number:	HT9425-23-1-0340
Current Contracting Organization:	Oklahoma, University of, Health Sciences Center
Current Performing Organization:	Oklahoma, University of, Health Sciences Center
Web Approval Date:	07-13-2023

Background: This proposal addresses three different focus areas (Understand, Prevent and Assess, and Treat). We seek to understand how exposure to traumatic stress prior to mild concussive TBI alters the appearance and severity of TBI and its outcomes; assess whether early detection of brain hypoxia with a novel non-invasive imaging agent predicts the severity of those outcomes; and treat within 12 hours and daily for 7 days with a novel drug to prevent and/or reduce severity of sensory, locomotor and cognitive dysfunction and anxiety-like behaviors after TBI or TBI+PTSD in males and females.

Rationale: TBI and PTSD are major causes of disability for military personnel and Veterans as well as civilians. TBI is the leading cause of death and disability in young adults (

Objectives: We will use a rat model of TBI that generates the most common type of TBI (mild TBI with no skull fracture or obvious damage). It produces physical symptoms related to injury severity (impaired balance, poor learning and memory, and anxiety-like behaviors) and can be combined with a model of PTSD. Our objectives are (1) to use a novel PET imaging agent to visualize reduced blood flow within the first 5 days following impact and to correlate that with increased brain peptide and decreased neurological function and (2) to test a novel drug for its ability to prevent or reduce the lack of blood flow that occurs early after injury as well as the symptoms produced by the TBI or TBI+ PTSD in male and female rats over a 30-day period. Our study is specifically designed to test for differences in symptoms and drug response in males and females, because females may present with different types and severity of symptoms than males and are often not included in initial drug development studies. We also will study, for the first time, how the severity of TBI symptoms is altered in rats that were subjected to both TBI and PTSD.

Outcomes: There are no FDA-approved treatments for TBI, and only two FDA-approved treatments for PTSD. We propose a new treatment for patients with TBI or TBI+PTSD that may be administered shortly after any type of TBI to prevent disrupted blood flow to the brain and the impairments that may follow. Benefits include identification of a new drug target and a drug to move forward for development to treat TBI and TBI with PTSD. A similar drug with the same drug target was recently in a clinical trial for depression and has a good safety profile. This drug class also is in development to improve movement for Parkinson’s patients and to prevent substance abuse (another common TBI and PTSD comorbidity). The holder of the patent to this drug is a consultant on this project and will work with us to improve drug efficacy or distribution, if necessary, to further develop it for commercialization and use in military and civilian populations for TBI and PTSD.

Proposal Title:	Retrospective Study of Treatment Outcomes: Understanding the Role of Personal, Organizational, and Treatment Factors in Army Alcohol Treatment Outcomes
Log Number:	TP220335
Current PI Name:	Elisa Borah
Award Number:	HT9425-23-2-0015
Current Contracting Organization:	Texas, University of, at Austin
Current Performing Organization:	Texas, University of, at Austin
Web Approval Date:	09-12-2023

Study Objectives: We want to understand what things make soldiers more successful in their alcohol misuse treatment as part of the Army Substance Use Disorder Clinical Care (SUDCC) program. This study will explore some of the differences between confidential and mandatory treatment options available to soldiers today. We specifically want to know more about how health, work, family, sleep, motivation, treatment options, and individual differences, might contribute to success.

Soldiers are considered to receive successful treatment when they achieve all their goals and maintain military readiness standards. Learning more about what things make soldiers more successful in treatment may improve the health and wellness of soldiers, families, and the Army community.

Study Focus: Our proposed study will address the Traumatic Brain Injury and Psychological Health Research Program (TBIPHRP) Focus Area 3a, as it will study the systems of care that can promote sustained functional recovery through Army Substance Use Disorder Clinical Care (SUDCC) treatment among U.S. Army Service Members (SMs). Our research seeks to close a gap in knowledge which will provide a better understanding of the intersection of risk and protective factors in long-term psychological health outcomes.

Potential Benefits, and Risks: This study promises to improve the delivery of care to Soldiers, leaders, and Army families impacted by alcohol misuse. Nearly a third of Soldiers are estimated to experience problems with alcohol. Indirectly the findings may lead to improvements in treatment and policy, which in turn promises to improve the readiness of the Army, military communities, and national security.

Risks from the research are no greater to study participants than issues faced in everyday life. Former patients and staff will be interviewed as part of the research. Other data will be gathered from anonymous health care records.

Projected Timeline: The study will collect data from 2023-2026 and present findings to military policy makers in early 2026. Treatment improvements may begin impacting individuals in care in 2026.

Proposal Title:	Amnion Cell Secretome-Mediated Therapy for Traumatic Brain Injury
Log Number:	TP220338
Current PI Name:	Benoit Mouzon
Award Number:	HT9425-23-1-0484
Current Contracting Organization:	The Roskamp Institute Inc
Current Performing Organization:	The Roskamp Institute Inc
Web Approval Date:	09-06-2023

Traumatic brain injury (TBI) is a major health concern for combat Veterans, Service Members, and the civilian population. Repetitive mild TBI (r-mTBI) is one of the primary causes of chronic morbidity in military personnel returning from the recent Middle East conflicts. Additionally, non-combat-related r-mTBI may contribute to long-term neurological and behavioral deficits in military and civilian populations. Following TBI, many patients never regain premorbid skills or functional responsibilities, despite intensive rehabilitation programs, and the burden on patients and caregivers can be enormous. This proposal addresses these specific concerns by using our clinically relevant translational mouse model to evaluate a novel potential r-mTBI therapeutic - ST266 - that has already been approved by the Food and Drug Administration (FDA) for other indications.

The objective of this application is to evaluate ST266, a novel neuroreparative cell-free therapeutic produced by Noveome Biotherapeutics, to treat the sub-acute/chronic consequences resulting from battlefield trauma. For this project, instead of a single drug that targets a specific target, we will use a rich, complex solution called ST266 that comprises molecules secreted from proprietary cells that have been shown crucial to neuroprotection, modulation of inflammation, cell recovery and healing. We will use our well-established preclinical mouse model of repetitive mild traumatic brain injury, in which we have demonstrated TBI-dependent behavioral and pathological dysfunction, consistent with human TBI patients. In this proposal we aim to specifically address two FY22 TBIPHRP IIRA focus areas, 1a “Understand” and 3a “Treatment” challenges, by investigating:

1. To evaluate the length and durability of treatment with ST266 and determine its potential benefit on impulsivity/disinhibition, sensory/motor dysfunction, and cognitive reserve and learning after brain injury.

2. To evaluate the length and durability of treatment with ST266, and determine its potential benefit on mitochondrial biogenesis, reduction of oxidative stress and neurodegenerative processes associated with brain injury.

By assessing nuanced aspects of neurobehavioral and pathological deficits, we will provide a framework from which informed decisions can then be made about the cellular and molecular mechanisms that are most important to improve the understanding, prevention, and treatment of psychological health conditions and TBI-related pathology. Within 30 months of the start date of this project, we will be able to determine: (1) which treatment, if any, provides neurological recovery based on the behavioral and neuropathological outcome markers; (2) this study will also provide novel insight into TBI-related pathology and neurobehavioral issues over time in both male and female mice; and (3) preclinical success in the study proposed here will enable Noveome to file an Investigational New Drug (IND) application to conduct a clinical trial specifically addressing this indication which will translate into the improvement of the health and/or quality of life for all Military Service personnel, Veterans, and civilians.

At the conclusion of this 3-year project, our findings could significantly impact the health of Service Members and Veterans with chronic consequences resulting from head trauma, and their families, by

offering them new therapeutic approaches to treat and potentially reverse their health problems. The aims of this study - to find both an effective treatment and an effective administration regimen - mean that the results can be useful for active-duty military, Veterans, and the civilian population to determine whether the negative sequelae of r-mTBI can be stopped with a defined period of treatment or will require a lifelong treatment. The sub-acute treatment paradigm having great translational relevance for our active-duty military and civilian population, and if proven effective, future studies could also investigate further delay treatments for Veterans who have previously sustained the traumatizing injury. We anticipate that positive results from this preclinical study could rapidly translate into clinical testing and implementation of an effective treatment.

Proposal Title:	Targeting Glucotoxicity to Improve Psychological Health After TBI
Log Number:	TP220357
Current PI Name:	Christoph Buettner
Award Number:	HT9425-23-1-0558
Current Contracting Organization:	Rutgers, New Jersey, State University of
Current Performing Organization:	Rutgers, New Jersey, State University of
Web Approval Date:	10-03-2023

This multidisciplinary proposal addresses the FY22 TBIPHRP IIRA Focus Areas to elucidate the role of traumatic brain injury (TBI)-induced impairments in glucose metabolism in the etiology of psychological and cognitive health conditions and the potential of targeting chronic impairments in systemic and cerebral glucose metabolism for treatment. TBI is a common cause of disability and, even in mild forms, can lead to several chronic sequelae such as post-traumatic stress disorder (PTSD), depression, anxiety and memory loss. While stress hyperglycemia is a known complication in the acute phase after TBI, the development of glucose intolerance and prediabetes in the chronic phase after injury is not currently a recognized consequence of TBI. Persistent brain inflammation is believed to underlie several of the long-term complications of TBI and is thus a promising therapeutic target, but its causes are poorly understood, especially in the context of mild injuries. Our proposal is based on four key findings by the Buettner Lab. First, rats subjected to mild repeated blast TBI, which mimics repetitive injury such as service members may experience in combat from repeated explosions, develop long lasting glucose intolerance leading to recurrent high glucose after meals. Second, in independent studies of acutely and chronically diabetic rats, we found that hyperglycemia drives microglial activation and brain inflammation. Third, administration of a diabetes drug that increases urinary excretion of glucose and normalizes blood glucose, reversed the brain inflammation and improved chronic memory loss and anxiety. Fourth, insulin directly acts on the brain to lower blood glucose after a meal, but brain inflammation can lead to persistently impaired action of insulin in the brain and high blood glucose. In TBI, impaired brain responses to insulin have been observed, suggesting that impaired insulin action in the brain after TBI may account for the glucose intolerance and glucose toxicity. Taken together, these findings support the central hypothesis that impaired brain control of glucose metabolism and elevated glucose levels maintain chronic neuroinflammation in TBI in a vicious cycle. A corollary is that interventions that normalize blood glucose levels may attenuate brain inflammation and thus alleviate cognitive and behavioral complications of TBI. This hypothesis will be examined in two specific aims in collaboration with Bryan Pfister from NJIT, who has strong expertise in rodent models of TBI. In Aim 1, we will test whether suppressing the glucose intolerance and glucose toxicity improves brain inflammation along with psychological health and memory in a rat model of TBI. In Aim 2, we will test whether restoring the action of insulin in the brain normalizes glucose levels thereby attenuating brain inflammation as well as psychological and cognitive sequelae of TBI.

Impact to the Research Field: The short-term impact to the field is to identify metabolic drivers of chronic brain inflammation in TBI and to refine the understanding of mechanisms of brain control of glucose metabolism that are important to the psychological and cognitive health conditions of TBI. The long-term impact is that this work will provide new insights into a role of peripheral metabolism in driving brain inflammation. Such knowledge may have broad clinical relevance that transcends TBI, and may apply to other neuropsychiatric conditions such as Alzheimer’s disease and chronic stress that are also associated with altered glucose metabolism.

Impact to Patient Care: The short-term impact of this research is to provide the medical field and the general population with the knowledge that an association between TBI and prediabetes exists and that brain toxicity driven by high glucose may increase vulnerability to psychological and cognitive complications after a brain injury. Subjects with a history of TBI may need to be counseled about their increased risk of diabetes and the need to implement lifestyle changes. The association is especially important to recognize in individuals who

suffered mild TBI and are often missed at long-term follow up. The long-term impact to patient care is that our studies may create the basis for clinical trials to test whether the diabetes drug tested in this proposal is effective to prevent prediabetes and improve psychological and cognitive health in TBI survivors.

Proposal Title:	Prevention of Post-Traumatic Stress: A Randomized Controlled Trial of Brief Prolonged Exposure Therapy for Injured Individuals Admitted to a Level I Trauma Center
Log Number:	TP220412
Current PI Name:	Ann Marie Warren
Award Number:	HT9425-23-1-0884
Current Contracting Organization:	Baylor Scott & White Research Institute
Current Performing Organization:	Baylor Scott & White Research Institute
Web Approval Date:	10-03-2023

Physical injury is a common occurrence, although sometimes these injuries are severe enough to result in a hospital admission, often to a trauma center. Injuries can occur through a variety of ways including falls, car wrecks, assaults such as a shooting or stabbing, or injuries from playing sports. In addition to the physical and medical effects of a serious injury, psychological and emotional changes can occur. Although it is common to have some symptoms after an injury such as anxiety, depression, memories of the injury that you can't stop thinking about and bad dreams, in some people these symptoms become more severe and long lasting, resulting in a condition we call Posttraumatic Stress Disorder or PTSD. We know there are several successful treatments to treat PTSD, and these treatments involve a type of talk therapy that helps you address the memory of the event and not avoid thinking about the event. The specific therapy that has evidence of helping with PTSD is called Prolonged Exposure therapy, or PE.

In this study, we want to use a brief version of the PE therapy, which usually takes 12 sessions, and shorten this to three sessions for people who are admitted to a Level 1 trauma center. A hospital that has a Level I trauma center means they are able to provide the highest level of trauma and emergency care available after an injury. We are planning to enroll people at two different Level I trauma centers in Texas, one in Dallas and one in Temple, and one Level I trauma center in Milwaukee, Wisconsin. The purpose of our study is to see whether this short version of the PE therapy can reduce or eliminate the chances of PTSD happening after an injury. This short form of PE has shown to be effective in another study of people who were admitted to the emergency department so we feel this could be beneficial for those admitted to a trauma center. People who consent, or agree, to be in our study will be randomized (like flipping a coin) into either the group that gets the short PE therapy or those that will get the usual treatment. The study activities will occur while people are admitted to the trauma center but will continue to follow people at three time points after injury, one month, three months and six months later to determine if the effects of the therapy have lasted. We think the people who are in the PE therapy group will have fewer PTSD symptoms; have fewer related symptoms such as depression, pain, and anxiety, and be able to complete the three sessions and actually find it helpful or satisfying. We hope that this study is beneficial to people who are admitted to a trauma center after an injury. In addition, we hope that the study is helpful to people who are either in the military or are Veterans because they often experience PTSD.

Proposal Title:	Inhaled Nitric Oxide for Treatment of Microvascular Dysfunction in Traumatic Brain Injury
Log Number:	TP220416
Current PI Name:	Samuel Shin
Award Number:	HT9425-23-1-0851
Current Contracting Organization:	Pennsylvania, University of
Current Performing Organization:	Pennsylvania, University of
Web Approval Date:	10-03-2023

Background: Traumatic brain injury is a devastating condition that afflicted 320,000 troops from military operations in the last two decades. Additionally, it affects 1.5 million people in the general American public. Despite thirty years of animal model research on various medications to treat traumatic brain injury, development of medications to treat it has been slow and ineffective. A major damage caused by traumatic brain injury is due to lack of sufficient blood flow in numerous regions of the brain that happens shortly after the initial impact. In this proposal, we suggest using inhaled nitric oxide in traumatic brain injury given that it can selectively dilate blood vessels in the brain that has reduced blood flow. It also has been shown to reduce damaging effects such as inflammation and chemical stress.

Focus Area: This proposal for a Research Level I Clinical Trial Award will address Focus Area 3: Treat, focusing on an intervention administered acutely, using personalized medicine approaches by tailoring treatments to biological elements present. By optimizing brain blood flow after traumatic brain injury using the innovative inhaled nitric oxide therapy, we hope to mitigate brain injury and improve outcomes.

Objective and Rationale: The objective of this study is to show that inhaled nitric oxide can improve blood flow in the brain and brain metabolism after traumatic brain injury, attenuating brain injury and resulting in improved long-term function. The rationale for this study is the abundance of animal model evidence that nitric oxide is helpful in reducing injury in traumatic brain injury and the evidence of its safety seen in current clinical use. Also, inhaled nitric oxide is a safe agent approved by the Food and Drug Administration, used in newborn babies for lung diseases since the year 2000. It has also been used in clinical trials for adults, and it is actively being tested in other clinical trials currently. In this study, we will use a spectroscopy device that sends light waves through the skull in order to detect blood flow and oxygen use in the brain tissue. This device has been validated by our group previously in several studies 63, 66. We will also monitor the levels of specific proteins that increase with severity of the injury in order to see if inhaled nitric oxide lowers their levels by reducing injury.

CBPR Approach: We will work in partnership with a TBI-survivor and an active member of a local community who will serve as a Lived Experience Consultant (LEC). Semi-annual meetings will be held to discuss the recruitment process, data analysis, and eventual dissemination of information at the end of the study.

Problem to be Addressed, Applicability, and Impact: We will optimize blood flow in the brain after traumatic brain injury by administering inhaled nitric oxide for the first three hours after injury. We are aiming to show that this is both a safe treatment and effective in reducing brain injury. As this agent is in current clinical use and there is a well demonstrated side effect profile, clinicians would be able to administer this agent with much ease if therapeutic potential can be demonstrated. Without the potential regulatory barriers that novel pharmacological agents would have to undergo and delays that would take place to build a new manufacturing infrastructure, inhaled nitric oxide is rapidly applicable to the traumatic brain injury patients. If the current trial can show that it is safe and effective treatment for TBI, it would have

an enormous impact in the clinical outcomes of these patients given there are currently no treatment regimen that can limit secondary injury after TBI. Since the blood flow improvement may lead to functional improvements weeks to months after the treatment, patient-related outcome may potentially show up at 6-month assessment period.

Benefit to the Military: The findings of study can directly benefit the military personnel who suffer an acute traumatic brain injury. During the early time period after traumatic brain injury when the blood flow to various regions of the brain is reduced, inhaled nitric oxide can be given to these personnel. Nitric oxide can be transported as a small gas tank and is easily deliverable to the front lines or field hospitals where they can be administered. It has a minimal side effect profile and there is no concerning long term health risk from its use at the doses we will test. Also, it can be used in numerous other individuals who suffer from traumatic brain injury in the civilian setting, such as motor vehicle crash. Given its safety and potential benefit, this is a promising agent to be studied for treatment of traumatic brain injury patients.

Proposal Title:	Inhaled Nitric Oxide for Treatment of Microvascular Dysfunction in Traumatic Brain Injury
Log Number:	TP220416P1
Current PI Name:	Ramon Diaz-Arrastia
Award Number:	HT9425-23-1-0852
Current Contracting Organization:	Pennsylvania, University of
Current Performing Organization:	Pennsylvania, University of
Web Approval Date:	10-03-2023

Background: Traumatic brain injury is a devastating condition that afflicted 320,000 troops from military operations in the last two decades. Additionally, it affects 1.5 million people in the general American public. Despite thirty years of animal model research on various medications to treat traumatic brain injury, development of medications to treat it has been slow and ineffective. A major damage caused by traumatic brain injury is due to lack of sufficient blood flow in numerous regions of the brain that happens shortly after the initial impact. In this proposal, we suggest using inhaled nitric oxide in traumatic brain injury given that it can selectively dilate blood vessels in the brain that has reduced blood flow. It also has been shown to reduce damaging effects such as inflammation and chemical stress.

Focus Area: This proposal for a Research Level I Clinical Trial Award will address Focus Area 3: Treat, focusing on an intervention administered acutely, using personalized medicine approaches by tailoring treatments to biological elements present. By optimizing brain blood flow after traumatic brain injury using the innovative inhaled nitric oxide therapy, we hope to mitigate brain injury and improve outcomes.

Objective and Rationale: The objective of this study is to show that inhaled nitric oxide can improve blood flow in the brain and brain metabolism after traumatic brain injury, attenuating brain injury and resulting in improved long-term function. The rationale for this study is the abundance of animal model evidence that nitric oxide is helpful in reducing injury in traumatic brain injury and the evidence of its safety seen in current clinical use. Also, inhaled nitric oxide is a safe agent approved by the Food and Drug Administration, used in newborn babies for lung diseases since the year 2000. It has also been used in clinical trials for adults, and it is actively being tested in other clinical trials currently. In this study, we will use a spectroscopy device that sends light waves through the skull in order to detect blood flow and oxygen use in the brain tissue. This device has been validated by our group previously in several studies 63, 66. We will also monitor the levels of specific proteins that increase with severity of the injury in order to see if inhaled nitric oxide lowers their levels by reducing injury.

CBPR Approach: We will work in partnership with a TBI-survivor and an active member of a local community who will serve as a Lived Experience Consultant (LEC). Semi-annual meetings will be held to discuss the recruitment process, data analysis, and eventual dissemination of information at the end of the study.

Problem to be Addressed, Applicability, and Impact: We will optimize blood flow in the brain after traumatic brain injury by administering inhaled nitric oxide for the first three hours after injury. We are aiming to show that this is both a safe treatment and effective in reducing brain injury. As this agent is in current clinical use and there is a well demonstrated side effect profile, clinicians would be able to administer this agent with much ease if therapeutic potential can be demonstrated. Without the potential regulatory barriers that novel pharmacological agents would have to undergo and delays that would take place to build a new manufacturing infrastructure, inhaled nitric oxide is rapidly applicable to the traumatic brain injury patients. If the current trial can show that it is safe and effective treatment for TBI, it would have

an enormous impact in the clinical outcomes of these patients given there are currently no treatment regimen that can limit secondary injury after TBI. Since the blood flow improvement may lead to functional improvements weeks to months after the treatment, patient-related outcome may potentially show up at 6-month assessment period.

Benefit to the Military: The findings of study can directly benefit the military personnel who suffer an acute traumatic brain injury. During the early time period after traumatic brain injury when the blood flow to various regions of the brain is reduced, inhaled nitric oxide can be given to these personnel. Nitric oxide can be transported as a small gas tank and is easily deliverable to the front lines or field hospitals where they can be administered. It has a minimal side effect profile and there is no concerning long term health risk from its use at the doses we will test. Also, it can be used in numerous other individuals who suffer from traumatic brain injury in the civilian setting, such as motor vehicle crash. Given its safety and potential benefit, this is a promising agent to be studied for treatment of traumatic brain injury patients.

Proposal Title:	Promoting Rapid Return to Functioning After Acute Stress Reaction: Assessing the Efficacy of the iCOVER Intervention
Log Number:	TP220430
Current PI Name:	Samuel McLean
Award Number:	HT9425-23-2-0032
Current Contracting Organization:	North Carolina at Chapel Hill, University of
Current Performing Organization:	North Carolina at Chapel Hill, University of
Web Approval Date:	10-03-2023

Rationale: One in six Service Members report experiencing an Acute Stress Reaction during a combat-related event. An Acute Stress Reaction is characterized by a shifting set of emotional, physical, and cognitive symptoms. These symptoms interfere with service member functioning, potentially endangering the individual Service Member, their team members, and the mission. Until recently, there were no interventions for Service Members to manage Acute Stress Reactions in team members. iCOVER is a new, brief (1-2 minute) intervention developed by the Walter Reed Army Institute for Research to rapidly restore functioning in Warfighters experiencing an Acute Stress Reaction. Research to date has demonstrated that Service Members feel that iCOVER is relevant, acceptable, and would be useful. iCOVER has recently been adopted by the militaries of allied nations (e.g., Canada, Germany, Mexico, Norway); however, to date the effectiveness of iCOVER has not been tested.

Approach: We will enroll 450 adults exhibiting substantial Acute Stress Reaction symptoms at four emergency departments/trauma centers in our AURORA Research Network: Washington University (St. Louis, MO), UMass Memorial Health (Worcester, MA), Henry Ford Health (Detroit, MI), and Cooper University Health (Camden, NJ). We have an outstanding track record of successfully performing research protocols at these sites. Study participants will be randomized to iCOVER, usual care (no specific intervention), or physical presence with reassurance. The effect of these interventions on the rapid restoration of neurocognitive function will be assessed using brief computer-based tests (performed on an iPad) 3, 10, and 20 minutes later. These neurocognitive tests will assess three domains critical to Warfighter performance: psychomotor vigilance, response inhibition, and working memory. If iCOVER is able to significantly improve performance on 2 of the 3 tests more than the two comparison interventions then iCOVER will be considered effective. Secondary analyses will compare distress symptoms 3, 10, and 20 minutes after intervention and neurocognitive function and psychological outcomes 1 hour, 2 days, and 7 days after intervention.

Focus Area 3a. Treat: The proposal is to test an immediate intervention to promote rapid and sustained functional recovery from an Acute Stress Reaction.

Problem to be Addressed: Despite rapid adoption, the efficacy of iCOVER is not known. This is because it is not feasible to conduct a randomized controlled trial of iCOVER during military deployment, when exposure to extreme stress is difficult to predict. The present study will perform a randomized controlled trial evaluating the effectiveness of the iCOVER intervention in an emergency department population experiencing an Acute Stress Reaction related to extreme stress. At the end of the award period, the results of this trial will inform decisions regarding the degree to which resources should be invested in iCOVER training and skill maintenance to enhance (1) U.S. warfighting capability and individual and unit resilience and (2) civilian performance in high-stress settings (e.g., first responders).

Benefit Service Members, Veterans, and/or Military Beneficiaries: iCOVER is potentially invaluable in supporting individual and unit performance under extreme stress on the battlefield. It provides small teams

with the capability to respond effectively to an Acute Stress Reaction, preventing that reaction from disrupting the mission. The long-term consequences of shifting the trajectory of an Acute Stress Reaction may benefit Service Members and Veterans by countering chronic adverse psychological outcomes. The results of this trial also have direct implications for other high stakes occupations and can be easily adapted for use by firefighters, police, and other first responders.

Proposal Title:	Long-Term Psychological and Physical Health Outcomes Following Military Deployment: The Veterans After-Discharge Longitudinal Registry (Project VALOR)
Log Number:	TP220455
Current PI Name:	Brian Marx
Award Number:	HT9425-23-1-0828
Current Contracting Organization:	Boston VA Research Institute, Inc. (BVARI)
Current Performing Organization:	Boston VA Research Institute, Inc. (BVARI)
Web Approval Date:	10-03-2023

Overall Project: Long-Term Psychological and Physical Health Outcomes Following Military Deployment: The Veterans After-Discharge Longitudinal Registry (Project VALOR)

Background: This proposal is designed to address Congressionally Directed Medical Research Program Traumatic Brain Injury and Psychological Health Research Program Understand Focus Area. This is research that aims to address knowledge gaps in foundational science, epidemiology, and etiology of psychological health conditions and/or traumatic brain injury.

Since 2001, more than 2 million U.S. Service Members have been deployed to Iraq and Afghanistan in support of post-9/11 conflicts. More than two decades of war have taken a staggering toll on Service Members; more than 7,000 U.S. Service Members have died in post-9/11 conflicts, more than 20,000 have been physically injured, and more than 30,000 U.S. Service Members and Veterans who served in post-9/11 conflicts have died by suicide. Costs to provide care for Veterans of post-9/11 conflicts are estimated at $2.2 to $2.5B.

Longitudinal cohort studies (i.e., research studies that follow the same group of research participants over an extended period of time) have been critically important to improving our understanding of the impacts of deployment experiences on psychosocial and physical health outcomes, as well as factors that amplify or diminish these impacts. However, numerous critical gaps remain, including poor understanding of a) long-term posttraumatic stress disorder (PTSD) symptom course, b) recovery from PTSD, c) long-term patterns in related psychosocial phenomena (e.g., suicide risk, functioning), d) sex differences in long-term deployment consequences, e) long-term impacts of deployment experiences on physical health, f) long-term impacts of military sexual trauma, harassment, and discrimination, and g) interactions among risk and resilience factors in predicting long-term outcomes.

The specific aims of this project are to address these gaps in our understanding of long-term patterns of adverse psychological and physical health outcomes following deployment, recovery from these outcomes, and predictors thereof among a nationwide sample of female and male Veterans. To accomplish this, we will extend prior research conducted using the Veterans After-Discharge Longitudinal Registry (Project VALOR), a Department of Defense-funded longitudinal cohort of 1,649 men and women who deployed to Afghanistan or Iraq as part of post-9/11 conflicts and subsequently utilized Veterans Health Administration (VHA) services. To date, Project VALOR participants have completed assessments on 5 occasions over ~7 years.

Research Plan: The proposed study would collect additional data in a series of additional, related projects to better understand:

A. Long-term impacts of deployment on psychosocial outcomes (e.g., PTSD, employment functioning, marriage quality, suicidal thoughts, suicide attempts)

B. Long-term impacts of deployment on physical health outcomes (e.g., coronary heart disease, cardiometabolic risk factors)

C. More accurately understand how pre-deployment (e.g., childhood abuse), peri-deployment (e.g., traumatic brain injury), post-deployment (e.g., reintegration experiences), and biological (e.g., polygenic risk scores) factors impact each other to more accurately understand the impact of risk and resilience factors

D. Understanding experiences reporting sexual assault and harassment and identifying barriers and facilitators to reporting

Impact and Relevance to Military Health: This proposal is relevant to the Understand focus area as this series of projects seeks to address knowledge gaps in foundational science, epidemiology, and etiology of psychological health conditions and traumatic brain injury. Results will have critical, direct implications for VHA policy and planning. The central focus of this application and each specific project are to better understand the long-term psychosocial and physical health impacts of military deployment and the potency of various risk and resilience factors.

Lay Abstract – Project 1 – Adverse Psychological Outcomes Following Deployment, Recovery from These Outcomes, and How These Patterns Differ for Female and Male Veterans

Overarching Challenge and Background: a) Long-term posttraumatic stress disorder (PTSD) symptom course, b) recovery from PTSD, c) long-term patterns in related psychosocial phenomena (e.g., suicide risk, functioning), and d) sex differences in long-term deployment consequences remain poorly understood. Most research on PTSD symptom course to date focused on either short-term (i.e., the first few years) or medium-term (i.e., 5–10 years) course following traumatic stressor exposure. Few studies have ever examined the longitudinal course of PTSD beyond 10 years. Better understanding the long-term PTSD symptom trajectories has significant implications for healthcare policy and planning. Many individuals who develop PTSD recover from the disorder (i.e., no longer meet diagnostic criteria and/or experience clinically significant reductions in symptom severity), either naturally or after receiving treatment. Although numerous indicators of risk for chronic PTSD have been identified, we continue to know very little about the factors that may determine recovery from PTSD (e.g., response to treatment or even natural recovery over time).

PTSD is not the only adverse psychosocial outcome associated with post-9/11 conflicts. Replicated evidence indicates Veterans of post-9/11 conflicts report elevated rates of mood disorders, anxiety disorders, substance use disorders, and suicidality. Military deployment and combat exposure have been directly linked to depression, among post-9/11 conflict Veterans. As with PTSD, early indications suggest many post-9/11 conflict Veterans are experiencing chronic patterns of other psychiatric symptoms. Long-term patterns in psychiatric symptomatology, psychosocial functioning, self-injurious thoughts and behaviors (SITBs), and cognitive functioning among Veterans remain poorly understood.

Research Plan:

The aim of this study is to examine long-term patterns of PTSD symptoms, comorbid psychopathology, psychosocial functioning, self-injurious thoughts and behaviors, and cognitive functioning among female and male Veterans who deployed in support of post-9/11 conflicts and examine sex differences in these patterns. We will measure PTSD diagnostic status, PTSD severity, romantic relationship quality, family functioning, occupational functioning, friendships, parenting, education, and self-care functioning, as well as suicidal thoughts and cognitive functioning (e.g., IQ). Using a number of data analytic strategies, we will

explore if Veterans can be organized into groups based on patterns of change in PTSD symptoms, comorbid psychopathology, psychosocial functioning, self-injurious thoughts and behaviors over time, and explore how these groups differ for female and male Veterans.

Impact and Relevance to Military Health: This project represents a meaningful extension of existing research in several ways. First, the proposal focuses on better understanding long-term patterns in health outcomes. Although nearly all research on PTSD and related psychosocial phenomena focuses on the decade following trauma exposure, many Veterans remain enrolled in Veteran Health Administration for the remainder of their lives. Accordingly, existing research has provided limited insight into long-term deployment-related psychosocial health outcomes. Second, the proposal aims to provide a more comprehensive picture of Veteran psychosocial and physical health. Although PTSD is one deployment-related condition, it represents one of many commonly reported psychosocial challenges. This proposal would obtain information about PTSD as well as comorbid psychiatric symptoms (e.g., depression, anxiety, and substance use), suicide risk, and numerous domains of psychosocial functioning (e.g., employment, romantic relationship, friendships, parenting, physical functioning). Third, the proposal aims to capitalize on the even sampling of female and male Veterans to understand unique longitudinal patterns in psychosocial health outcomes among female Veterans, an under-studied population.

Lay Abstract – Project 2 – Adverse Physical Health Outcomes Following Deployment, Links to Psychosocial Outcomes, and How These Patterns Differ for Female and Male Veterans

Overarching Challenge and Background: Deployment experiences and subsequent psychiatric symptomatology (e.g., PTSD, depression) are linked to numerous adverse physical health outcomes. Among Veterans, PTSD has been linked to increased risk for numerous chronic health conditions, including cardiovascular disease, gastrointestinal conditions (e.g., irritable bowel), musculoskeletal (e.g., arthritis), type II diabetes, and metabolic syndrome. Additionally, many Veterans, particularly older Veterans struggle more frequently with chronic illnesses than non-Veterans.

However, the impact of deployment experiences (e.g., combat, MST) on long-term patterns in physical health, and factors that mitigate these risks, remain poorly understood. A long-standing challenge in the field has been to disentangle the causal pathways of a) direct effects of deployment experiences, b) indirect effects of subsequent psychiatric symptomatology, and c) indirect effects of subsequent health factors (e.g., weight gain, sedentary behavior) on physical health outcomes. Complicating this challenge, sex differences in each of these risk factors are well-documented. Accordingly, any identified intervention targets to reduce adverse health impacts may not function similarly for female and male Veterans; this distinction has critical implications for VHA policy application. Post-deployment psychiatric symptoms are linked to numerous health factors that are, in turn, associated with adverse physical health outcomes (e.g., tobacco use, alcohol use, substance use, physical inactivity, poor diet). The direction of the effect between psychiatric symptoms and health factors remains unresolved and has important implications for intervention efforts. If psychiatric symptoms or health factors function as a primary buffer, they may represent a high priority intervention effort. Alternatively, a tightly synergistic association would indicate simultaneous intervention might be more effective in reducing risk for adverse health outcomes.

Research Plan:

Aim 1. Examine longitudinal patterns of physical health outcomes among female and male Veterans who deployed in support of post-9/11 conflicts and examine sex differences in these patterns.

Aim 2. Examine longitudinal patterns of diet, physical activity, and exercise as they relate to psychosocial and physical health outcomes.

The VHA electronic medical record includes detailed information on Veteran medical condition diagnoses and treatment, including prescribed medication, vital signs, blood test data (e.g., complete blood count,

metabolic panel, cholesterol/triglyceride tests), ordered consults, procedures (e.g., surgery), events (e.g., heart attack).

Additionally, we will use validated questionnaire measures to assess diet, exercise, and physical activity.

For Aim 1, we will descriptively identify diagnosed physical health conditions, including conditions linked to deployment and subsequent psychopathology. We will then model the association between time since return from deployment and disease onset risk using survival analysis. Additionally, we will enter sex as a predictor in the survival analysis models. For Aim 2, we will descriptively explore patterns of diet, physical activity, and exercise, then use latent difference score modeling and regress a) change between the two measurement occasions and b) level at each occasion on sex. We will enter diet, physical activity, and exercise as predictors of physical health outcomes in the survival models described in Aim 1. Additionally, we will use bivariate latent difference score modeling to examine a) the correlation between change in diet, physical activity, and exercise relative to change in psychosocial health outcomes, and b) how level of each construct predicts subsequent changes in the other over time.

Impact and Relevance to Military Health: Veterans with PTSD are at elevated risk for numerous adverse physical health outcomes, including Veterans, cardiovascular disease, gastrointestinal conditions, musculoskeletal and metabolic conditions. This proposal focuses on a) better understanding the long-term physical health forecast for veterans, b) exploring how these patterns differ for female and male veterans, and c) understanding how these patterns relate to psychiatric symptoms. Results from this study have direct clinical implications for VHA care and policy.

Lay Abstract – Project 3 – Pre-, Peri-, and Post-Deployment Predictors of Adverse Psychosocial Outcomes Following Deployment, Recovery from these Outcomes, and How these Predictors Differ for Female and Male Veterans

Overarching Challenge and Background: Previously established risk and resilience factors include demographic, psychological, and social factors. Research examining the predictive values of these factors has historically focused primarily on PTSD rather than related adverse outcomes (e.g., trauma-related deficits in obtaining and sustaining employment, divorce, suicide attempts). Although we have learned much about how individual risk and recovery factors predict long- term outcomes, we know little, if anything, about how risk and recovery factors impact each other over time.

Examining interactions among risk and recovery factors holds the potential to more realistically capture naturally occurring patterns in risk and resilience factors and potentially, more accurately account for their effect on symptom course. Project 3 is focused on better understanding risk and resilience factors for PTSD, as well as other long-term psychosocial and physical health outcomes, sex differences therein, and how these factors interact to forecast these long-term outcomes.

Research Plan:

Aim 1. Examine pre-, peri-, and post-deployment predictors of long-term patterns of psychological and physical health outcomes among female and male Veterans who deployed in support of post-9/11 conflicts and explore sex differences in these effects.

Aim 2. Examine the impact of psychosocial and pharmacological interventions on long-term patterns of psychological and physical health outcomes, and sex differences therein, among female and male Veterans.

Aim 3. Evaluate validity of the current Veteran Health Administration suicide risk classification system.

Aim 4. Examine genetic markers of long-term patterns of psychological and physical health outcomes and sex differences therein.

We will include previously collected data (e.g., PTSD diagnostic status, childhood trauma exposure, deployment experiences, employment history, genetic data) as well as additional data collection (e.g., updated symptom assessment, medical records on treatment utilization) for prediction models. For Aim 1, we will use machine learning, an advanced data analytic technique used to better understand complex research questions involve many variables. In this application, we will use machine learning to better understand a) how a large number of risk and resilience factors impact each other over time and b) harness the value of multiple predictors to more accurately forecast long-term outcomes. We will use this approach to understand of these risk and resilience factors predict long term patterns in psychosocial health (e.g., PTSD, suicidal thoughts, ability to sustain employment, marriage quality, parental functioning) and physical health (e.g., cardiovascular disease, type II diabetes). We will also use this method to examine how therapy and psychiatric medication impact each other and predict long-term patterns in psychosocial and physical health. For Aim 3, we will classify each participant into acute and chronic risk levels during a clinical interview and examine the association between risk classification and probability of subsequently attempting suicide. For Aim 4, we will examine polygenic risk scores for each outcome and conduct planned contrasts to explore sex differences in these predictive effects.

Impact and Relevance to Military Health: This project focuses on several prominent research priorities for both DoD and VHA, including utilizing diverse data sources to more accurately forecast long-term psychosocial and physical health risk, examining current practices in suicide risk classification. Results have direct implications for VHA care and policy.

Lay Abstract – Project 4 – Experiences Reporting Sexual Assault, Harassment, Discrimination, and Barriers to Reporting

Overarching Challenge and Background: Military sexual trauma (MST) - sexual assault or repeated, threatening sexual harassment that occurred while a Veteran was serving on active duty or active duty for training - is a pervasive problem among Service Members and Veterans, with survivors being at increased risk of both mental and physical health difficulties. Reporting MST (i.e., filing a formal complaint), which is the first step to providing survivors with the support they need and holding the perpetrators responsible, is complicated by both system- and individual-level barriers. Studying the facilitators and barriers to MST reporting is complicated by the reluctance of MST survivors to disclose these experiences at all.

Research Plan:

Aim 1. Examine experiences reporting sexual assault, sexual harassment, and discrimination and identify barriers to reporting.

Aim 2. Examine perceptions of culture and climate as they relate to assault, harassment, and discrimination.

Research Question 1: What were Veterans' perceptions of culture and climate as they relate to assault, harassment, and discrimination?

Research Question 2: Do these perceptions differ between male and female Veterans?

This project will involve development a new self-report measure designed to assess barriers and facilitators to MST reporting, including those specific to workplace culture and climate, that is reflective of both male and female MST survivors' experiences; and data from this measure which can be used to understand the association between barriers and facilitators and disclosure, reporting, and healthcare utilization among a large sample of OEF/OIF Veterans. This effort will prioritize content validation. The content validation process will involve four key parts: a review of the literature, the definition and refinement of key constructs, interviews with Veterans, and the development and refinement of test items. Following initial psychometric examination, including factor structure, internal consistency, and construct validity, we will utilize the newly validated measure to better understand these factors, and sex differences therein, among Veterans.

Impact and Relevance to Military Health: The proposed project adds uniquely to the literature by developing a measure that reflects the experiences of both male and female Veterans who are MST survivors and who have demonstrated differential levels of comfort with disclosing these experiences. Whereas the study of factors which influence MST disclosure is not new, most studies focus on barriers and facilitators for solely female survivors. Indeed, a recent qualitative examination of Service Members' perceptions of male MST and barriers to reporting highlighted that, despite the high rates of male MST, this phenomenon remains understudied, The authors conclude with a call for additional research to focused on male MST to better understand barriers to reporting and care seeking. Our earlier work demonstrates that there are Project VALOR participants who are male MST survivors and are willing to disclose their experiences to us even if they will not disclose to others (e.g., the VHA). We will therefore be able to conduct interviews with both male and female MST survivors, stratified by VHA screen disclosure, to gather unique information about their experiences. Based on this, at the conclusion of the proposed study we will have two important deliverables: 1) a new self-report measure designed to assess barriers and facilitators to MST reporting, including those specific to workplace culture and climate, that is reflective of both male and female MST survivors' experiences; and 2) data from this measure which can be used to understand the association between barriers and facilitators and disclosure, reporting, and healthcare utilization among a large sample of OEF/OIF Veterans.

Proposal Title:	A Cluster Randomized Controlled Trial of X-Core: A Multilevel Sexual Assault Prevention Intervention for Active-Duty Airmen
Log Number:	TP220460
Current PI Name:	Belinda Hernandez
Award Number:	HT9425-23-1-0763
Current Contracting Organization:	Texas, University of, Health Science Center at Houston
Current Performing Organization:	Texas, University of, Health Science Center at Houston
Web Approval Date:	09-12-2023

For the past 3 years, sexual assault (SA) and sexual harassment (SH) within the military have continued to increase, making them significant public health problems. SA can have serious consequences for the survivors and negatively affect productivity, mission readiness, and overall Service Members' well-being. Both SH and SA can be prevented by implementing interventions that promote healthy relationship skills; however, there is currently no such intervention for active-duty Service Members. We developed Code of Respect (X-CoRe) to fill this gap. X-CoRe is an innovative, web-based, multi-level SA and SH prevention intervention for active-duty Airmen. Consisting of three components (one for Junior Enlisted, Leadership, and the installation), X- CoRe increases Airmen's knowledge and skills to build and maintain healthy, respectful relationships, ultimately reducing SAs and enhancing Airmen's overall well-being. The goal of this 4-year cluster randomized controlled trial (RCT) is to determine the efficacy of X-CoRe in preventing SA and SH among Junior Enlisted Airmen, those most at risk for SA and SH. This proposal specifically addresses FY22 TBIPHRP CTA sub-focus areas (2d) development, evaluation, and implementation of crosscutting prevention approaches targeting upstream factors to address adverse outcomes and (2f) solutions to address aspects of workplace culture and climate that are associated with increases in harmful behaviors. This study builds on our strong partnership with our current military advisory group, which will guide the study, and preliminary studies that established X- CoRe's feasibility and favorability. At the end of this study, we expect to establish the efficacy of X-CoRe in reducing SA and SH among a sample of Junior Airmen while also improving their overall health, well-being, and mission readiness. If effective, X-CoRe can be scaled-up and tested in a Level 3 clinical trial to determine its effectiveness in real-world settings at installations across the U.S.

Proposal Title:	Mechanisms of Seizure-Induced Death of TSC Model Mice
Log Number:	TS220001
Current PI Name:	Ian Wenker
Award Number:	HT9425-23-1-0378
Current Contracting Organization:	Virginia, University of
Current Performing Organization:	Virginia, University of
Web Approval Date:	09-15-2023

This proposal addresses Tuberous Sclerosis Complex Research Program's Focus Area "Preventing epilepsy, improving treatment, and mitigating neurodevelopmental outcomes associated with TSC-related seizures." Compared to healthy control patients, those with Tuberous Sclerosis Complex (TSC) have an up to fivefold higher incidence of death. This is due to a number of factors, but the most substantial is sudden unexpected death in epilepsy (SUDEP). According to a recent analysis of the Tuberous Sclerosis Alliance Natural History Database, SUDEP is the most common cause of death for TSC patients. SUDEP is defined as the sudden, unexpected, nontraumatic, and nondrowning death of a person with epilepsy for which postmortem examination does not reveal another cause of death. As implied in this definition, how SUDEP occurs is not presently understood. Transgenic mouse models of TSC also experience high mortality rate; however, no research has been performed on the mechanism(s) of death in these mouse models (i.e., How do these seizures produce death?).

Although a number of mechanisms have been proposed for SUDEP, they all culminate with eventual failure of breathing and/or heart function. My research program is focused on understanding how the brain controls breathing and cardiovascular function in health and disease. I have expertise in recording breathing and heart function and have already published results concerning SUDEP in other models of epilepsy, where I uncovered that respiratory arrest is the primary driver of fatality.

Based on previous reports monitoring seizures in TSC mouse models, and my previous data in other SUDEP models, I hypothesize that seizures produce respiratory arrest, and fatality occurs when breathing does not recover immediately after the seizure.

In Aim 1, I will record breathing and heart function in addition to seizure monitoring in two commonly used mouse models of TSC. The primary goals of this aim are to determine whether seizures initiate death and whether breathing or heart function fail to produce death.

In Aim 2, I will record multiple lower brain regions that are important for control of breathing in addition to recording breathing and heart function. I will also administer the drug vigabatrin in one group of mice, which has been shown to nearly eliminate seizures, but modestly effect fatality in TSC mice. The goals of these experiments are to understand how mice die when vigabatrin is administered (e.g., as in Aim 1: from seizures; heart or breathing dysfunction), and to see what lower brain regions are activated during seizures and fatality.

Although the experiments proposed are technically challenging and will have great impact for the field, they are straightforward and feasible. My approach allows for detailed recording of numerous mice 24 hours a day, 7 days a week. This ensures the success of my proposed experiments. When fatality occurs, I will be able to assess what happens to breathing and heart function: what fails first and how this relates to seizures.

Results from these studies will be entirely novel, as work of this nature has never been attempted in TSC mouse models. It will provide future directions for understanding and preventing SUDEP in TSC mouse models. Ultimately, identifying the cause of death and neural mechanisms will produce therapies that prevent aspects of severe seizures and death in TSC patients. This would affect a large fraction of TSC

patients and their families, as many with TSC have intractable convulsive seizures, putting them at high risk for SUDEP.

Proposal Title:	Can EVs Expand the Therapeutic Effect of Gene Replacement for Tsc1 in Brain?
Log Number:	TS220004
Current PI Name:	Xandra Breakefield
Award Number:	HT9425-23-1-0338
Current Contracting Organization:	Massachusetts General Hospital
Current Performing Organization:	Massachusetts General Hospital
Web Approval Date:	04-04-2023

These studies are designed to increase the effectiveness of gene therapy in a TSC1 mouse model. This model focuses on brain lesions that underlie neurologic symptoms in TSC1 patients. These lesions, which are mimicked in our mouse model, include overgrowth of the cells lining the ventricles, which leads to hydrocephalus in patients, and increased size, signaling, and proliferation of brain cells, which may contribute to epilepsy. Current therapies for hydrocephalus and, to some extent seizures, rely on neurosurgery and treatment with analogues of the drug rapamycin. Neurosurgery is invasive and can lead to complications, and drug treatment has to be continuous and is associated with compromise of the immune system and potentially interferes with brain development in children. This defines an unmet medical need.

We envision our therapy, which involves gene replacement therapy using adeno-associated virus vectors, which are now used for treatment of several neurologic diseases in patients, can be expanded by engaging membrane vesicles released by cells throughout the body to carry the therapeutic cargo over a broader biodistribution, not only in the brain but in other tissues. In clinical practice, gene therapy using these vectors is carried out once by injection into the bloodstream with the vector spreading throughout the body, including across the blood-brain barrier. Replacement therapy lasts for many years without further treatment. This approach could be used in children or adults to prevent new lesions and decrease the size of existing lesions.

This therapy has the potential to prevent hydrocephalus and decrease epilepsy in children and to reduce size of lesions in peripheral overgrowths, such as renal angiomyolipoma and lymphangioleiomyomatosis (LAM), although a much larger dose of vector would be needed in adults as compared to children if given through the bloodstream. Although these viral vectors are used clinically in all ages, they may run the risk of being taken up mostly by liver cells with the possibility of toxic effects there. Also, the modifications to the replacement protein, hamartin, to increase its transfer by membrane vesicles among cells, may elicit a negative immune response. This will be monitored carefully in our animal model. Abnormally high expression of the replacement protein may elicit some toxicity, which will be evident in our mouse model, but to date has not been a problem.

Our gene replacement therapy using these virus vectors may reach clinical trial within 2 years. Adding in the modification of the hamartin protein to increase its biodistribution in the brain and other organs would take another 2 years to accumulate data for the U.S. Food and Drug Administration (FDA) to review prior to clinical trials.

Based on the amazingly positive response in our mouse model of TSC1 to vector-mediated gene replacement therapy – extending lifespan from 50 days to over 200 days, and the extensive expertise of our laboratory in using membrane vesicles in the body to expand the range of gene replacement, we anticipate that this combined approach can be paradigm shifting and improve symptoms in organs throughout the body of TSC1

patients. Our initial focus is on the brain as that is our area of expertise, and we will make our results available to the scientific community, share all vectors, and seek collaborations with investigators focused on the brain and other organs.

Proposal Title:	Treatment of Epilepsy in Tuberous Sclerosis Complex by Interneuron Progenitor Transplantation
Log Number:	TS220011
Current PI Name:	Masaaki Torii
Award Number:	HT9425-23-1-0208
Current Contracting Organization:	Children's Research Institute at CNMC
Current Performing Organization:	Children's Research Institute at CNMC
Web Approval Date:	03-08-2023

This project will address the Focus Area: Preventing epilepsy, improving treatment, and mitigating neurodevelopmental outcomes associated with tuberous sclerosis complex (TSC)-related seizures, by providing key data on the efficacy of inhibitory cell transplantation-based therapy for intractable focal epilepsy associated with TSC. Since neural excitatory-inhibitory imbalance also underlies TSC-Associated Neuropsychiatric Disorders (TAND), this project may also address the Focus Area: Understanding and treating the features of TAND and reducing their impact.

More than 80% of patients with TSC exhibit epilepsy. For many of these patients, the only therapeutic option is surgical resection, but less than 50% are able to achieve proper postsurgery seizure management. Recent advances in TSC research have led to the promise of new drugs, but there are significant concerns and limitations in their clinical use, particularly in young patients. The goal of our research is to develop an effective alternative approach that is minimally invasive and offers better seizure control and postoperative morbidity. Toward this goal, in the proposed project, we will test out hypothesis that local transplantation of inhibitory neural cells targeted to the site of epileptogenesis will ameliorate seizure activities in TSC, using a unique mouse model that recapitulates the focal epilepsy in TSC.

Our proposed study is based on recent successes of interneuron progenitor transplantation by other groups to reduce epileptic activity in different types of epilepsy (i.e., temporal lobe epilepsy and generalized epilepsy) in animal models. A clinical trial has been started for the treatment of drug-resistant unilateral mesial temporal lobe epilepsy. We will test whether a similar approach can reduce highly refractory epilepsy with more focal epileptogenic sites, as seen in TSC. Considering the highly focal nature of epilepsy in TSC, we will also test the potential benefit of targeted transplantation using a micron-scale device, which our laboratory has recently developed, expecting that it will concentrate the inhibitory function of transplanted cells at the focal domain.

If these results prove that epilepsy is suppressed in mice with TSC, they are expected to eventually lead to clinical trials as a treatment for TSC patients with intractable focal epilepsy for which surgical resection is the only treatment option. The benefits of this approach would include potential permanent cure of epilepsy, improvement of otherwise intractable epilepsy, and provide better postsurgical control than surgical resection. Potential risks would include the possibility of tumorigenicity, host inflammatory response, and sensorimotor impairments. To avoid or reduce such risks, approaches such as the induced cell death method, which destroys the transplanted cells, have already been considered.

Given the successful initiation of a clinical trial for drug-resistant unilateral mesial temporal lobe epilepsy with a similar interneuron progenitor cell transplantation approach, we anticipate that the proposed study, if successful, will lead to clinical trials in 10 years. The results of this exploratory study are expected to provide essential data for further research on the application of cell transplantation-based therapies for intractable focal epilepsy associated with TSC.

Proposal Title:	Optimizing Therapeutic Control of Epilepsy in Tuberous Sclerosis Complex Using a Novel Biosensor
Log Number:	TS220017
Current PI Name:	Edward Chaum
Award Number:	HT9425-23-1-0410
Current Contracting Organization:	Vanderbilt University Medical Center
Current Performing Organization:	Vanderbilt University Medical Center
Web Approval Date:	05-04-2023

This proposal addresses the Fiscal Year 2022 (FY22) Tuberous Sclerosis Complex Research Program (TSCRP) Focus Area: Preventing epilepsy and improving treatment of tuberous sclerosis complex (TSC)-related seizures. Some of the most important health issues in patients with TSC are neurological disease: autism, intellectual disability, and seizures. More than half of all patients with TSC will develop uncontrolled seizures (epilepsy). The importance of seizure control in TSC cannot be overstated; chronic epilepsy in children is a major risk factor for impaired brain developmental and lifelong disability. The clinical decision-making when a patient is not responding to anticonvulsant medications is complex. To increase the dose to try to achieve a better effect may put the patient at risk for drug toxicity. Non-compliance may be unrecognized and to abandon a medication to begin a trial of a different drug may result in loss of seizure control. There is a significant, unmet clinical need to improve and optimize the dosing of anticonvulsant medications in patients with TSC.

Scientific Objective and Rationale: Biosensors act at the intersection between biology and electronics to convert chemical information into electronic signals. We have recently shown that we can accurately measure the level of many drugs used for the treatment of epilepsy directly from the blood using an innovative biosensor. Therapeutic drug monitoring is the clinical practice of measuring medication levels to optimize dosage regimens for patient benefit. It is used to monitor drugs with narrow therapeutic windows and potential toxicity and is based upon on a definable relationship between the concentration of the drug in the blood and its therapeutic effect. An office-based, non-invasive point-of-care medical device that measures the patient’s medication level, from a drop of saliva, during a clinic visit would have a significant impact on patient care to improve seizure control in patients with TSC. Current turnaround time to measure anticonvulsant levels by commercial labs is 2-4 days. The specific aims of this project are to (1) show that we can measure anticonvulsant and other TSC medication levels directly from blood and saliva in the lab and in a large animal model and that the measured levels are as accurate as current laboratory methods, (2) prototype a hand-held medical device to do this, and (3) show that the device can measure the anticonvulsant levels in patients from a drop of saliva taken during a clinic visit.

What types of patients will it help, and how will it help them? Sirolimus is a medication used in many patients to reduce tumor growth and to control epilepsy; however, its optimal dose is unknown. Similarly, recent studies have shown that cannabidiol [CBD] can reduce seizures in children with refractory epilepsy syndromes, and a new CBD drug (Epidiolex) is approved for this use in children. However, little is known about the metabolism of CBD or the optimal dosing of the drug to prevent seizures, and there is no commercial test to measure CBD in the blood. In fact, the therapeutic range for CBD is currently unknown.

Management of these patients is challenging and evolving. TSC-associated neuropsychiatric disorder (TAND) is a recent term that describes the clinical spectrum of brain dysfunction in patients with TSC including aggressive behavior, autism spectrum disorder, intellectual disabilities, and psychiatric disorders that require the use of additional medications and treatment strategies to. Current treatment for TAND is expanding to include anti-psychotics, tricyclic antidepressants, and other medications that can be measured

using the biosensor. An office-based platform that informs the clinician of the medication level at the time of neurologic and behavioral assessment would improve patient care for seizure control and TAND.

What are the potential clinical applications and benefits? The impact of this technology on patient care is significant. In the short term, real-time therapeutic drug monitoring in the clinic could be easily adopted and play an important role in optimizing treatment regimens to improve clinical management of these medications and reduce the risk of seizures. Long term, larger clinical trials that demonstrate improved control of epilepsy by routine drug monitoring in the clinic would lead to significant changes in clinical practice, not only for TSC but in the fields of neuropsychiatry and emergency medicine (e.g., drug overdose, poisoning). Sirolimus is used in clinical trials for cancer, transplantation, and Alzheimer's, but drug levels are not routinely obtained or known. The applicability of the biosensor is broad and can also be applied to inform and optimize responses in clinical trials for other diseases in addition to TSC.

What is the projected time to achieve a patient-related outcome? Completion of the specific aims of this project will yield a hand-held medical device that is shown to accurately measure CBD, anticonvulsant, and sirolimus levels in saliva within 2 years. The data obtained from the large animal and pilot human studies proposed will support an investigative new device application to the U.S. Food and Drug Administration (FDA) for the biosensor.

Proposal Title:	Estrogen Promotes Lymphangioleiomyomatosis (LAM) Indirectly Through Stimulation of Innate Immunity
Log Number:	TS220024
Current PI Name:	Stephen Hammes
Award Number:	HT9425-23-1-0281
Current Contracting Organization:	Rochester, University of
Current Performing Organization:	Rochester, University of
Web Approval Date:	04-02-2023

Lymphangioleiomyomatosis (LAM) is a rare lung disorder that consists of multiple “smooth muscle-like” tumors that progressively grow until they interfere with normal lung function. Many patients with LAM will have a lung collapse or develop fluid in their lungs, and some will require lung transplantation. Even after transplantation, many patients find that tumors return to the lungs. In addition to the suffering that accompanies the progressive suffocation due to the loss of functional lung, the ultimate fate for a large number of patients is death.

The goal of the research proposed here is to find better treatments for LAM. Importantly, LAM has some unusual properties that, if better understood, could be used against it when it comes to treatment. First, LAM is found almost exclusively in women, suggesting that the LAM tumors may originate from or be stimulated by factors found primarily in women. Second, LAM tumors develop after puberty, grow more during pregnancy or on birth control pills, and stabilize after menopause, suggesting that the female hormone estrogen might be driving LAM tumor growth. Third, LAM tumors contain mutations in one of the two tuberous sclerosis (TSC) genes, which are the genes that are altered in the disease “tuberous sclerosis.” Finally, LAM tumors re-appear in transplanted lungs, suggesting an origin of the LAM cell that is outside of the lungs.

Putting all of this information together, we proposed that LAM tumors may come from or behave like smooth muscle cells from the uterus, an estrogen sensitive organ found only in women. We shut off TSC2 expression only in the mouse uterus, and females developed LAM tumors in the uteri that metastasized to the lungs. Recent work looking specifically at genes expressed in individual LAM cells from human patients showed that in fact the LAM cells look just like uterine cells, confirming that LAM tumor cells might indeed originate from or share a similar origin as uterine cells.

Interestingly, reflecting what is seen in LAM patients, the LAM tumors in the aforementioned mouse model required estrogen for significant growth. However, uterine cells isolated from these TSC2 knockout mice, as well as other existing LAM cell lines, had little estrogen sensitivity in the lab. These observations suggest that estrogen may not be directly stimulating LAM tumors cells – in fact, estrogen may be stimulating other cells that in turn promote LAM progression. In support of this concept, we find that estrogen stimulates the production and actions of immune cells called neutrophils, which in turn release a molecule called neutrophil elastase that then promotes LAM tumor cell proliferation. In the project described here, we will study how this estrogen to neutrophil to LAM cell pathway functions, which will reveal novel new therapeutic targets for LAM, including potentially blockade of estrogen signaling or neutrophil actions. We have already discovered some possible novel treatments that have been tested in animal models (e.g., blockers of neutrophil elastase or of neutrophil migration into tumors) and expect more to come out of these studies.

We predict that within 3 years we will have some new treatment options that have been tested in our preclinical mouse models, and that within 5 years we will have novel therapies being tested in human patients with LAM.

Proposal Title:	Defining the Cell Cycle Phase-Specific Regulation and Function of mTORC1 to Identify New Therapeutic Targets in TSC Tumors
Log Number:	TS220030
Current PI Name:	Alexander Valvezan
Award Number:	HT9425-23-1-0288
Current Contracting Organization:	Rutgers, New Jersey, State University of
Current Performing Organization:	Rutgers, New Jersey, State University of
Web Approval Date:	04-27-2023

People with Tuberous Sclerosis Complex (TSC) are at risk of developing tumors in many organs, including the brain, heart, lungs, kidneys, and skin. The current standard-of-care drugs that are used to treat TSC tumors (rapamycin and its analogs) can halt or shrink tumors, but do not kill the tumor cells. Thus, tumors are not eliminated, and they can re-grow rapidly when the treatment is discontinued, underscoring the urgent need for new and improved therapies. This project will provide new understanding of the molecular events that occur in tumor cells in TSC and test a new strategy to selectively kill those cells, with the potential to eliminate tumors, thereby advancing the Fiscal Year 2022 Tuberous Sclerosis Complex Research Program Idea Development Award Focus Area of gaining a deeper mechanistic understanding of TSC signaling pathways and developing new strategies for eradicating TSC tumors.

At the molecular level, tumor cells in people with TSC typically contain genetic mutations that inactivate a critical group of proteins called the “TSC complex.” The TSC complex normally blocks the activity of another group of proteins called mTOR complex 1 (mTORC1), so mutations in TSC patients cause uncontrolled mTORC1 activation. Uncontrolled mTORC1 activation drives tumor growth in TSC and is blocked by rapamycin to halt tumor growth. Much effort has been focused on understanding the regulation and function of the TSC complex and mTORC1 to develop new treatments for TSC tumors, but many critical questions remain.

This project will study the TSC complex and mTORC1 in a way that has seldom been done in the field, but nevertheless is essential to understanding their regulation and function, as well as tumor growth, in TSC. All proliferating cells, including tumor cells in TSC, go through a “cell cycle” comprised of different phases in which cells accomplish different tasks so they can grow and divide into new cells. Most common experimental techniques combine thousands of cells from various phases of the cell cycle and therefore lose any information about what happens in specific cell cycle phases. For this reason, very little is known about how the TSC complex and mTORC1 differ from one cell cycle phase to another and whether they have functions that are uniquely essential only in specific phases. Discoveries of phase-specific functions could provide a wealth of new opportunities for targeting TSC tumors, so this represents a fundamental and highly significant gap in our understanding of the TSC complex, mTORC1, and Tuberous Sclerosis Complex. We hypothesize that the TSC complex and mTORC1 have differential functions throughout the cell cycle that could provide new therapeutic targets for treating TSC tumors.

This hypothesis is strongly supported by our extensive preliminary data, in which we studied the TSC complex and mTORC1 throughout the cell cycle and discovered two new molecular events that only occur in specific cell cycle phases. One of these events is an important modification of the TSC complex that only occurs when cells are dividing, strongly suggesting that it plays a critical, but currently unknown, role during that time. The other key discovery is a new role for mTORC1 in controlling cellular metabolism to support cell growth and proliferation, which provides a compelling new target for eliminating TSC tumors. In this project, we will rigorously and systematically determine how these events are controlled and what their functions are in order to determine whether they could be exploited to develop new therapies for TSC.

As proof of principle, our previous studies demonstrated that targeting metabolic pathways controlled by mTORC1 can induce potent and selective TSC tumor cell death, indicating that this strategy could have significant advantage over the current therapies. However, we have only scratched the surface with regards to identifying and exploiting targetable “metabolic vulnerabilities.” Thus, we will also use powerful genetic and biochemical techniques to rigorously determine whether blocking our newly discovered metabolic function of mTORC1 in multiple TSC tumor models can prevent tumor growth and kill tumor cells. We anticipate that this project will establish this new strategy for selectively killing TSC tumor cells while sparing normal cells. By making significant new inroads into the cell cycle phase-specific effects of the TSC /mTORC1 network and how they influence TSC tumor growth, this project will also fill critical gaps in our current knowledge and could bring cell cycle studies to the forefront of TSC research to open up numerous avenues for the development of new treatments.

Proposal Title:	Cardiac Rhabdomyomas as Biomarkers of TSC Disease Severity
Log Number:	TS220039
Current PI Name:	David Ritter
Award Number:	HT9425-23-1-0212
Current Contracting Organization:	Children's Hospital, Cincinnati
Current Performing Organization:	Children's Hospital, Cincinnati
Web Approval Date:	03-27-2023

Tuberous sclerosis complex (TSC) is a genetic disorder that leads to tumors throughout the body, seizures, autism, and intellectual disability, among other things. The combination of tumors and other findings are present in a patient is not able to be predicted with current knowledge. Currently, the first detectable tumor is a cardiac rhabdomyoma that can be seen on prenatal ultrasounds. Rarely these tumors can threaten the life of the fetus or newborn by causing heart failure or fatal arrhythmias. Few studies have tried to study rhabdomyomas in a way that determines the best imaging protocols for identifying and monitoring rhabdomyomas so that we know which babies need treatment and when to begin treatment before the baby is ultimately in trouble. Our prospective, patient-centered study addresses this critical gap in knowledge so that we might be able to use detection and characterization of cardiac rhabdomyomas, especially during the prenatal period before the baby is born, to identity who is at risk of cardiac complications or at risk for developing other major manifestations of TSC. Only then will we be positioned to use rhabdomyomas as an identifying feature for determining who might benefit from pre-emptive, protective treatments early in life, even prenatally.

Proposal Title:	Regulating Together in Tuberous Sclerosis Complex: A Pilot Feasibility Study in Children and Adolescents with TSC-Associated Neuropsychiatric Disorder (TAND)
Log Number:	TS220041
Current PI Name:	Jamie Capal
Award Number:	HT9425-23-1-0344
Current Contracting Organization:	North Carolina at Chapel Hill, University of
Current Performing Organization:	North Carolina at Chapel Hill, University of
Web Approval Date:	04-27-2023

Scientific Objective and Rationale: Approximately 90% of individuals with Tuberous Sclerosis Complex (TSC) are affected by difficulties in behavioral, psychiatric, intellectual, academic, neuropsychological, and psychosocial areas, collectively termed TSC-Associated Neuropsychiatric Disorder (TAND). Individuals with TSC exhibit higher rates of TAND-associated behavioral challenges throughout life compared to the general population, which results in significant caregiver burden and for which there are no specific treatments. Emotion dysregulation (ED), defined as a general deficit in one's ability to modulate the intensity or duration of emotional responses in an adaptable and contextually appropriate manner, is commonly associated with multiple psychiatric disorders and maladaptive behaviors seen in TSC. A treatment for ED has been developed called Regulating Together (RT), which is an evidence-based group treatment program, targeting both children and their caregivers, designed to improve ED regardless of underlying etiology and is effective, feasible, and accepted in children with features and behaviors similar to TAND. Our overall objective is to understand ED in children and adolescents with TSC and TAND-associated challenging behaviors. We first aim to characterize ED in children and adolescents with TSC and TAND. Our second aim is to pilot RT delivered remotely to school-aged children with TSC and TAND to determine if ED-specific behaviors improve after a 5-week group treatment intervention, followed by a 10-week period consisting of utilizing skills at home. Lastly, we aim to understand facilitators and obstacles to RT feasibility and implementation through caregiver interviews.

Project Impact: Short-term positive benefits for the participants and caregivers participating in the research groups include providing behavioral treatment for challenging behaviors in an easily accessible manner. Long-term benefits include using what we learn from the pilot study to inform larger treatment trials, which will facilitate future adoption and incorporation of RT into clinical practice for children with TSC and TAND and their caregivers.

Fiscal Year 2022 (FY22) Tuberous Sclerosis Complex Research Program (TSCRP) Clinical Translational Research Award Focus Area: The proposed research proposal aligns closely with FY22 TSCRP Clinical Translational Research Award Focus Area for understanding and treating the features of TAND and reducing their impact, including pharmacological, behavioral, and surgical interventions.

Proposal Title:	Role of the Secreted Factor CTHRC1 in the Pathogenesis of TSC
Log Number:	TS220049
Current PI Name:	Nicola Alesi
Award Number:	HT9425-23-1-0215
Current Contracting Organization:	Brigham and Women's Hospital, Inc.
Current Performing Organization:	Brigham and Women's Hospital, Inc.
Web Approval Date:	03-22-2023

This project is focused on a protein, CTHRC1 (collagen triple-helix repeat containing 1), that has never before been studied in tuberous sclerosis complex (TSC). In other diseases, CTHRC1 is linked to the rate of cellular growth, and in several types of cancer, a high level of CTHRC1 is associated with a poor clinical prognosis.

In a new line of investigation in our lab, we have discovered that levels of CTHRC1 are strikingly elevated in cells that lack the TSC proteins. At the messenger RNA level, CTHRC1 is elevated 15-fold in TSC1-deficient cells vs. controls, and in TSC2-deficient cells CTHRC1 is elevated 100-fold. These levels of elevation are remarkable – very few TSC-associated genes show levels of elevation in this range. Interestingly, in cells, these high levels of CTHRC1 are not suppressed by Rapamycin. Levels of CTHRC1 are also increased in human angiomyolipomas and in lymphangioleiomyomatosis (LAM) cells.

In TSC2-deficient cells, CTHRC1 is a major determinant of cellular growth. If we inhibit CTHRC1, the growth of the cells decreases about 2.5-fold in the conventional growth system (on plastic dishes) and decreases about 5-fold in a three-dimensional assay called “colony formation.”

These data suggest that CTHRC1 is a newly recognized and important driver of tumor cell growth in TSC. Because it is not affected by the mTOR inhibitor Rapamycin (which is similar to everolimus/Afinitor), CTHRC1 could help to explain why tumors in TSC are not eliminated during therapy with mTOR inhibitors.

The critical next step, and a major part of this proposal, is to test the importance of CTHRC1 in mouse models of TSC.

If CTHRC1 promotes the growth of tumors in TSC, this could pave the way to a completely new concept for treatment, perhaps combining drugs or antibodies that “target” CTHRC1 with mTORC1 inhibitors. This could also help us understand the fundamental reasons why mTORC1 inhibitors do not eliminate tumor cells in TSC.

Identifying therapeutic strategies to eliminate tumor cells in TSC, a key goal of this work, is a critical step toward a “cure” for TSC.

Proposal Title:	Wireless, Implantable Interface to Visual Cortex
Log Number:	VR220001
Current PI Name:	Kenneth Shepard
Award Number:	HT9425-23-1-0758
Current Contracting Organization:	Columbia University
Current Performing Organization:	Columbia University
Web Approval Date:	09-29-2023

Blindness is a devastating condition affecting millions of patients worldwide. Many of these patients have damage to the retina or early visual pathways but have an intact visual cortex. The key to improving the lives of these patients is restoring some visual perception with the action of a visual cortical prothesis (VCP), a device that is implanted directly in the visual cortex. The most significant limitations in the VCPs that have been introduced to date is the very limited number of electrodes in these devices. The most advanced device to date, the Orion, from Second Sight Medical Products, has only 64 electrodes. Members of our team are involved in the clinical trials of Orion; we are, therefore, very familiar with this state of the art. No system has more than 100 electrodes. While patients with these devices are able to perform some visually guided behaviors, the overall quality of visual perception created by these devices is quite poor.

Here, we are developing a device with three orders of magnitude higher electrode count and density, allowing a richness of stimulation patterns that has never before been possible. While we simply do not know the quality of visual perception that may be possible here, the expectation is that with this dramatic increase in the number of electrodes, this should be considerably enhanced. While the number of electrodes on a single chip increases to more than 65k, the implant displaces a volume of only 3 mm^3, allowing the device to sit entirely in the subdural space with wireless powering and communication. This means that the implantation of the device can be made with only a small "slit" in the cranium that quickly heals, making the insertion of the device much less invasive that than of other implants, which require removal of large regions of the skull or leave the patient with wires and leads from the electrodes to large implanted electronics. This is made possible by taking advantage of the latest advances in complementary metal-oxide-semiconductor (CMOS) electronics, the same technology that has fueled the revolution of computing and communication technology. For 100 electrodes, the Orion device, in contrast displaces a volume almost three orders of magnitude more than this.

In this project, we will prove the safety and efficacy of the device with a series of experiments in pigs with simulation and recording from V1. Information for recording with known visual stimuli will be used to inform the requisite stimulation patterns. We will also perform basic biocompatibility and safety studies required to get approval by the FDA to perform the first clinical trial in humans.

Proposal Title:	Sustained Therapeutic Protein Cocktail Delivery to Prevent Vision Loss After Ocular Trauma
Log Number:	VR220024
Current PI Name:	Katelyn Swindle-Reilly
Award Number:	HT9425-23-1-0782
Current Contracting Organization:	Ohio State University, The
Current Performing Organization:	Ohio State University, The
Web Approval Date:	09-29-2023

The rationale for the proposed studies is that controlled release of anti-inflammatory therapeutics coupled with an injectable, biodegradable drug delivery system will significantly improve visual outcomes after traumatic ocular injury. A novel combination of therapeutics, with evidence of trauma mitigation in other systems, will be investigated for application in ocular models of retinal detachment, proliferative vitreoretinopathy, and traumatic optic neuropathy. The therapeutic cocktail contains haptoglobin, hemopexin, and transferrin, which have potential to reduce inflammation and cell death at the site of injury. The responsive nanoparticle delivery system further has the potential to scavenge stressors and release more therapeutic at injured sites.

The objectives of this study are to evaluate the capability of the therapeutic loaded nanoparticles to (1) scavenge high levels of oxidative stressors after trauma, (2) controllably release novel anti-inflammatory therapeutics, and (3) extend therapeutic duration and release.

The three aims are: (1) Quantify therapeutic release and efficacy in vitro and in vivo, (2) Determine treatment safety and efficacy in an in vivo model of retinal detachment and proliferative vitreoretinopathy, (3) Determine treatment safety and efficacy in an in vivo model of torsionally induced traumatic optic neuropathy.

A new treatment strategy will be developed to provide sustained release of anti-inflammatory therapeutics for vision restoration and/or maintenance after traumatic ocular injury. In the short term, this study will measure how the therapeutic cocktail impacts inflammation and visual outcomes after ocular injury. Identification of a way to prevent complications associated with retinal detachment and optic neuropathy has the potential to significantly improve long-term visual outcomes and quality of life for injured civilians, Warfighters, and Veterans. Localized suppression of inflammation and oxidative stress by the therapeutics and delivery system tested in these studies will demonstrate safety and efficacy sufficient to warrant continued investigation in preclinical and then clinical trials for treating ocular injuries. Any long-term impacts will also translate directly to visual system care in the civilian sector. The proposed research also has the potential to inform treatment strategies for other vision-threatening diseases in which inflammation and oxidative stress are implicated.

Proposal Title:	RGC Transplantation as a Treatment for TBI-Related Optic Nerve Injury
Log Number:	VR220053
Current PI Name:	Donald Zack
Award Number:	HT9425-23-1-0589
Current Contracting Organization:	Johns Hopkins University
Current Performing Organization:	Johns Hopkins University
Web Approval Date:	09-13-2023

Ocular injuries account for up to 13% of all injuries sustained by Soldiers in present-day warfare. These injuries cost the United States economy $25 billion in health care, work lost, and family support. More importantly, these injuries impact the vision and quality of life of Soldiers, Veterans, and their Families. Blast injury can cause damage to the optic nerve, the "telephone cable" that carries visual information from the eye to the brain. The nerve cells that carry information in the optic nerve are called retinal ganglion cells (RGCs). Blast-induced injury to the optic nerve and resulting death of RGCs can cause vision loss. Since the eye does not have the ability to regenerate or significantly replace lost RGCs, restoring lost vision to our Soldiers and Veterans suffering from optic nerve damage is, unfortunately, not possible. However, there is increasing hope that replacing such lost vision may be possible in the foreseeable future. Research strategies that are being developed include prosthetic retinal implants, reactivation of intrinsic regeneration mechanisms, whole eye replacement, and RGC transplantation for optic nerve regeneration. The latter approach, optic nerve regeneration, has become closer to a feasible reality in recent years due to developments in axon regeneration and stem cell biology, with new techniques permitting the differentiation of human pluripotent stem cells into RGCs for cell replacement therapy.

Cell replacement in the retina holds great promise for restoring lost sight. In an attempt to restore RGCs and visual function lost secondary to optic nerve injury and disease, we and others have been working to replace lost cells by transplantation of stem cell-derived RGCs. Our ongoing experiments have shown partial survival of grafted cells in healthy and damaged retinas following transplantation of RGCs in animal models. However, as promising as these studies are, there are still major challenges that need to be addressed. Among the challenges are that transplanted RGCs often do not survive well in their new hosts, and the cells that do survive the initial transplantation tend to die over time. An additional and deeper challenge is that we need to develop better methods to help the fibers growing from the RGCs (which are called axons) find their way to the correct sites in the brain. And once the axons arrive in the brain, we need to find ways to help them form functional connections (which are called synapses) to the right cells in the brain.

In the work described in this application we propose to employ a collaborative team-based approach with investigators experienced in the study of optic nerve injury and regeneration to simultaneously address these and other challenges in our continuing efforts to help patients regain lost vision by means of RGC transplantation-based optic nerve regeneration. In the proposed studies we will optimize the generation of RGCs from human stem cells, explore the role of intrinsic and extrinsic factors in RGC survival and integration, develop improved methods to get transplanted cells integrated into the retina, explore how microenvironmental factors influence RGC survival, and test these improvements using mouse and large animal (pig) models of traumatic optic neuropathy. These complementary efforts, taken together, will, hopefully, help advance the field and get us closer to the point where the dream of restoration of lost vision by optic nerve regeneration transitions from a dream to a reality.

Proposal Title:	The Role of the Rho-Kinase Pathway in Proliferative Vitreoretinopathy
Log Number:	VR220059
Current PI Name:	Leo Kim
Award Number:	HT9425-23-1-0527
Current Contracting Organization:	Schepens Eye Research Institute
Current Performing Organization:	Schepens Eye Research Institute
Web Approval Date:	09-03-2023

The overarching goal of this proposal is to address the lack of effective treatment for proliferative vitreoretinopathy (PVR), a primary cause of severe and irreversible visual loss in ocular trauma patients. The incidence of blindness from PVR is especially high among active Service Members due to the combat-related risks of exposure to explosive devices, blast, and projectiles.

PVR occurs when breaks and tears in the retina, caused by trauma-induced retinal detachment and perforating injuries, disrupt the retinal pigment epithelial cells (RPE) and neighboring cells, and place them in direct contact with the intraocular vitreous fluid. To date, the only treatment for PVR is the surgical removal of the membranes and reattachment of the retina. However, success of corrective surgery for PVR is low as invasive treatment and surgical peeling of the membranes further damage the retina promoting recurrent PVR and low visual outcomes. The development of safe and efficient adjunctive medical treatments for PVR is therefore critical to reduce the prevalence of blindness following ocular injuries. Targeting the key cellular processes involved in the activation of cells involved in the formation of PVR is a promising strategy for the treatment of PVR.

The Kim laboratory has identified that PVR membrane formation is associated with the activation of the Rho-Kinase pathway. Using a model of PVR, we found that cells derived from human PVR membrane samples show a higher activation of Rho-Kinase in our PVR model. We found that Rho-Kinase inhibitors have therapeutic potential in blocking the contractile and migratory properties of PVR cells. Importantly, the Rho-Kinase inhibitor, netarsudil, is already commercially available for glaucoma and could be repurposed and readily deployed for PVR treatment.

To ensure the success of this project, we have assembled a team of scientists and clinicians who are experts in the molecular mechanisms of retinal injury as well as experimental and clinical PVR. Completion of this proposal has strong potential to determine the efficacy of Rho-Kinase inhibitors to combat PVR and identify novel therapeutic targets, surrogate biomarkers, and strategies for efficient prevention or treatment of PVR.

We expect that these findings will provide the necessary validation for the subsequent evaluation of Rho-Kinase inhibitors for clinical applications and have already established collaborations with clinicians to facilitate translational development.

Our proposed work will have an important beneficial impact on active Service Members, Veterans, and civilians by reducing the risk of vision loss from ocular trauma thereby improving their quality of life and well-being.

Proposal Title:	Rapid Delivery of SIRT1-Mediated Neuroprotective Treatment for Traumatic Optic Neuropathy
Log Number:	VR220086
Current PI Name:	Kenneth Shindler
Award Number:	HT9425-23-1-0725
Current Contracting Organization:	Pennsylvania, University of
Current Performing Organization:	Pennsylvania, University of
Web Approval Date:	09-26-2023

The main objective of this proposal is to evaluate the therapeutic potential and mechanisms of a novel therapy to prevent nerve damage in animal models of traumatic optic neuropathy (TON). TON can result from direct optic nerve injury, but more commonly occurs indirectly due to optic nerve stretching from blunt head trauma, in patients with traumatic brain injury. TON results in damage to the retinal ganglion cells (RGCs), the nerve cells that comprise the optic nerve and bring visual signals from the eye to the brain, with associated permanent visual deficits. Attempted medical and surgical treatments have all failed to improve visual outcomes. Thus, novel neuroprotective therapies are needed to prevent RGC loss and preserve vision following TON, and a potential therapy that can be deployed rapidly in the field will be studied. Previous studies showed that activation of the SIRT1 deacetylase by the polyphenol resveratrol, as well as genetic overexpression of SIRT1, reduces RGC loss in experimental optic neuropathies. However, high oral resveratrol doses that increase risk of side effects are required; thus, alternate strategies to upregulate SIRT1 are needed. In addition, mechanisms underlying SIRT1-mediated neuroprotection are not well understood, including whether SIRT1 signaling is required directly within RGCs, and which downstream signals play key roles. Development of novel experimental tools to deliver the SIRT1 gene, and innovative use of intranasal drug administration to target RGCs, place us in a unique position to address both the therapeutic potential of SIRT1-mediated RGC neuroprotection, and mechanisms underlying these effects.

TON often occurs in patients with traumatic brain injury, a frequent sequela of blunt head trauma or blast-related injuries. Sixty percent of traumatic head injuries result in neuro-ophthalmic abnormalities, and 50% of those involve the optic nerves or visual pathways. TON was among the most common ocular injuries requiring specialized ophthalmic care during U.S. operations in Iraq, as nearly 20% of ocular combat injuries involved TON. The prevalence of these injuries, together with lack of available treatments, suggests TON is a devastating cause of vision loss in military and civilian settings. One major impediment making it difficult to develop treatments for TON is that trauma-induced damage to the optic nerve occurs at the time of injury, with a limited window of time available to deliver therapies prior to the damage becoming permanent. It is likely that any successful therapy will need to be delivered shortly after trauma and, ideally, would be available to be administered by first responders/medics during the initial encounter at the site of the trauma. Having an easily administered, rapid-acting therapeutic available for medics to use in the field during military operations or first responders to use in civilian settings, will represent a critical advancement in development of neuroprotective therapies for TON.

Outcomes of the current proposal can directly affect these critical needs. Short-term outcomes will demonstrate key molecular mechanisms underlying the ability of SIRT1 to prevent RGC damage following trauma, and will confirm the ability of intranasal administration of a SIRT1 activating compound, resveratrol, to prevent RGC loss and preserve vision. Long-term outcomes will build off the short-term outcomes through the development of enhanced therapeutics targeting downstream signals of SIRT1, and through translational studies that will assess feasibility and efficacy of intranasal dosing of resveratrol in TON patients. Due to significant anatomic differences between mice and humans, following proof-of-concept intranasal delivery studies in mice in the current proposal, evaluation of doses and intranasal

delivery methods for human use will need to be performed. Importantly, even if anatomical differences prove too significant to allow direct translation and use of intranasal drug delivery as a feasible delivery method of resveratrol for human TON, successful data generated in this proposal would suggest that the ability to deliver resveratrol to ocular tissues is indeed a potent neuroprotective strategy, and would support future studies of alternate methods to delivery therapy to specific cell targets in the human eye or optic nerve.

Finally, the current studies will also open important new avenues of research that hold tremendous promise for addressing other key health issues faced by military, Veteran, and other civilian populations. Gene delivery methods will be used extensively in the current proposal to investigate critical mechanisms underlying SIRT1-mediated RGC neuroprotection. It is not expected that gene delivery will increase SIRT1 expression quickly enough to serve as a viable treatment for TON and thus it is used mainly as an experimental tool in the current proposal. However, gene delivery can serve as an important therapeutic approach for more chronic diseases, and the focus here on modulating a conserved pathway involved in RGC damage suggests that findings may be applicable to other optic neuropathies, including glaucoma. Indeed, in preliminary studies (publication pending) we found similar RGC neuroprotective effects of SIRT1 gene therapy in a mouse model of glaucoma. Glaucoma affects approximately 6% of the population and is a frequent cause of vision loss in both civilian and Veterans Administration eye clinics. Successful results of the current proposal may provide logical mechanisms to explore in future investigations of the role of SIRT1 gene therapy in treatment of glaucoma.

Overall, this proposal is poised to make a significant impact on the development of a potential new therapeutic strategy for neuroprotection in TON and advance our understanding of this therapy. Demonstrating an ability of SIRT1 gene therapy to prevent RGC damage in experimental TON models will reveal the potential role of this therapeutic strategy to fulfill unmet needs in the treatment of TON patients.

Proposal Title:	Transitioning the Minimally Invasive Artificial Cornea (mi-KPro) from Bench to Bedside: A Safer Alternative for the Treatment of World's Corneal Blindness
Log Number:	VR220088
Current PI Name:	Eleftherios Paschalis Ilios
Award Number:	HT9425-23-1-0906
Current Contracting Organization:	Massachusetts Eye and Ear Infirmary
Current Performing Organization:	Massachusetts Eye and Ear Infirmary
Web Approval Date:	09-29-2023

Severe ocular injuries, as frequently encountered in the military, can lead to blindness even if promptly treated. Excessive scarring, abnormal blood vessel formation (neovascularization), and loss of limbal stem cells significantly reduce that success of standard transplantation surgery. In these patients, vision restoration can be achieved only by using an artificial cornea, typically made of plastic and titanium, positioned in the front part of the eye as a window. Indeed, 20,000 patients have already benefited from such artificial corneas, which are developed in our laboratory and have been approved by the U.S. Food and Drug Administration (FDA) and the European Union. The Boston Keratoprosthesis artificial cornea is a collar button window that is implanted surgically in the eye and functions as a regular human cornea. Although short- and mid-term outcomes are excellent, in terms of device retention and vision restoration, long-term complications often compromise vision. Two major post-operative complications often encountered clinically are glaucoma and the intraocular infection, both attributed to the penetrating nature of the device. These complications, however, can be eliminated or minimized by redesigning a less invasive artificial cornea.

To this end, we developed a minimally invasive artificial cornea, the mi-KPro, that is implanted within the corneal tissue with a non-penetrating surgical procedure. The optical cylinder is then inserted inside the eye through a very small incision, while the remaining device is placed on the surface of the eye. This results in minimal post-operative complications, while providing excellent vision restoration, unobscured from corneal opacities. Animal studies with the mi-KPro have shown that it is well retained in the eye for more than a year, can replace the injured cornea after chemical burn, and most importantly, does not induce glaucoma nor infection even without providing any eyedrops medication for a year. The encouraging preclinical results provides groundwork to transition to a pilot human study.

Successful results from a pilot study in humans would help to treat Service Members that incurred severe ocular injuries, but also to treat existing traumas in Veterans that have resulted in corneal blindness. We also expect significant public benefit given that 1.5 - 2 million patients become corneal blind every year, and many of them require artificial cornea to restore their vision. Although 1,500 artificial corneas are implanted every year, this number is expected to increase significantly if we develop an artificial cornea with low post-operative complications.

We believe that this is an important project for the military and public. According to recent reports, the number of eye trauma between 2000 and 2010 among active military members was approximately 186,555 (ambulatory and hospitalized cases) with an annual cost of $382,905,615 due to blindness. The projected lifetime costs to military members with eye injuries or vision impairment is estimated to be $24 billion. Corneal blindness is a leading cause of blindness with 1.4 million blind people per year in the general population. In recent wars, civilian injuries have shifted from bullets to burns, with one out of four burn

injuries caused by explosives. Likewise, during peace-time, industrial accidents are responsible for two-thirds of civilian burns. Ocular burns comprise about 7%-18% of all ocular traumas presented to the emergency room in the United States with 84% of them being chemical burns. These numbers depict the impact of ocular trauma to the military and to the public. The proposed artificial cornea may significantly improve the outcomes of severe ocular traumas and reduce complications, which together will vastly bring down the medical cost to the military and the public. Both the military and civilian patients will benefit from the mi-KPro, due to the ease of implantation, its long-term safety and efficacy, and the simple post-operative management.

During the 3 years of this study, we expect to obtain safety and feasibility data to transition to phase 2 and 3 clinical trials. Our team is composed of dedicated physicians engaged in artificial corneal surgery, and our laboratory, which manufactures the only two FDA-approved artificial cornea, is equipped with all necessary equipment, clean rooms, and expertise to materialize this project. Out team of basic scientists and physicians at Mass Eye and Ear share the same goal, to provide a solution to an unmet clinical need and reduce corneal blindness. This study aims to provide a safer and effective therapy for treating corneal blindness in military and public hospitals.

Proposal Title:	Multifunctional Bandage Lens and Bioadhesive for the Treatment of Corneal Wounds
Log Number:	VR220100
Current PI Name:	Tannin Schmidt
Award Number:	HT9425-23-1-0566
Current Contracting Organization:	UConn Health
Current Performing Organization:	UConn Health
Web Approval Date:	09-13-2023

Rationale: Damage to the eye occurs in a large number of military-related injuries, with >275,000 eye injuries in the U.S. armed services from 2000 to 2017, which are often associated with loss or diminished sight. How quickly these injuries are repaired are important factors that affect long-term eyesight. We propose below a specialized adhesive bandage lens that can reconstruct the eye contour after an injury and minimize inflammation, adhesions, and infection. Therefore, this research directly addresses the FY22 Vision Research Program Focus Areas "Eye injury …as related to military exposure, including blast, … thermal, or chemical trauma" as well as "treatment of eye injuries in prolonged field care settings."

Our proposed research is advancing more mature research, based on our experience and expertise with proteoglycan 4 (PRG4), gelatin methacryloyl (GelMA), and ophthalmic biomaterials. We co-discovered PRG4 on the ocular surface and demonstrated it has lubricating function there and on ophthalmic biomaterials. PRG4 has anti-inflammatory and anti-fibrotic properties; we showed recombinant human PRG4 (rhPRG4) dampens inflammation-induced cytokine expression in corneal epithelial cells. Finally, we showed rhPRG4 contributes to tissue regeneration and wound healing. We now have strong preliminary data showing GelMA, which has previously been studied for the treatment of corneal injuries, effectively delivers rhPRG4 and antibiotics. However, GelMA use for corneal wounds in emergent settings has been limited by imprecise eye contour recreation. We propose to overcome this by in situ crosslinking of GelMA under a bandage lens, leveraging the multifunctional PRG4 gradually released under the bandage lens to promote healing, and by slowly releasing antibiotics to prevent infection. Our novel lens can be used by first responders in prolonged field care settings.

Objective: The objective of this work is to develop a bandage lens lubricated by rhPRG4 together with rhPRG4- and antibiotic-loaded GelMA that helps the proper filling of corneal defects followed by GelMA in place crosslinking with light. We believe that the rhPRG4-lubricated lens together with rhPRG4- and antibiotic-loaded GelMA, that is crosslinked directly on the eye, will lead to rapid closure and prevent adhesions, infection, and scarring, enhancing corneal wound healing. This will be tested by the following Aims:

Aim 1: Assess and optimize the lubricating properties of rhPRG4 on bandage lenses, the physical/adhesive properties of GelMA, and rhPRG4 and antibiotic release kinetics from GelMA.

Aim 2: Confirm the activity of rhPRG4 and antibiotic eluted from bandage lens and GelMA, respectively, as assessed by modulating inflammation and scarring, as well as preventing infection in cells.

Aim 3: Evaluate the ability of the rhPRG4-lubricated bandage lens together with rhPRG4- and gentamicin-loaded GelMA adhesive, crosslinked in place, in rabbits to modulate inflammation, prevent scaring and infection, and improve corneal wound healing.

Impact: The expected short-term impact of this work on the field of eye trauma care is data supporting a new rhPRG4-lubricated bandage lens combined with properly contoured rhPRG4- and antibody-loaded adhesive GelMA to treat corneal wounds. This has the potential to open up and help create new avenues of research in the context of PRG4 being used together with GelMA to treat other types of wounds. The potential long-term impact of this work lies in paving the way toward testing this specialized lens in people, thus translating a new, rapidly deployable treatment tool for cornea wounds in field settings. Given that our rhPRG4 has been successfully tested clinically for treating dry eyes in people, and has been shown to be both safe and effective, the potential for faster clinical translation, i.e., getting our new lens into the clinic to help people, is significant.

Proposal Title:	Understanding and Treating TBI-Associated Photophobia with Botulinum Toxin Type A and Its Impact on Visual Function
Log Number:	VR220131
Current PI Name:	Anat Galor
Award Number:	HT9425-23-1-0608
Current Contracting Organization:	Miami, University of, Coral Gables
Current Performing Organization:	Miami, University of, Coral Gables
Web Approval Date:	09-15-2023

It is estimated that up to 19% of U.S. Veterans may have traumatic brain injury (TBI), with over 260,000 Operation Iraqi Freedom/Operation Enduring Freedom Veterans diagnosed with the condition. TBI can impart devastating lifelong consequences. Photophobia, or extreme sensitivity to light, is one of the most distressing as its occurrence results in high morbidity without effective treatment. Individuals most severely affected become prisoners in their own homes due to intolerance to even small amounts of light. Even in less severe cases, most individuals have significant functional limitations and often require sunglasses indoors and outdoors. There are few therapeutic options for individuals with photophobia, beyond the use of tinted lenses. We have focused our investigation on understanding and treating TBI-associated photophobia and its impact on visual function. Specifically, our research topic includes studying whether Botulinum Toxin Type A (BoNT-A) will promote sustained functional recovery during the chronic phase of injury. Given our interest in photophobia, we have selected the fiscal year 2022 VRP Investigator-Initiated Research Award Focus Area to investigate the pathobiology underlying TBI-associated visual dysfunction and its treatment.

Proposal Title:	Artificial Cornea and Sensor-Based Continuous IOP Monitoring System to Treat Patients with Cornea Blindness Secondary to War-Related Ocular Injuries
Log Number:	VR220135
Current PI Name:	Ramesh Ayyala
Award Number:	HT9425-23-1-0502
Current Contracting Organization:	South Florida, University of
Current Performing Organization:	South Florida, University of
Web Approval Date:	09-03-2023

Eyes are very susceptible to blast injuries and blunt trauma. In both situations, the front lens of the eye, called the cornea, along with the white part of the eye and the drainage pathways of the eye can be damaged resulting in loss of vision from corneal scar formation (corneal blindness) and blockage of drainage pathways resulting in elevated eye pressure (glaucoma, which can lead to blindness from irreversible damage to the connection between the eye and the brain, i.e., the optic nerve). Active-duty Service Members are exposed to eye trauma from blast injuries and chemical burns that can leave them with permanent loss of vision from corneal scars and glaucoma. Routine corneal transplant surgeries using eye bank tissue has a high risk of failure in this set-up. Artificial cornea (or keratoprosthesis) may be the only answer to restore vision in this patient population. The current U.S. Food and Drug Administration-approved artificial cornea models (e.g., Boston KPRO) were designed 30 years ago and are successful in fewer than 50% of the cases. Two of the main issues that complicate the current devices are the inability to monitor eye pressure (leading to blindness from undetected glaucoma) and infections from the lack of biocompatibility and wound healing (mostly from the rigidity and hydrophobic nature of the biomaterial used). Also, Boston KPRO requires assembly along with eye bank cornea tissue at the time of surgery.

The objective of our proposal is to resolve these two issues using modern polymer technology. Our aims are to (1) Create a one-piece flexible artificial cornea that is hydrophilic and biocompatible to facilitate better wound healing using modern polymer technology and without the need for eye bank corneal tissue, and (2) Build a membrane-based implantable sensor to self-monitor the intraocular pressure by the patient.

If successful, the implantable sensor will be the first of its kind and has the potential to change the way we approach glaucoma management, not only in these difficult cases but also in routine glaucoma patients. Continuous intraocular pressure measurement of the human eye using an implantable device inside the eye will open doors to better understanding of the pressure fluctuations and will establish the accuracy of the current technology that measures the eye pressure externally. It will also empower patients to monitor their own eye pressure at home, thus decreasing the number of visits to doctors' offices.

The proposed design modification will decrease the chance of extrusion and promote wound healing. The use of modern technology to resurface the artificial cornea to make it more hydrophilic will mean that the proposed keratoprosthesis will be coated by patients' tear film leading to improved tolerance. This technique, which was developed in our lab, can be applied to modify the surface of other types of prostheses that are currently used in the human body. Since the prototype versions of these devices are already made in our lab, we anticipate clinical translation within a 5-year period.