The Gulf War Illness Landscape

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I. Gulf War Illness Primary Features and Prevalence

I.1. Symptoms of Gulf War Illness

Within a short time after the 1990-1991 Gulf War (GW), some Veterans who served in and around the Southwest Asia theater of operations developed enduring, chronic health conditions and constellations of symptoms and illnesses that could not be explained by established medical or psychiatric diagnoses or standard laboratory tests.

Symptoms experienced and reported by GW Veterans vary widely. However, the reported symptoms are similar clinically and usually include combinations of widespread pain, muscle aches, headache, persistent cognitive issues, fatigue, respiratory disorders, stomach and intestinal symptoms and skin abnormalities. In addition to the physical symptoms, changes in behavior and mood and problems with interpersonal relationships are reported.

Initially, this constellation of disorders was referred to as "Gulf War Syndrome." Other names given to these problems included chronic multi-symptom illness (CMI), undiagnosed illness, Gulf War illness (GWI), and other terms. Currently, "Gulf War illness" is the term recommended by the National Academy of Medicine and is used by scientists, clinicians, Veterans organizations, and the U.S. Department of Defense (DOD).

I.2. Prevalence

GWI is estimated to affect 175,000 to 250,000 of the nearly 700,000 troops deployed to the 1990-1991 GW theater of operations. Twenty-seven of the 28 Coalition country members participating in the GW conflict have reported GWI in their troops. Epidemiologic studies indicate that rates of GWI vary in different subgroups of GW Veterans. GWI affects Veterans who served in the U.S. Army and Marines Corps at higher rates than those who served in the Navy and Air Force, and U.S. enlisted personnel are affected more than officers. Studies also indicate that GWI rates differ by where Veterans were located during deployment, with the highest rates among troops who served in areas in proximity to combat.

II. Risk Factors

II.1. GW Exposures

During the GW, Service Members were exposed to low levels of chemicals, including chemical warfare agents released by the destruction of Iraqi munitions and storage facilities, widespread spraying and personal use of pesticides, prophylactic medications to protect against hazardous exposures, constant dust and sandstorms, and effluent from oil well fires ignited by Iraqi troops.

Cholinergic agents represent the class of compounds with the broadest exposures experienced by Service Members deployed to the GW. Of these, organophosphates, including the chemical warfare agents sarin, cyclosarin, soman, and the pesticides permethrin (PER) and chlorpyrifos (CPF), have received considerable attention. Other cholinergic agents to which GW Veterans were exposed include pyridostigmine bromide (PB) pills, which were given as a prophylaxis against nerve agents, and the insect repellant N,N-diethyl-meta-toluamide (DEET). Virtually all

deployed troops were exposed to the pesticide PER, which was used on clothing to kill insects, the area pesticide CPF, which was used in no-pest strips in mess and residential areas, and the insect repellant DEET, which was applied directly to skin. Many troops were given PB pills regularly in anticipation of a nerve agent attack, and many troops were likely exposed to vapor plumes resulting from destruction of chemical weapons, including sarin, cyclosarin, and possibly mustard gas and soman.

Exposures to other agents that may be related to development of GWI include airborne particulates and emissions from Kuwaiti oil well fires, desert dust, multiple vaccinations (including anthrax vaccination), depleted uranium (DU), chemical agent-resistant coating (CARC) paint, psychological and physiological stress, heat, and miscellaneous petroleum products such as cleaners, lubricants, and fuels. It is generally assumed that individuals meeting the criteria for GWI were likely exposed to multiple agents and stressors.

II.2. Other Etiologic Considerations

Uncertainties regarding types and doses of chemical exposures, as well as a lack of scientific knowledge about the synergistic effects of combined agent exposures, have impeded the development of a consistent theory of GWI etiology. Genetics, epigenetics, and gene-environment interactions are being investigated for potentially contributing to GWI.

Multiple studies have examined the role of Paraoxonase 1 (PON1), an enzyme capable of hydrolyzing several organophosphates, a number of which are believed to be contributors to GWI. A polymorphism in codon 192 of the PON1 gene (PON1-Q192R) determines which form of the enzyme (glutamine [Q] or arginine [R]) is produced and impacts enzymatic activity. The R allele enzyme metabolizes chemical weapon agents like sarin and the Q allele enzyme metabolizes insecticides (reviewed in Haley, 1999). The combination of the PON1-192R genotype coupled with hearing chemical alarms (a proxy for sarin exposure) was recently associated with GW Veterans having GWI, providing strong evidence for an etiologic role of low-level nerve agent in GWI (Haley et al., 2022). The combination of certain less common genotypes of the enzyme butyrylcholinesterase (BChE), another gene involved in organophosphate detoxification, with PB pill use (common during the GW) has been shown to confer greater risk for developing GWI (Steele et al., 2015). Studies are underway to assess DNA damage from exposures present in the GW theater by measuring somatic mutation frequency, overall genome instability and chronic alterations in global DNA methylation.

Traumatic brain injury (TBI) was not initially considered to be common in the 1990-1991 GW. Therefore, the relationship between TBI and chronic health symptoms experienced by GW Veterans has only recently been undertaken by the GWI research community. GW Veterans' self-reported experience of TBI has been shown to be related to increased rates of chronic health symptoms and CMI (Yee et al., 2016 and 2017). Preclinical research studies funded by the Gulf War Illness Research Program (GWIRP) are now investigating the influence of TBI on outcomes following exposure to GW agents. Clinical studies have included known TBI injury in their exposure surveys. Steele et al. reported that experiencing mild traumatic brain injury during deployment was one of only two deployment experiences/exposures reported by Veterans where statistical analysis found a significant risk factor for GWI (Steele et al., 2021).

III. Pathobiology of Gulf War Illness

GWI and its associated symptoms impact a number of physiological systems. Because exposures to various neurotoxicants were known to occur during the GW and many of the symptoms of GWI clearly relate to nervous system dysfunction, a considerable portion of GWI research has focused on nervous system pathobiology. Other physiological systems that have been and are actively being investigated include the immune/inflammatory and gastrointestinal (GI) systems and respiratory system. Other efforts include more molecular-focused studies on respiration, management of oxidative potential, and the gut-brain axis, which has emerged as a potential source of GWI pathology. Studies that have included female GW Veterans suggest that sex differences may play a role in the underlying pathobiology of GWI, even suggesting that GWI is more severely experienced by women (Shastry et al., 2022).

III.1. Preclinical Investigations

Preclinical models of human physiology are a primary component of the research landscape. Preclinical research allows us to learn about pathobiology and test potential treatments without applying dangerous or invasive methods on living humans. The most basic form of preclinical research is on cultured cells in vitro. An advanced example of this is human induced pluripotent stem cell cultures (hiPSCs). GWIRP-funded investigators have generated hiPSCs from skin fibroblast cells and peripheral blood mononuclear cells from deployed GW Veterans who have GWI symptoms, as well as those who did not develop GWI. These cells are being made available to the research community with the intent to foster rapid-throughput studies of novel therapeutic approaches (Qiang et al., 2017). HiPSCs derived from Veterans with or without GWI were differentiated into forebrain glutamatergic neurons and then exposed to a GW-relevant toxicant regimen consisting of DFP and cortisol, a human stress hormone. Elevated levels of total and phosphorylated tau, reduced microtubule acetylation, altered mitochondrial dynamics/transport, and decreased neuronal activity were observed in neurons exposed to the toxicant regimen (Yates et al., 2021).

Several animal models have been developed to elucidate possible molecular and physiological mechanisms underlying GWI. These models have been used to characterize molecular, cellular, and functional effects associated with chemical exposures similar to those encountered by Veterans during the GW. These animal studies have provided evidence on brain, autonomic, behavioral, neuroendocrine, immune, and epigenetic abnormalities and support the conclusion that relevant chemical and non-chemical (stress induction, heat) exposures are associated with the physiological and behavioral characteristics of GWI in Veterans.

White et al. summarized several rat and mouse studies evaluating the effects of exposures including combinations of PB, PER, CPF, sarin, diisopropyl fluorophosphates (DFP, a sarin surrogate), and stress. In many cases, exposures were administered at dosage levels that do not produce overt symptoms of toxicity (White et al., 2016). Beginning with the work of Abou-Donia and colleagues with a rat model of PB, DEET, and CPF exposures (Abou-Donia et al., 1996), these models recapitulate some features of GWI patients, including cognitive dysfunction and immune and inflammatory disruption. Furthermore, these preclinical studies have shown that combinations of chemicals impact absorption, metabolism, and biological pathways differently when compared to single-exposure studies (RAC-GWVI Scientific Findings and Recommendations, 2008). Importantly, preclinical studies are now directed toward investigating

the chronic outcomes, and underlying pathobiology, from relatively acute exposures to GW agents, which holds greater translational relevance for identifying effective treatments for the current human patient population.

The findings from research utilizing several rodent GWI models are described in this current research landscape; however, this is not a comprehensive list of GWI models. Further animal model development supported by the DOD GWIRP can be found at (https://cdmrp.health.mil/terp/default). Additionally, Dr. Laxmikant Deshpande recently published a review of preclinical models for Gulf War illness in the journal *Pharmacology & Therapeutics* (Ribeiro et al., 2021).

Several investigators have exposed rodents to a combination of PB, PER, DEET, and restraint stress (used as a surrogate for combat stress) in doses that do not immediately produce observable toxic effects. However, animals ultimately displayed depressive behavior, lack of motivation, and memory defects (Parihar et al., 2013; Hattiangady et al., 2014); abnormal lipid metabolism and increased immune signaling (Abdullah et al., 2012); and long-term epigenetic alterations (Pierce et al., 2016). Other rodent models have shown various types of delayed central nervous system (CNS) abnormalities that appear sometime after exposures to combinations of CPF with DEET or PB or PB plus PER (Torres-Altoro et al., 2011; Ojo et al., 2014; Nutter et al., 2015; Cooper et al., 2016).

Evidence of CNS inflammation was reported early on (Bozkurt et al, 2010) and, recently, has been the subject of extensive animal research. A series of studies has established a model based on dual exposure to PB and PER (Abou-Donia et al., 2004). Using this model, researchers have documented neurobehavioral, neuropathological, and neuroinflammatory effects, as well as evidence for mitochondrial dysfunction, in the short-, mid-, and long-term post-exposure (up to 22 months). Genomic and proteomic studies were used to discern many features of neuroinflammation (Abdullah et al., 2011 and 2013; Zakirova et al., 2015 and 2016).

O'Callaghan et al. reported compelling results using a single chemical agent, DFP, preceded by pretreatment with corticosterone (CORT), a stress hormone that would normally be expected to suppress inflammatory responses produced by external stressors (O'Callaghan et al., 2015). Exposure to a single dose of DFP resulted in inflammation in the brain, but pretreatment with CORT, and subsequent intermittent exposure, exacerbated the CNS inflammatory response and produced a persistent "priming" of the immune system, continuing to generate exacerbated responses to subsequent irritant challenges. Priming was maintained for months in the mouse model (equivalent of 20 years in humans) by periodic low-dosing with CORT. This model has been further utilized in two research consortia supported by the GWIRP to identify new features of GWI pathobiology and new targets for treatment (Morris & Klimas, FY12; Sullivan, FY12). In 2017, O'Callaghan and team showed that the model's neuroinflammatory effects do not appear to be related to the AChE inhibition induced by these organophosphate agents, but these exposures may exert their effects on the brain through the "organophosphorylation" of other neuroimmune targets (Locker et al., 2017). This model has ultimately been refined to a 5-week regimen of post-DFP CORT exposure and has proven to provide appropriate chronic inflammatory signaling deficits in an abbreviated time period.

Other studies have focused on additional cellular and subcellular targets of GW chemical agents and suggest abnormalities associated with cholinesterases, tubulin (Grigoryan et al., 2008 and

2009), axonal transport (Rao, 2017), and mitochondrial function (Middlemore-Risher et al., 2011). Microtubule dysfunction has also been investigated (Rao, 2017), including decreased microtubule width in neurons of mice exposed to chlorpyrifos (Jiang et al., 2010). Mitochondrial defects have been the target of experimental treatment approaches with the antioxidant vitamin Coenzyme Q10 (ubiquinone) (Golomb et al., 2014).

III.2. Clinical Investigations

III.2.1. Case Definitions

Research on GWI has relied on a number of differing definitions of the disorder, including CMI, also known as the Centers for Disease Control and Prevention (CDC) definition described by Fukuda in 1998 (Fukuda et al., 1998), the Kansas case definition (Steele, 2000), the Haley Research Factor case definition (Haley et al., 1997 and 2001; Iannacchione et al., 2011), and adaptations of these approaches. In 2013, the U.S. Department of Veterans Affairs (VA) convened the Institute of Medicine (IOM) (now the National Academy of Medicine [NAM]) to "develop a case definition for CMI as it pertains to the 1990–1991 Gulf War Veteran population." This panel was not able to define a consensus case definition for GWI but recommended the use of the CMI definition in clinical settings, as it is somewhat inclusive. In the same report, the IOM recommended use of the Kansas definition in research settings because it is more selective and includes exclusionary criteria (Institute of Medicine of the National Academies, 2014). Current best practice in research is to use one of the two IOM-recommended case definitions (CMI or Kansas) for primary analyses that best fit a study and code participants using the other definition to facilitate cross-comparison of study results. Recent research upholds the relevance of recommended case criteria by Fukuda and Steele based on longitudinal analyses. The study notes that these could be further refined and updated regarding exclusionary criteria and symptom inclusionary criteria based on reported exposure-outcome relationships (Krengel et al., 2022). A recent study of the Ft. Devens Cohort (FDC) of GW Veterans in Massachusetts found that rates of both the CDC and Kansas case criteria have increased substantially (by 20%), over the course of 20 years in these largely non-treatment-seeking Veterans (Zundel et al., 2020). An additional criticism of these criteria is that some of the symptoms may be sensitive to changes that are associated with normal aging (i.e., joint pain and sleep dysfunction), and thus may not reflect the actual deployment-related illness (Dursa et al., 2019).

III.2.2. Imaging Studies

Consistent differences between GWI cases and controls have been demonstrated using imaging technologies that measure brain structure and function.

Structural magnetic resonance imaging (MRI) techniques have been employed in GW Veteran populations to define structural changes in the brain, such as a reduction in brain size, that are associated with specific exposures in theater or are associated with GWI diagnosis. MRI-based measurements of specific brain areas and their volumes (segmentation and volumetry techniques) have revealed frank reductions of white and gray matter volumes in Veterans with suspected sarin/cyclosarin exposure when compared to controls (Chao et al., 2010, 2011, and 2014). Using diffusion tensor imaging, which assesses the integrity and connectivity of white matter structures to other parts of the brain, Rayhan and Stevens reported increased axial diffusivity in subjects with GWI compared to controls. These results suggest that the white

matter in GWI patients functions less effectively. Furthermore, they reported that the increased diffusivity seen in GWI patients was associated with increased fatigue, pain, and hyperalgesia (Rayhan, Stevens, Timbol et al., 2013). Chao et al. also observed increased axial diffusivity in GWI patients and found that this increased diffusivity correlated with poorer neurobehavioral performance (Chao et al., 2015). In functional MRI (fMRI) studies, where activation of brain structures in response to cognitive and other behavioral challenges can be visualized, Calley et al. reported case/control differences in specific brain regions during a Semantic Object Retrieval Test (Calley et al., 2010).

fMRI studies using a pre-/post-exercise protocol have shown that brain regions activated in response to innocuous heat stimulus following exercise were different among Veterans diagnosed with the three subtypes of GWI defined by the Haley criteria (Haley et al., 2001) and that, as a whole, the GWI group had distinct responses post-exercise when compared to the control group (Gopinath et al., 2012). Furthermore, the Haley-defined GWI subgroups showed atrophy in different brain regions and exhibited compensation in different brain regions during a verbal working memory task following exercise. Another MRI study revealed case/control differences in regional brain activation during functional tests involving memory encoding and recall (Hubbard et al., 2013).

Brain functional patterns measured by magnetoencephalography showed that patterns of synchronous neural interactions (SNI) were distinctly different in participants with GWI compared to healthy controls. Moreover, GWI-SNIs did not differ significantly from known immune-related diseases (rheumatoid arthritis, Sjogren's syndrome), but did differ significantly from Alzheimer's, schizophrenia, and post-traumatic stress disorder (PTSD) SNIs (Georgopoulos et al., 2017).

Loggia et al. has provided the first direct evidence of imaged brain neuroinflammation in Veterans with GWI. Positron Emission Tomography (PET) imaging with [^{11}C]PBR28, a marker of inflammation that binds to the translocator protein (TSPO), a protein upregulated in activated glia and macrophages, identified widespread elevations of the PBR28 signal in cortical brain regions. This finding strongly supports exploration of neuroinflammation as a therapeutic target for GWI (Alshelh et al., 2020) (Loggia, FY13).

III.2.3. Neurocognitive Findings

Because the neurocognitive and affective symptoms reported by GW Veterans commonly include problems in memory, concentration, and mood, psychological tests are often used to quantify neurobehavioral function in this Veteran group.

A large study comparing deployed GW Veterans to non-deployed GW-era Veterans found that deployed participants performed worse than their non-deployed counterparts on tests that assess short-term memory attention, visuospatial abilities, executive function, and fine motor coordination and speed (Toomey et al., 2009). Differences in performance on specific cognitive tasks were associated with self-reported exposures to specific chemical agents in theater. Self-reported exposure was found to predict poorer performance outcomes on measures of short-term memory, attention, and affective functions (White et al., 2001) and was also associated with poorer executive function and greater mood complaints (Sullivan et al., 2003). In a number of studies, researchers reported poorer visuospatial and memory functions and greater dysphoria in Veterans meeting the criteria for GWI versus controls (Axelrod et al., 1997; Anger et al., 1999;

Binder et al., 1999; Storzbach et al., 2000 and 2001; Bunegin et al., 2001; Lange, Tiersky et al., 2001; Sullivan et al., 2003; Odegard et al., 2013). One study showed little difference between cases and controls in cognitive domains but did find significantly poorer reports of mood and quality of life (QoL) in those with GWI (Wallin et al., 2009). The author noted that this study involved a very small sample of GW-deployed Veterans and lacked the statistical power to detect subtle but significant differences in cognitive outcomes.

III.2.4. Autonomic and Neuroendocrine Systems

Studies have linked autonomic dysregulation to symptoms experienced by GW Veterans. In these studies, important differences in function among ill GW Veterans, including subgroups, and controls were not apparent during resting, but rather emerged following some type of physiological challenge. The challenge used in these studies was most often physical exercise but could take other forms. In animal models, pharmacological challenges (e.g., drugs that increase heart rate) like corticosterone and dobutamine have been used to achieve similar outcomes.

Evaluation of parasympathetic and sympathetic nervous system regulation in GW Veterans have demonstrated that some symptoms, such as chronic diarrhea, dizziness, and fatigue, as well as changes in cardiovascular indices, may be caused by subtle autonomic system dysfunction (Haley et al., 2004; Rayhan, Stevens, Raksit et al., 2013).

Due to the extreme conditions of deployment and possible exposure to pathogenic agents during the GW, it has been suggested that the neuroendocrine control system may have been pushed beyond its normal operating capacity. Thus, neuroendocrine dysregulation resulting from GW deployment has been reported, including demonstrations of pronounced differences between GWI Veterans and controls after exercise and other challenges. Specific patterns of altered hypothalamic-pituitary-adrenal (HPA) axis functioning that are distinct from other conditions such as PTSD have been identified (Golier et al., 2007; Golier et al., 2009; Ben-Zvi et al., 2009). A GWIRP-funded project (Craddock et al., 2014) found that regulation of sex hormones through the hypothalamic-pituitary-gonad (HPG) axis and components of innate and adaptive immunity undergo distinct and significant changes following exercise challenge in Veterans with GWI (Broderick et al., 2011).

Research under a GWIRP-supported consortium has integrated basic and clinical research to identify the metabolic signaling mechanisms involved in disruption of autonomic cardiovascular function and endocrine functions in GWI (Morris, Klimas, FY12). Results suggest there are changes in cardiac regulation associated with GW-era chemical exposures (Carrera et al., 2021).

III.2.5. Neuroimmune Response

Multiple channels of communication between the brain and the immune system have been identified in prior neuroscientific research, and brain-immune interrelationships have been investigated in GWI. In the short term, inflammatory responses generated by the immune system are helpful to organisms in responding to infectious agents and other physiological insults, eliciting self-preserving metabolic responses; however, chronic inflammation can be maladaptive. This observation led to interest in chronic neuroinflammatory activation of the brain's glial cells as a potential cause of chronic symptoms in GWI. Chronic glial activation results in the synthesis and release of pro-inflammatory cytokines and chemokines (O'Callaghan

et al., 2008) and is particularly relevant to GWI because the effects are seen in both gray and white brain matter. Gray and white matter volumes have both been shown to be reduced in neurotoxicant-exposed and symptomatic GW Veterans (see Imaging Studies, above). In addition to lower white matter volumes, studies have shown reduced information processing speeds in symptomatic GW Veterans exposed to low-dose sarin (Proctor et al., 2006). Taken together, the findings of reduced white matter volumes and poorer information processing suggest that glial cells may have an important role in the development of (and ongoing) health symptoms and the cognitive complaints of GW Veterans.

A GWIRP-funded project (Klimas, FY08) used comprehensive molecular profiling combined with control theory to link a stress-potentiated neuroinflammatory response with symptom severity of GWI. The project identified changes in immune cell abundance, function, and signaling (Broderick et al., 2013). Further investigation into whether GWI is related to chronic brain-immune activation and inflammation was the basis of a GWIRP-supported research consortium (Sullivan, FY12). A pilot study conducted by this group showed that serum antibodies for a series of neuronal and glial-specific proteins were significantly elevated in a GWI cohort. The results must be validated further, but they support continued study into glial signaling, white matter alterations, and neuronal degeneration (Abou Donia et al., 2020). These findings may also contribute to development of a panel of objective diagnostic biomarkers of GWI.

III.2.6. Mitochondrial Dysfunction

Exposures linked to GWI are known to impair cell energy, and adverse cell energetics have been shown to contribute to symptoms consistent with GWI. Given these observations and because mitochondria are the source of chemical energy for the cell, the potential relationship between mitochondrial dysfunction and GWI has been subject to investigation. A GWIRP-funded pilot study provided the first objective evidence of mitochondrial dysfunction in Veterans with GWI. Compared to controls, Veterans with GWI exhibited prolonged post-exercise recovery of phosphocreatine, a compound used as a backup energy store and a robust index of mitochondrial function (Koslik et al., 2014). This finding supports the presence of mitochondrial pathology in GWI.

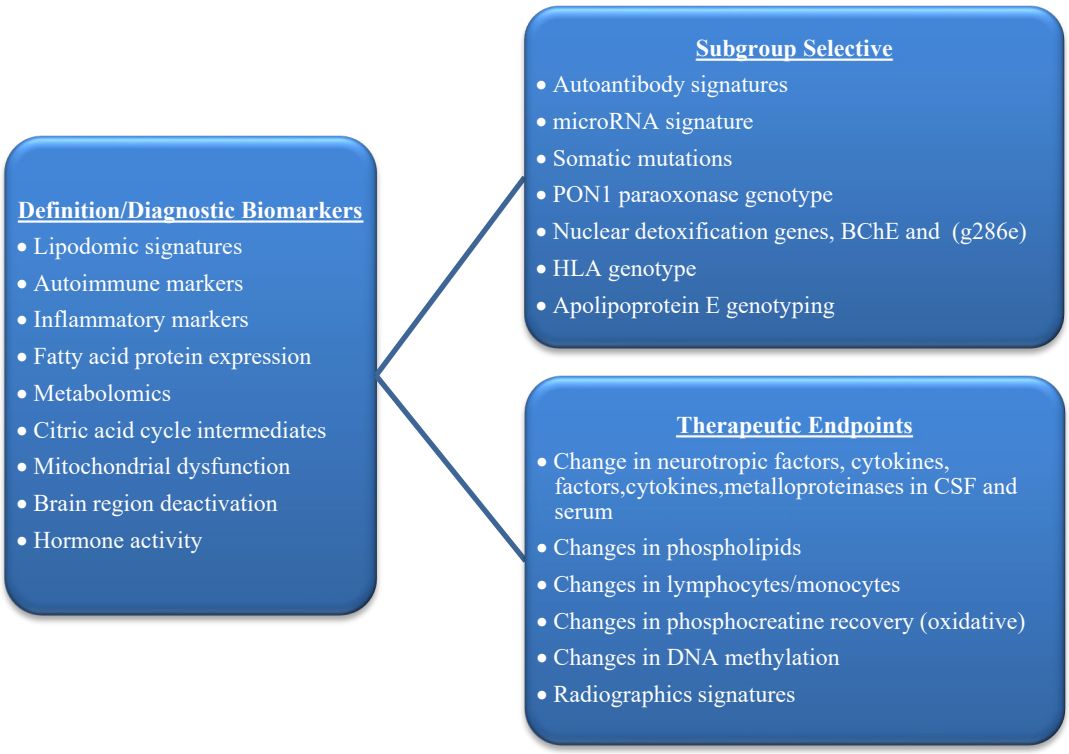
IV. Clinical Biomarkers

Objective diagnostic biomarkers are used to quantify the link between measurable altered biological processes and their clinical manifestations. According to the National Institutes of Health (NIH) (FDA-NIH Biomarker Working Group, 2016), a biomarker can be defined as a "characteristic that is objectively measured as an indicator of normal biological processes, pathogenic processes, or biological responses to a therapeutic intervention." Biomarkers of exposure and biomarkers of definition/diagnosis are two biomarker categories that may become critical in the treatment of a disease and could provide information about the mechanism(s) of GWI leading to the clinical manifestation of its symptoms.

Exposure biomarkers can be defined as measures of a chemical or toxicant in various physical media (e.g., blood, hair, urine, teeth, nails) or in the environment (e.g., air, water) (FDA-NIH Biomarker Working Group, 2016). Diagnostic biomarkers can be used to detect or confirm the presence of a specific disease or condition of interest, or to identify individuals with a subtype of

a disease (FDA-NIH Biomarker Working Group, 2016). This category can be further divided into cohort/subgroup selective and therapeutic efficacy clinical biomarkers. Research efforts have focused on discovering definition/diagnostic biomarkers to characterize and identify GWI Veteran subgroups and therapeutic endpoint biomarkers to evaluate treatments/therapeutics for GWI patients. A list of some of these biomarkers can be found below (Figure 1).

Figure 1. GWI Clinical Biomarkers



IV.1. Development of Biomarkers for GWI

Biomarkers of exposure refer to biological measurements of an exposure agent in the body of an individual. They can be detected in blood, urine, hair, fat, teeth, feces, toe- or fingernails, bone, and other bodily specimens. For these types of biomarkers, the exposure agent itself must be detected in a biosample. In many cases, the detection level can help identify illness etiology and/or quantify the severity or dose of exposure, especially if the exposure has been recent. GWI has long been thought to be related to exposures that GW Veterans experienced in theater; however, obtaining valid exposure biomarkers in this population has been difficult for several reasons. These include (1) lack of any monitoring of exposure in theater, (2) the time lapse between the war and identification of the problem of GWI symptoms in deployed Service Members, (3) most of the agents of concern do not remain detectable in the body over extended periods, and (4) the wide range of exposures of concern. Because of these complications, other indicators have been emphasized in GWI research in attempts to identify the exposures that precipitated the illness. Initially, these attempts focused on self-reported exposure, including knowledge about areas in theater of particular risk (e.g., sarin, oil well fire smoke, pesticides,

CARC paint) and then expanding to air-monitoring data on levels of an agent or agents in specific areas of the Gulf War theater. Self-report of hearing chemical alarms has been used as a proxy for neurotoxin exposure. Other considerations included exposures due to job assignments. Of the chemical agents of concern, DU associated with embedded shrapnel is one of the few that can be studied and positively associated with exposures during the Gulf War. While there has been continued interest in a causative role for DU in GWI, Dr. Robert Haley published findings in 2021 that suggest that even the highest likely levels of DU inhalation played no role in the development of GWI (Parrish et al., 2021). Antibodies associated with infectious agents and indirect measures of other types of exposures are less easily associated directly with the Gulf War due to difficulty identifying the timing of exposure. Therefore, research efforts have been focused on studying the underlying biological effects in Veterans post-deployment through markers of pathology and directly relating these to GWI, rather than identifying toxic exposures per se still evident in ill GW Veterans.

GWI is a chronic, multisymptom illness resulting from unknown exposures to a variety of agents that occurred more than three decades ago; hence, the need for biomarkers of GWI to aid in the diagnosis and treatment of ill GW Veterans is a major priority in the field. Approaches traditionally used to address disease biomarkers can introduce fatal flaws or confounding factors when applied to GWI investigations. Underpowered studies have many implications since identifying early detection biomarkers of heterogeneous medical conditions requires larger sample sizes than if the disease was homogeneous. Bearing this in mind, some previous biomarker discovery studies may have failed because they were underpowered for a heterogeneous disease. For this reason, fully powered biomarker studies that address the heterogeneity of GWI are needed.

IV.2. Biomarkers of Definition/Diagnosis

- Chronic Inflammation/Neuroimmune

Several GWI theories and studies have centered on inflammation, the immune system, and related hormonal systems, with many particularly addressing neuroinflammation. Researchers have observed priming of the neuroimmune system in animal models, and an overabundance of inflammatory cytokines, hormones, and other markers of inflammation in Veterans with GWI. Thus, molecular species indicative of abnormal immune/inflammatory activity, including cytokines, hormones, and other molecules that regulate the immune system and inflammatory response have long been targeted as potential markers of GWI, as they could serve as diagnostic targets for GWI or be used to identify GWI disease subtypes.

One theory surrounding the cause for GWI pathobiology is that chemical insult to the nervous system allowed leakage of CNS proteins from the blood-brain barrier (BBB) into the peripheral bloodstream, where exposure to the immune system then resulted in a persistent immune/ inflammatory response. This theory has been supported by the finding of autoantibodies to CNS proteins in plasma from Veterans with GWI but not in healthy controls, Veteran controls with back pain, or civilian controls suffering from myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) or Irritable Bowel Syndrome (IBS) (Abou-Donia et al., 2020). Moreover, this finding was extended by summing all means of CNS autoantibodies for each group into a new index score called the Neurodegeneration Index (NDI). Further investigation of autoantibody activity revealed sex-based differences in levels of two of these plasma

autoantibodies (Abou-Donia et al., 2021). These findings support the notion that use of plasma autoantibodies to CNS proteins may distinguish between both male and female Veterans with GWI and other healthy and symptomatic control groups.

In a complex study, thousands of measurements were made using microarray, ELISA technology, and flow cytometry technology both before, during, and after an exercise challenge. The resulting data were used to construct networks of regulatory immune and hormonal control in Veterans with GWI, healthy Veterans, and Veterans with ME/CFS. Control networks differed in Veterans with GWI versus controls, and different network connections could be related to specific symptom domains (Broderick, 2011 and 2013).

- Cell Types and C Reactive Protein (CRP)

A diagnostic classifier scheme for GWI consisting of levels of lymphocytes, monocytes, and CRP was reported by Johnson et al. The classifier was probabilistic and had a positive predictive accuracy of 90% when a probability threshold of 70% was used as the cutoff. Bach et al. found that lymphocyte, monocyte, neutrophil, and platelet counts were higher in GWI subjects compared to controls. A diagnostic model of three biomarkers — lymphocytes, monocytes, and CRP (a marker of inflammation) — had a predicted probability of 90% for diagnosing GWI when the probability of having GWI was above 70%. (Johnson et al., 2016). Georgopoulos and colleagues investigated the association between inflammation and GWI symptoms in a sample of GW Veterans and found a highly significant positive association between CRP and GWI symptom severity. These results highlight the role of inflammation as well as CRP in GWI and point to the potential benefit of interventions for GWI aimed at reducing inflammation (James et al., 2019).

- Oxidative Stress/Mitochondrial Dysfunction

Mitochondrial dysfunction and oxidative stress have long been investigated as contributing to the pathobiology of GWI, and have been observed in several animal models (Shetty et al., 2017; Repine et al., 2016) and in a human cell line exposed to GWI toxicants (Yates et al., 2021).

In human studies, both mitochondrial DNA lesion frequency and mitochondrial DNA copy number were elevated in peripheral blood mononuclear cells (PBMCs) of Veterans with GWI relative to controls (Chen et al., 2017). Several studies of cellular metabolism have observed shifts in metabolism that suggest oxidative stress and reduced availability of cellular energy. Researchers have addressed the question of reduced respiratory capacity and mitochondrial DNA damage repair capacity in PBMCs from Veterans with GWI. Investigators analyzed plasma samples from subjects from a prior acupuncture study and found clear differences in plasma proteome markers between GWI and healthy controls, mainly related to immune system and metabolic function. The set of metabolites altered in ill Gulf War Veterans was found to correctly classify subjects with GWI from controls, and thus was dubbed a "high confidence network" (Abou-Donia et al., 2017).

Studies of metabolomics, lipidomics, and other features related to energy availability have indicated increased oxidative stress in Veterans with GWI compared to healthy controls. Subsequent studies found that mitochondrial dysfunction in GWI may at least partially explain this increase. Changes in metabolism, lipid synthesis, mitochondrial function, and chemical

energy stores have revealed differences between Veterans with GWI and healthy controls, some of which have been exploited as targets for development of GWI biomarkers (Naviaux et al., 2019; Emmerich et al., 2017).

One seminal observation in the study of mitochondrial dysfunction in GWI was the finding that the recovery time of the energy-storage species phosphocreatine was prolonged in muscle after exercise in Veterans with GWI relative to matched controls (Koslik et al., 2014). While providing a significant key to understanding GWI pathobiology, the diagnostic potential of such testing might be limited as the study required comparison between Veterans and closely matched controls to reveal the differences.

- Genotype/Gene Expression/Epigenetics

Researchers have observed differential expression of various proteins in GWI Veterans versus controls. Proteins involved in immune and hormonal systems have been most frequently identified as potential biomarkers. Efforts to exploit these differences as biomarkers have addressed not only levels of proteins themselves but also include the genes and regulatory elements that control them. Studies of genotype have revealed some genetic variants in detoxification enzymes contributing to susceptibility/resistance GWI (discussed above). Other studies have addressed epigenetic changes, though most of those studies have been limited to animal models of GWI. Epigenetic mechanisms respond to external stimuli, and the effects of such exposures can become chronically embedded in the genome. One type of epigenetic insult is histone modifications, which are being investigated in animal models of GWI for their impact on pathobiology and as a novel target for therapeutics.

Epigenetic alteration has been suspected as a contributing factor in the persistence of GWI and may serve as a possible diagnostic biomarker. Differential DNA methylation in specific genes was observed in GWI animal models (Pierce et al., 2016; Ashbrook et al., 2018) and in Veterans with GWI compared to matched controls (Trivedi et al., 2019). In addition, in an animal model, investigators identified miRNAs that had been epigenetically silenced with differential expression of specific PIWI-interacting RNAs (piRNAs) (Pierce et al., 2016). Another study showed decreased acetylation in the promoter of the gene for brain-derived neurotrophic factor (BNDF), with a corresponding decrease in BNDF protein in the hippocampus in animals. These results suggest a potential use for epigenetic alterations as a diagnostic marker for GWI.

One study examined DNA methylation in PBMCs of ill GW Veterans and healthy controls and uncovered epigenetic dysfunction in GWI. Global DNA methylation levels were similar in GWI patients and controls using ELISA-based assays. However, microarray technology detected over 10,000 differentially methylated (mostly hypermethylated) CpG sites across gene regulatory elements and within coding regions. Results confirmed the presence of hypermethylation alterations in promoters that regulate expression of genes that impact immune and inflammatory responses in ill GW Veterans. In one example, the promoter for interleukin 1 receptor type 1 (IL1R1), involved in inflammatory processes, was found to be significantly hypermethylated in GWI patients. These results are consistent with previously reported dysregulation of the immune system in GWI. However, it provides a molecular basis in the form of epigenetic modification for such dysregulation and contribution toward the pathobiology of GWI (Trivedi et al., 2019).

One study showed that symptomatic GWI patients continue to exhibit significantly elevated levels of nuclear excision repair genes despite being decades removed from their wartime exposure. This might represent a persistent hormetic effect or that this induced state is maintained in the subpopulation by ongoing exposures in post-war life (Grant et al., 2021).

An important component of the exposures documented in GW Veterans was smoke from oil well fires set by retreating Iraqi forces. The products of organic combustion created in these fires are known to damage DNA, and such lesions are substrates for the DNA nucleotide excision repair (NER) pathway. Grant and colleagues analyzed NER capacity and gene expression in Veterans with GWI, finding a significantly elevated of level DNA repair. Both total gene expression and NER gene expression successfully differentiated individuals with GWI from unaffected controls. The observed functional increase in DNA repair capacity was accompanied by an overexpression of genes in the NER pathway (Latimer et al., 2020).

- Brain Imaging

Early imaging studies found only subtle differences between Veterans with GWI and controls, which included reduced white and gray matter volumes (Chao et al., 2010). More sophisticated techniques have been developed that are able to distinguish features in GWI brain images that might be used to distinguish them from controls and even distinguish GWI subtypes. However, more recent work has shown long-term effects of GWI in this population. Now, decades after early imaging studies showed few differences, frank differences between GWI Veterans and controls can be observed using standard clinical imaging techniques.

Using magnetic resonance imaging (MRI), diffusion tensor imaging (DTI), and blood oxygen level-dependent (BOLD) flow imaging, Rayhan et al. were able to detect patterns of abnormal differential regional activation and deactivation after exercise and abnormalities in white matter volume that differed not only between cases and controls but also between two physiological subtypes (Rayhan, Stevens, Raksit, et al., 2013; Rayhan, Stevens, Timbol, et al., 2013). Other BOLD imaging studies revealed abnormal patterns of connectivity in language network components, and visual processing and sensorimotor components in GWI Veterans versus controls (Gopinath et al., 2012). In Veterans with GWI, brain microstructural alterations observable by MRI have been found to correlate with upregulation of proinflammatory cytokines in blood and self-reported fatigue and sleep disturbances (Cheng et al., 2020).

Recently, an automated GWI classifier was developed based on features of MRI images using T1-weighted magnetic resonance imaging (T1W-MRI), DTI and novel neurite density imaging (NDI) data, independently. The NDI-based classifier proved to be the most sensitive, with an accuracy of 90% (F-score = 0.941) for classifying GWI cases from controls (Guan et al., 2020). While this supports the concept of using brain imaging to diagnose GWI, this approach may be limited because it requires a highly sophisticated imaging modality and extremely rigorous normalization of image data to match those used in the training set. Nonetheless, these researchers found that NDI has been shown to be sensitive to the microstructural and macrostructural changes in the brains of Veterans with GWI. When applied within a machine learning framework, this could be a sensitive marker for detecting GWI pathology and distinguishing cases from controls.

Brain inflammation has been imaged by PET using an 11C tracer that selectively binds the 18 kilodalton (kDa) translocator protein (TSPO) which is upregulated in activated microglia/macrophages and astrocytes. The tracer signal was greater overall in Veterans with GWI than controls and spiked in specific brain regions. The authors state the spatial distribution is similar to that observed in fibromyalgia (Alshelh et al., 2020). In ongoing studies, investigators are imaging the CNS in Veterans with GWI employing the 11C TSPO-ligand neuroinflammation tracer described above and complementing this with spectroscopic imaging and blood-based analyses of energy/oxidation related metabolites to uncover diagnostic biomarkers for GWI. (Loggia, FY13)

In a mouse model of persistent GWI, Liquid Chromatography/Mass Spectrometry (LC/MS) was used to quantify over 1,000 proteins in brain lysates. Statistical analysis showed that 31 proteins were differentially expressed in response to exposure, of which 8 were upregulated and 23 were downregulated (Abdullah et al., 2011). The most affected pathways involved included nuclear factor kappa B (NFκB) signaling, integrin signaling, and G protein signaling. Since these changes were detected in brain lysate, they could not be used directly as clinical markers in Veterans. However, they do suggest systemic alteration that might be detected and used as GWI biomarkers after further development.

- Respiratory Effects

Though respiratory symptoms are one of the symptom domains listed in the Kansas case definition for GWI, lung disease related to GWI has not been well investigated. Current studies have demonstrated an excess of lung disease in GW Veterans, and lung disease has been demonstrated in animal models of exposure to GW toxicants (Powers et al., 2021). In preliminary studies, deployment-related chronic (or "constrictive") bronchiolitis (DR-CB) has been characterized in Veterans with GWI and post-9/11 Soldiers using computed tomography-parametric response mapping (CT-PRM), which compares lung tissue density in image voxels in inspiration and exhalation. In ongoing studies, researchers observed distinct PRM signatures in Veterans with GWI, suggesting possible disease subtypes. While not diagnostic of GWI, this non-invasive technique for assessing DR-CB may represent a significant improvement in methods for diagnosing DR-CB, which currently requires lung biopsy (Osterholzer, FY16).

- Hormonal Activity

Studies of hormonal activity have most often been one part of studies attempting to measure a wide variety of substances including hormones, cytokines, cell populations, metabolites, and other potential markers. Studies suggest that markers of pro-inflammatory activity may be biomarkers of GWI, and certainly these molecules are players in the regulatory network governing the interplay between hormonal and immune systems. Yet, despite several studies addressing a wide variety of analytes, including hormones, abnormal levels or changes in hormone levels specifically have not been observed to be a distinguishing characteristic of GWI in Veterans.

In one study targeting growth hormone deficiency, investigators found that some Veterans who had completed a growth hormone stimulation test met objective criteria for the presence of adult growth hormone deficiency (AGHD). Within that AGHD-positive group, an association was found between hormone deficiency and the occurrence of GWI (Jorge, FY16).

IV.3. Biomarkers to Identify GWI Veteran Subgroups

- Autoantibody Signatures

Researchers have found blood levels of autoantibodies to CNS proteins that could be used to distinguish Veterans with GWI from healthy Veterans or civilians with other inflammatory conditions (ME/CFS, IBS) (Abou-Donia et al., 2017 and 2020). Moreover, sex-based differences in these levels have also been observed in levels of two plasma autoantibodies (Abou-Donia et al., 2021). Further investigation may reveal autoantibody "signatures" that might be used to identify GWI subtypes.

- Response to Exercise Challenge: Autonomic Response and MicroRNA signatures

In a study addressing the response of Veterans with GWI to exercise, Veterans with GWI segregated into two distinct clusters: one exhibited postural orthostatic tachycardia (Stress Test Activated Reversible Tachycardia, START) and the other did not but instead experienced exercise-induced hyperalgesia (Stress Test Originated Phantom Pain, STOPP) (Rayhan, Stevens, Raksit, et al., 2013). Moreover, these subtypes showed distinct differences in brain regional activation and deactivation and white matter connectivity using BOLD flow imaging and DTI and distinct signatures of miRNA expression (Washington et al., 2020). Because of the considerable cost and effort involved in such physiological assessments, it is unclear whether they would be of practical clinical use.

miRNA levels were also examined in this cohort and were comparable to those in healthy controls before exercise. However, after an exercise challenge, 12 miRNAs were diminished and 6 miRNAs were elevated relative to healthy controls. In addition, levels of two specific miRNAs were significantly different in the START and STOPP subgroups and might be used to distinguish between them (Baraniuk et al., 2017). Alteration in expression of specific species of miRNAs and piRNAs has also been reported in animal models of GWI (Pierce et al., 2016).

- Somatic Mutations

In a study of somatic mutations at the glycophorin A (GPA) gene, Grant et al. found that Veterans with GWI had overall significantly higher frequency of mutations compared to controls. They further identified a group of Veterans with GWI, deemed "outliers," with much higher levels of GPA mutations than other Veterans with GWI, suggesting that overall genomic instability may also contribute to a higher frequency of mutations (Grant et al., 2021). It is currently unclear whether this high mutational level could be used to define a distinct GWI subtype.

In a study of chromosome condensation defects in cultured lymphocytes, researchers again found higher levels of defects and genome instability in Veterans with GWI and a subgroup of GWI Veterans with approximately twofold higher levels of defects compared to other Veterans with GWI. Different types of chromosomal aberrations have been observed in different GWI patients, and such aberrations may be used for classifying GWI patients into different subgroups (Liu et al., 2018).

- Paraoxonase (PON1) Genotype

Investigators have studied the association of Q192R polymorphic alleles in organophosphate detoxifying enzyme paraoxonase 1 (PON1) and the occurrence of GWI in deployed Veterans. One study found that individuals who reported hearing many chemical alarms (as a proxy for chemical weapon exposure) were more likely to have GWI if they possessed the 192R variant and that individuals who reported excessive pesticide use were more likely to have GWI if they possessed the 192Q variant (Haley et al., 1999) (see also Other Etiological Considerations). Conversely, an earlier study failed to show evidence of association between GWI and PON1 or BChE genotypes (Haines et al., 2017), but this may be because potential exposures were not considered.

- Nuclear and mitochondrial detoxification genes, butyrylcholinesterase (BChE)

The enzyme BChE present in plasma acts as a detoxifying scavenger of nerve agents, pyridostigmine, and certain pesticides. One study found that the association of pyridostigmine use with GWI was greater in individuals bearing less-common variants of BChE that are less active as scavengers than more common BChE variants (Steele et al., 2015). Individuals bearing the less common BChE alleles may represent a more susceptible subtype of GWI.

Nuclear detoxification genes such as the cytochrome P450 family and mitochondrial haplotypes are known to influence the risks associated with oxidative stress. When specific nuclear detoxification genes and the mitochondrial genome were sequenced from Veterans with GWI and matched controls, researchers found that nuclear detoxification genetics related significantly to GWI symptom severity, using a self-reported symptom score (Bui et al., 2020).

- HLA Phenotype

Investigators have revealed that copy number of six specific human leukocyte antigen (HLA) Class II genes could distinguish Veterans with GWI from controls with 84% accuracy (Georgopoulos et al., 2015). Using magnetoencephalography, investigators were able to tease out significant associations between HLA gene copy number and specific symptom domains and map these to particular brain regions (James et al., 2016). These data suggest that further characterization of HLA genetic signatures as they pertain to symptomatology might help identify subgroups of Veterans with GWI when compared to Veterans who are not deficient in HLA copy number. More recent research by this group further supports a significant effect of HLA status on GWI, identifying both protective and susceptible alleles and suggesting that anthrax vaccination may be one environmental exposure that contributes to inflammation and GWI in the absence of protective HLA alleles.

- Changes in DNA methylation

DNA methylation has traditionally been thought to be a more long-term pathologic effect. However, some studies describe changes in human DNA methylation as a result of short-term exposure to air pollution or particulate matter (Li et al., 2018). Epigenetic changes have largely been observed in animal models of GWI and have been noted for specific genes and miRNAs. Studies have shown that Veterans with GWI have a similar overall methylation rate as controls, but there are significant differences in the population of genes affected in ill Veterans with GWI,

with genes involved in inflammation representing the largest group of differentially affected genes. One ongoing GWIRP-funded project will use genome-wide epigenetic DNA methylation profiling in Veterans with GWI and controls to identify biomarkers of GWI. While this project is an attempt to discern GWI or its subtypes, the results may also be applicable to the development of therapeutic endpoints (Paris, FY19). Epigenetic changes associated with GWI are also described in more detail above (see Genotype/Gene Expression/Epigenetics).

- Permethrin metabolite

Antibodies to an albumin-bound permethrin metabolite, 3-phenoxybenzoic acid (3-PBA), have been detected in Veterans with GWI and in animals exposed to permethrin at chronic post-exposure timepoints. Preliminary results suggest that detection of antibodies related to exposure type might have utility in identifying GWI subtypes. These studies further suggest that pesticide exposure associated with GWI may have resulted in the activation of the peripheral and CNS adaptive immune responses, possibly contributing to an autoimmune-type phenotype in Veterans with GWI (Joshi et al., 2019).

IV.4. Biomarkers as Therapeutic Endpoints

- Changes in Neurotropic Factors, Cytokines, Metalloproteinases in CFS and Serum

Studies in a rat model of persistent GWI found that elevated levels of High Mobility Group Box Protein 1 (HMGB1), inflammatory cytokines, and complement-activated related proteins were observed in neuron-derived extracellular vesicles (NDEVs) and astrocyte-derived extracellular vesicles (ADEVs), respectively. These changes correlated with changes in cytokines, complement activation, and activation of microglia and astrocytes in brain. Taken together, these data demonstrate the relevance of these markers and their availability in blood-borne extracellular vesicles, suggesting potential use as therapeutic endpoints (Madhu et al., 2019).

In GWI Veterans, but not in healthy Veteran controls, levels of fatigue correlated with levels of IL-1β and IL-15 (Parkitny et al., 2015), and levels of CRP in blood correlated with a composite symptom severity score calculated across the six symptom domains specified in the Kansas definition of GWI (James et al., 2019). These data imply that these parameters could provide objective means to confirm self-reported levels of symptom severity.

In a complex study, thousands of measurements were made using microarray, ELISA technology, and flow cytometry technology both before, during, and after an exercise challenge. The resulting data were used to construct networks of regulatory immune/hormonal control in Veterans with GWI, healthy Veterans, and Veterans with ME/CFS. Control networks differed in Veterans with GWI versus controls, and different network connections could be related to specific symptom domains (Broderick et al., 2011 and 2013).

In a separate study, investigators examined plasma samples from GWI subjects using laser-induced breakdown spectroscopy (LIBS) to distinguish blood plasma metabolites, using subjects with chronic low-back pain as symptomatic controls. A subset of atomic and ionic transitions emissions peaks that provided 70% correct diagnosis was evaluated in a blinded fashion on 10 additional samples and was found to yield 90% classification accuracy, 100% sensitivity, and 83.3% specificity (Gaudiuso et al., 2021).

- Changes in Phospholipids and Lipid Metabolism

Lipidomic analysis in animal models and in Veterans has revealed changes in phospholipid composition that suggest aberrations in molecular pathways. Phospholipid analysis is an attractive candidate for therapeutic endpoints because the analytes are readily measured and can provide a window into a wide variety of cellular systems that are responsive to changes on a reasonably short timescale. Moreover, some of the systems assayed could be mechanistic targets for experimental treatments.

Studies in mice have shown lipidomic changes consistent with alterations in peroxisomal pathways, steroyl-CoA desaturase, and increased levels of phosphocholine ethers in brain and plasma, suggesting both peroxisomal and lysosomal dysfunction (Abdullah et al., 2012 and 2013).

In Veterans with GWI, researchers have found abnormal levels of phosphoethanolamine ether, arachidonic acid, and docosahexanoic acid, but these changes were subtle and perhaps not sensitive enough to use as treatment endpoints. Lipidomic signatures across many analytes may prove a more useful choice for treatment endpoints. In an analysis of 358 metabolites assayed representing 46 biochemical pathways in Veterans with GWI, the metabolomic signature of GWI was dominated by increases in ceramides, sphingomyelins, and phosphatidylcholine lipids, along with a decrease in plasma purines (Naviaux et al., 2019). Many of these pathways have ties to inflammation and brain function, and the changes observed were distinct from metabolic changes known to be associated with ME/CFS.

Studies have shown that altered lipid profiles in the brain and blood following GW pesticide exposure in rodents accompany neurobehavioral and bioenergetics deficits and inflammation (Abdullah et al., 2012). The ε4 allele of apolipoprotein E (APOE) is a major genetic risk factor for neurodegenerative diseases, including Alzheimer's Disease (AD); however, there is currently no direct evidence that it is a contributor for GWI. Veterans with GWI who carry the APOE ε4 allele may be considered a subtype associated with some symptomology, including cognitive decline and a higher risk of developing neurodegenerative disease. Current studies hypothesize that blood lipid/metabolite profiles and the presence of the APOE ε4 allele will correlate with more severe cognitive impairment in GWI (Abdullah et al., 2012).

- Changes in Lymphocyte and Monocyte Populations

Changes in immune cell populations have implications for the state of the immune system and inflammatory activity and, for GWI, may correlate directly with symptom severity. As such, immune cell profiles can serve as endpoints for therapeutics that target inflammation and components of the immune system. In a study by Broderick et al., measurement of cellular subtypes in GW Veterans pre-and post-exercise challenge showed changes in CD2+ T and NK (natural killer) lymphocyte populations, which correlated positively with symptom severity. Likewise, IL-10 levels and changes in intracellular perforin in cytotoxic T cells (CD3+, CD8+) aligned with IL-2 and IL-5 levels and physical function scores (Broderick et al., 2013). Therefore, changes in these cytokines may be a useful a surrogate for changes in immune cell populations that correlate with GWI symptom severity.

Another study examining immune cell populations before, during, and after an exercise challenge found that, at baseline, GWI patients had a higher proportion of monocytes as compared to healthy controls. In addition, proportions of both dendritic cells and eosinophils in GWI patients were decreased at baseline compared to controls. The dysregulation of these cells in GWI patients compared to healthy controls demonstrates a dysfunction of the innate immune system (Van Booven et al., 2021).

- Radiographic Signatures

Abnormally high levels of the 18 kDa translocator protein (TSPO) have been imaged by PET in brains of Veterans with GWI using a ^{11}C-TSPO binding tracer (Alshelh et al., 2020). Such imaging could be used as an endpoint for experimental therapeutics targeting neuroinflammation since one expects disappearance of excess TSPO as inflammation subsides. Other brain imaging studies of Veterans with GWI have found morphological abnormalities and abnormal regional activation (and deactivation) during mental tasks.

- Heart Rate Variability

Accumulating evidence suggests that GWI is characterized by autonomic nervous system dysfunction, including higher heart rate and lower heart rate variability. Heart rate and heart rate variability is an easily measured parameter that could be used as an endpoint for restoration of normal autonomic function in GWI. Studies have investigated heart rate and other autonomic functions. While some studies found no differences between Veterans with GWI and controls during waking hours without exercise challenge (Haley et al., 2004), others report elevated heart rate variability in Veterans with GWI using 24-hour electrocardiograms (Blanchard et al., 2019). Additionally, while heart rate variability normally decreases during sleep in healthy controls, this normal drop was absent in Veterans with GWI (Haley et al., 2013). Investigators have begun to evaluate the potential for using restoration of the normal drop in heart rate variability during sleep as a measure of therapeutic effectiveness (Mathersul et al., 2021).

Many of the therapeutic efficacy biomarkers discussed are currently or will be confirmed in larger clinical studies.

GWI clinical trial investigators are regularly challenged to complete enrollment of both symptomatic and healthy GW Veterans and GW-era Veterans. The GWIRP has prepared a document to assist investigators in this process. The document, General Guidance for Gulf War Veteran Outreach and Recruitment, can be found on the GWIRP website

General Guidance for Gulf War Veteran Outreach and Recruitment

V. Treatments

Clinical trials with the potential to have significant impacts on the health and lives of Veterans with GWI continue to be an ongoing priority. A primary focus of the GWIRP has been to fund research that aims to identify treatment targets and evaluates interventional approaches to alleviate symptoms.

While many studies remain in progress, several have already shown varying levels of promise for ill GW Veterans.

V.I. Treatments Using Alternative or Mind-Body Interventions

V.1.1. Why This Strategy for GWI

Alternative or mind-body interventions utilize the interactions among brain, mind, body, and behavior, with the intention to use the mind to affect the body and its physiological responses to positively influence health. In theory, these interventions could act by quelling inflammatory processes by way of effects on the HPA homeostatic hormonal axis. These types of interventions are symptom-based and target improvement in the QoL of the patient. Alternative or mind-body interventions include acupuncture, acupressure, tai chi, yoga, and detoxification protocols, to name a few. These techniques have been previously used to treat general pain, back and neck pain, headaches, and sleep disturbances. Since many of these manifestations are also experienced by GW Veterans, researchers believe that they may improve the QoL of this population.

V.1.2. Potential Impact

Despite self-reported evidence in reduction of fatigue, headaches, and general pain, there has been no definitive evidence for the effectiveness of this type of intervention, mainly due to the lack of objective markers of improvement. Such treatments have the potential to be widely applicable since they are relatively non-invasive, free of side effects, and do not require special technology. Studies funded by the GWIRP and the VA address these types of interventions to identify markers of improvement as well as further evidence in support of these techniques as effective treatment options for Veterans with GWI.

V.1.3. Completed Clinical Trials

Effectiveness of Acupuncture in the Treatment of Gulf War Illness

Veterans with GWI report suffering from multiple chronic symptoms including, but not limited to, headaches, muscle and joint pain, and fatigue. This study aimed to identify whether individualized acupuncture could be used as a cost-effective treatment for reduction of GWI symptoms. Acupuncture has been used successfully to treat key symptoms experienced by Veterans with GWI, and several studies have shown this technique to be generally beneficial, safe, and cost-effective. This study found positive improvements in the severity of Veterans' self-reported health complaints, overall health, and fatigue. A positive improvement in the bi-weekly treatment group was statistically significant when compared to the weekly treatment group and controls. In addition to these outcomes, the Principal Investigator also reported significant improvements on pain scores on the McGill Pain Scale from the 2-month to the 6-month time point. Finally, study participants reported a favorable experience and would recommend acupuncture to family and friends. The primary outcome was the Physical Functioning Subscale of the Quality of Life Scale, from the Short Form 36 Health Survey (SF-36). (Conboy et al., 2012) NCT01305811

Gulf War Illness: Evaluation of an Innovative Detoxification Program

This study hypothesized that a detoxification program can improve the general physical and mental health and/or the QoL of Veterans with GWI. The detoxification program is a rehabilitative therapy that uses a combination of exercise, nutritional supplements, and sauna sessions to improve the QoL of individuals diagnosed with multi-symptom illnesses. This detoxification program has been used as a treatment protocol for individuals affected by environmental exposure to toxic substances. Researchers assessed improvements in pain and fatigue levels and overall symptom burden post-treatment in subjects who met the Kansas case criteria for GWI. Published results from this study noted that the patient-reported outcomes indicated improvements in pain and fatigue scores (Kerr et al., 2019). The procedure was deemed safe and well-tolerated by the participants. This research offers preliminary data to advise future efforts to improve the health of Gulf War Veterans. Clinical outcome measures used include the SF36-V Forms of Quality of Life, Physical and Mental Status; the Multidimensional Fatigue Inventory; the McGill Pain Questionnaire; the Trail Making Test A and B; the Grooved Pegboard Test; the Wechsler Memory Scale III-a Test, the Stroop Color Word Test, the Symptom Checklist 90 Test; and the State-Trait Anxiety Inventory Test. NCT01672710

Investigating Clinical Benefits of a Novel Sleep-Focused, Mind-Body Program on Gulf War Illness Symptoms: An Exploratory Randomized Controlled Trial

Sleep problems are a common concern for GW Veterans. Given the diversity of this population, data that could enhance our knowledge of the impact of sleep disturbances and other comorbid symptoms are valuable. This study sought to evaluate the impact of mind-body bridging (MBB) in GW Veterans that suffer from these conditions. Upon completion of the study, the results showed MBB to be more efficacious in improving sleep quality compared to the control condition, sleep education (Nakamura et al., 2017). Additionally, GW Veterans reported an improvement in PTSD, depression, and fatigue symptoms post-treatment. The primary outcome measure used was the Medical Outcomes Study - Sleep Scale (MOS-SS). NCT01543997

Effectiveness of Acupressure Treatment for Pain Management and Fatigue Relief in Gulf War Veterans

Acupressure is a traditional Chinese medicine therapy that uses localized massage that provides relief from pain, nausea, and fatigue. Published data indicate that this intervention has been proven effective in reducing fatigue in cancer patients and those with neck pain. Given the existing scientific support of acupressure in relieving these symptoms, a study evaluating this intervention and its potential impact on GW Veterans would have a powerful impact on the field if successful. This study sought to assess the efficiency of acupressure combined with routine care versus reiki as routine clinical care for the management of pain and fatigue. Though the cohort was small, clinical outcomes showed fatigue reduction and pain relief for those receiving acupressure. The data provide further basis for the potential implementation or refinement of this treatment for alleviation of specific symptoms presented by Veterans with GWI. Clinical outcome measures included the SF-36 for QoL evaluation; revised Piper Fatigue Scale (rPFS) for fatigability evaluation; and Brief Pain Inventory (BPI) for pain evaluation. NCT02075489

A Randomized, Multi-Center, Controlled Trial of Multi-Modal Therapy in Veterans with Gulf War Illness (Exercise and Behavioral Therapy Trial)

This project studied GW Veterans who present unexplained pain, fatigue, or cognitive difficulties. Cognitive behavioral therapy (CBT), aerobic exercise, and their combination were evaluated to determine their impact on the unexplained physical symptoms reported by these subjects. Published outcomes from this project showed that exercise alone and in combination with CBT significantly improved cognitive and mental health functioning, fatigue, and distress, while CBT alone only improved cognitive and mental health functioning. However, neither treatment, either alone or in combination, resulted in a positive impact to pain (Donta et al., 2003). NCT00007748

Testing the Feasibility of MC CBT for Veterans with IBS Alliance

GI disorders are among the unexplained illnesses reported by many Veterans with GWI. In order to find an effective treatment for IBS, in particular, this trial sought to test the feasibility of minimal contact Cognitive Behavioral Therapy (mcCBT). This psychological therapy attempts to teach patients skills for managing and controlling negative thoughts and the ability to cope with daily stressors.

A Multimodal Evaluation of the Comparative Efficacy of Yoga Versus a Patient-Centered Support Group for Treating Chronic Pain in Gulf War Illness

Chronic pain is one of the most common and debilitating symptoms reported by Veterans suffering from GWI. Yoga is a practice that involves breathing and relaxation techniques, physical exercise, and positive thinking to promote well-being. There is evidence indicating that this technique is a safe, cost-effective, and self-sustaining practice that has the potential to alleviate chronic pain. This study assessed yoga vs. CBT for amelioration of pain, fatigue, and mood symptoms in Veterans with GWI. The primary outcome of pain severity improved significantly in the yoga condition but not in the CBT condition. Similarly, fatigue was reduced significantly more in the yoga group than in the CBT group. Yoga may be an effective and easily administered treatment for core GWI symptoms, making it one of the few treatments for GWI with empirical support (Bayley et al., 2021). NCT02378025

Pilot Test of Telephone-Delivered Cognitive Behavioral Therapy for Insomnia for Veterans with Gulf War Illness

One of the common symptoms reported by Veterans who suffer from GWI is sleep disturbances such as insomnia. Published reports associate insomnia with fatigue and psychological disorders. This trial sought to investigate the efficacy of a telephone-delivered CBT with Insomnia (CBT-I) for sleep in GW Veterans. CBT-I produced significant improvements in overall GWI symptom severity, individual measures of fatigue, cognitive dysfunction, depression and anxiety, insomnia severity, subjective sleep quality, and sleep diary outcome measures. The beneficial effects of CBT-I on overall GWI symptom severity and most individual GWI symptom measures were maintained 6 months after treatment. This trial may be a significant first step toward identifying a non-invasive treatment to improve the QoL for the estimated 175,000 Veterans with GWI (Chao et al., 2021). NCT02782780

Impact of Exercise Training on Pain and Brain Function in Gulf War Veterans

Reports show that resistance exercise training has a moderate impact on the improvement of pain, tenderness, and muscle strength in women with fibromyalgia (FM). Both FM and GWI patients are known to suffer from widespread chronic musculoskeletal pain. This study evaluated the potential benefit of resistance exercise training on Veterans with GWI, and the brain's response to pain during training. Results showed that 54 Veterans with chronic muscle pain who participated in resistance exercise training saw improvement in their condition and exhibited increased muscular strength without symptom exacerbation or reductions in total physical activity (Stegner et al., 2021). NCT01350492

Telemedicine Treatment for Veterans with Gulf War Illness

Despite evidence showing CBT as an effective treatment for the alleviation of GWI symptoms, the number of Veterans seeking this treatment is limited. One of the main issues raised by Veterans include the requirement for in-person treatment sessions, which makes it challenging for some. This study aims to determine if CBT delivered by phone could be an efficient delivery method for this treatment by eliminating in-person sessions. If successful, this delivery method could reduce the resources committed by the VA and provide readily available resources to GW Veterans, regardless of geographic location. While conduct of the study has been completed, no findings have been reported to date. NCT00129454

Evaluation of a Mindfulness-Based Intervention for Gulf War Illness (A Randomized Controlled Trial of a Mindfulness-Based Intervention for Gulf War Syndrome)

Mindfulness-based interventions (MBIs) use meditation techniques to increase moment awareness, manage physical pain, and relieve stress, among others. This VA study sought to evaluate two MBIs, Mindfulness-Based Stress Reduction (MBSR) and treatment as usual, to determine which intervention results in a greater improvement on chronic muscle pain. Investigators found that MBSR in addition to treatment as usual was associated with improvements in self-reported symptoms of Gulf War illness, including pain, fatigue, cognitive failures, and depression. NCT01267045

Problem-Solving Therapy for Gulf War Illness (Cognitive Rehabilitation Therapy for Gulf War Veterans)

While the etiology of GWI remains unknown, GW Veterans continue to report unexplained and life-limiting health issues. This VA study seeks to determine whether problem-solving therapy, a patient-centered cognitive rehabilitation treatment that teaches patients tactics to address real-life problems in hopes to improve day-to-day life, can reduce disability by compensating for problem-solving deficits. The study was completed, and the investigators published findings related to the role of illness beliefs and behaviors in coping with medically unexplained symptoms. (Sullivan et al., 2019) NCT02161133

Complementary and Alternative Medicine (CAM) in Veterans with Gulf War Illnesses

Integrative restoration (iRest®) yoga nidra is a type of meditation that induces deep relaxation through breathing, body sensing, imaging, and relaxation techniques. This VA trial aims to determine whether combined auricular acupuncture and iRest yoga nidra will lead to improved overall health functioning, sleep quality, and stress in Veterans with GWI, compared to GW Health Education. Positive outcomes from this trial would provide the basis to include CAM

interventions in the standard of care for GWI. In addition, the benefits of these interventions would also have a potential positive impact on Veterans of all wars. This study has completed recruitment and results are being analyzed (Hull et al., 2014). NCT02180243

V.1.4. Ongoing Clinical Trials

Novel Interventions for Gulf War Veterans' Illnesses

Tai chi is a traditional Chinese mind-body therapy that can improve both physical health and psychological well-being in patients with a variety of chronic conditions. This study seeks to determine the effectiveness of tai chi on Veterans with GWI. If this therapy has a positive impact on the reduction of GWI symptoms, it could be an easy-to-implement, non-pharmaceutical treatment that Veterans could practice in clinic and/or independently within their own homes. Providing Veterans some relief from the debilitating symptoms of GWI would have a significant impact on the QoL of these Veterans. This trial is currently recruiting. No data are available at this time. NCT02661997

Predictors of Response to Insomnia Treatments for Gulf War Veterans

While alternative treatments for insomnia, such as CBT, have been identified, the specific target population that would benefit from this intervention remains unclear. The purpose of this study is to evaluate the efficacy and effectiveness of sleep restriction (SR) and cognitive therapy (CT) in GW Veterans suffering from insomnia. If successful, this trial would address the need for non-pharmacological treatments and/or tools for clinicians to identify the best insomnia treatment for individual GW Veterans. This study is currently recruiting. No data are available at this time. NCT03208049

Pilot Test of Apnea and Insomnia Relief for Veterans with Gulf War Illness

Insomnia is a common symptom of GWI that can exacerbate other non-sleep GWI symptoms such as fatigue, pain, mood, and cognitive dysfunction. It has been shown that Veterans with GWI are at greater risk for obstructive sleep apnea compared to Veterans without GWI. This pilot study in the San Francisco area aims to treat obstructive sleep apnea and insomnia in tandem to reduce GWI symptoms in Veterans with GWI, and obstructive sleep apnea and insomnia, utilizing an innovative behavioral intervention. This study is currently recruiting; no data are available at this time. NCT05137743

V.2. Anti-Inflammatory/Immune Effector Therapies

V.2.1. Why This Strategy for GWI

Chronic neuroinflammation is the sustained activation of glial cells, the resident innate immune cells in the CNS, and recruitment of other immune cells into the brain. It is initiated in response to a variety of cues, including infection, TBI, toxicant exposure, or autoimmunity. Typically, the CNS is an immunologically privileged site because peripheral immune cells are blocked by the BBB. However, sustained glial cell activation can compromise the BBB, allowing circulating immune cells to pass through, perpetuating the immune response. Published findings of reduced white matter volumes in ill GW Veterans compared to controls suggest that activated glial cells may play an important role in the development of, and ongoing, health symptoms (Heaton et al.,

2007). This has led to interest in neuroinflammatory chronic glial activation as both a potential cause of chronic symptoms in GWI and a target for therapeutic intervention strategies.

V.2.2. Potential Impact

A successful trial with improved clinical outcomes and reduced proinflammatory biomarkers would validate the hypothesis that chronic inflammation is part of the underlying pathophysiology of GWI. This could lead to a new paradigm for the diagnosis and treatment of GWI – targeting the underlying cause of disease and not just ameliorating symptoms.

V.2.3. Completed Clinical Trials

A Randomized, Double-Blind, Placebo-Controlled, Crossover Trial of Mifepristone in Gulf War Veterans with Chronic Multisymptom Illness

Distinct biological alterations reflective of disturbances in central processes that regulate neuroendocrine systems have been associated with GWI pathobiology. In particular, enhanced negative feedback inhibition of the HPA axis and lower 24-hour plasma adrenocorticotropic hormone (ACTH) levels have been found in deployed GW Veterans compared to controls (Golier et al., 2006). Since dysregulation of the HPA axis can have deleterious effects on multiple systems including the immune system, the autonomic nervous system, and the CNS, this axis is a useful treatment target. A GWIRP-supported trial sought to determine whether mifepristone, a glucocorticoid receptor antagonist, could reverse the neuroendocrine alterations described in GWI by reducing glucocorticoid sensitivity and, in so doing, improve the health of these Veterans. Mifepristone has previously been safely used to treat physical symptoms and disturbances of memory and mood in other medical and neuropsychiatric disorders associated with disturbances in the HPA axis. The primary outcome measure was improvement in physical health as measured by the Veterans Rand 36-item health survey. While the study was negative with respect to all clinical outcomes, the data suggest a moderate dose of mifepristone may have circumscribed cognitive-enhancing effects in ill GW Veterans (Golier et al., 2016). Additional studies have further explored neuroendocrine approaches to treating GWI with a focus on cognitive outcomes.

Nasal Irrigation for Chronic Rhinosinusitis and Fatigue in Patients with Gulf War Syndrome

Symptoms of chronic rhinosinusitis (CRS) and fatigue are the first and third most common symptoms of GWI, respectively, and are biologically characterized by interrelated proinflammatory cytokines. This three-arm trial compared saline nasal irrigation (S-NI) or xylitol nasal irrigation (X-NI) plus routine care with routine care alone on outcomes including sinus symptoms, fatigue, overall QoL, cost-effectiveness, and proinflammatory biomarkers and cell types. S-NI was hypothesized to improve sinus symptoms by thinning and clearing mucus and inflammatory mediators, decreasing mucosal edema, and improving ciliary function. X-NI has been shown to change the salinity of the mucosal surface, resulting in enhanced antimicrobial properties. Outcomes included self-rated, disease-specific quality of life ratings (SNOT-20), which improved in both NI groups, approaching clinically important difference compared to controls (Rabago et al., 2020). The results suggest that nasal irrigation can provide effective adjunct therapy for CRS and fatigue in Veterans with GWI. NCT01700725

Botanical Microglia-modulators for Treatment of GWI

Inflammatory processes may be critical in the maintenance of multisymptom illnesses characterized by pain and fatigue, and microglia modulators may be a novel and effective approach to treating those disorders. The overall objective of this GWIRP-supported Phase 1/2 screening trial was to screen nine botanical microglia-modulating and central anti-inflammatory agents to identify those that would be most promising for further study in treating GWI. To accommodate the various symptom profiles, the primary outcome was a single item measure of overall GWI severity. Out of the nine botanical agents tested, four showed a significant impact on GWI symptoms over both baseline and placebo conditions. These agents are resveratrol, stinging nettle, pycnogenol, and CurcumaSorb. The other botanical agents (epimedium, luteolin, boswellia, fisetin, reishi) showed no appreciable effect on GWI symptoms (Hodgin et al., 2021; Younger et al., 2021). This screening study identified four botanical agents that are currently being studied in a subsequent trial (Younger, FY21).

Gulf War Illness Inflammation Reduction Trial

Pilot studies comparing blood samples from GW Veterans with and without multiple symptoms of pain, fatigue, and cognitive dysfunction demonstrate plasma from symptomatic Veterans has significantly higher levels of inflammatory proteins and blood cells, indicating the presence of chronic inflammation. The efficacy of glucocorticoids (GCs) as anti-inflammatory and immunosuppressive drugs is clearly established. Their pleiotropic effects on immune system function make them attractive as potential treatments for the inflammation associated with GWI. Prednisone is an effective and widely prescribed synthetic GC. This recently completed, GWIRP-funded proof-of-concept clinical trial aimed to determine whether reducing inflammation with prednisone could be an effective treatment for GWI. The treatment group received a low dose of modified-release prednisone for 8 weeks while the control group received a placebo. Veterans with more severe GWI, those with lower VR-36 physical component summary scores, a measure of physical health-related quality of life, saw significant improvement in those scores compared to Veterans receiving placebo. These results, which support both the efficacy of prednisone as a treatment for GWI and the GWI inflammation hypothesis, are pending publication (Bach, FY13).

V.2.4. Ongoing Clinical Trials

Testing the Model: A Phase I/II Randomized Double-Blind Placebo Control Trial of Targeted Therapeutics: Liposomal Glutathione and Curcumin

Using data from previous studies in a dynamic computational model of GWI, investigators identified five prime targets, with NF-κB being "upstream" of several others. This GWIRP-supported trial will evaluate two nutraceuticals known to downregulate NF-κB, comparing each to placebo. It is anticipated that this intervention will impact many cellular functions, including the antioxidant and methylation-related metabolic function of PBMCs. The results of this study could support a Phase III clinical trial of the more effective of these treatments. The trial is currently recruiting participants. NCT02848417

The Use of B-Cell Suppression Therapy (BCST) in Gulf War Illness: A Phase I/II Study

Immune inflammatory biomarkers, which have been implicated in GWI, can be used to identify targeted therapeutic interventions or biologic response modifiers. This GWIRP-supported trial aims to target two different immune pathways: the pro-inflammatory cytokine cascade and

autoantibody production. Using the B-cell suppressing therapy intravenous immunoglobulin (IV-IG), investigators not only hope to decrease the presence of autoantibodies but also decrease pro-inflammatory cytokine expression and reset underlying mechanisms of disease by reducing B-cell memory cells to prevent future autoantibody production, thus restoring immune balance. The primary outcome variable in this trial is changes in SF-36 vitality and physical function subscores. This study may provide an understanding of disease onset and progression and provide a targeted therapy for at least a subgroup of patients with GWI. The study is currently ongoing, with anticipated recruitment of South Florida and a virtual option for Gulf War Veterans nationwide (Klimas, FY16).

A Randomized, Double-Blind, Placebo-Controlled Clinical Trial of Oleoylethanolamide for Targeting Lipid Metabolism in Gulf War Illness

Recent research has shown that one of the pathogenic mechanisms in GWI involves impaired lipid metabolism, which corresponds with brain glial activation and inflammation. These lipid alterations are detected in the blood of Veterans with GWI and point to an abnormal function of peroxisomes, which regulate lipids required for cellular signaling. Preclinical studies targeting peroxisomal lipid metabolism with oleoylethanolamide (OEA), a natural dietary supplement that activates peroxisome proliferation, found that OEA treatment reduced glial activation, inflammation, and neurobehavioral deficits and promoted mitochondria biogenesis in GWI mice (Joshi et al., 2018). This GWIRP-supported pilot clinical trial will test whether OEA can correct the underlying lipid disturbances and inflammation associated with GWI in a Veteran cohort. Although the primary objectives are based on biological outcomes related to lipid parameters associated with GWI, data collected on general health and symptoms of GWI will help guide the design of a future Phase III clinical trial to develop OEA as a treatment of GWI. This trial is ongoing. NCT05252949

Understanding Gulf War Illness: An Integrative Modeling Approach

Computer models of the neuroendocrine system suggest that tumor necrosis factor (TNF)-α silencing followed separately by glucocorticoid receptor blockade might be able to shift the neuroendocrine system from an abnormal state of dynamic equilibrium characterized by the GWI phenotype to a normal, healthy state. Initial pilot trials used a combination treatment strategy employing a tumor necrosis factor receptor (TNFR) antagonist followed by a glucocorticoid receptor blockade in a Phase 1 study. The team plans to repeat the dynamic modeling before treatment and during the trial to further inform the computational model and evaluate the impact of the intervention. Results of this pilot study will be expanded in a subsequent Phase 2 trial, accessing the GWIRP-supported Gulf War Illness Clinical Trials & Interventions Consortium (GWICTIC), see Section VI, Research Resources and Collaborative Efforts). NCT04255498

V.3. CNS Stimulants or Depressants

V.3.1. Why This Strategy for GWI

Exposures to various neurotoxicants were known to occur in the GW. Neurotoxicants can cause adverse effects and major functional changes in the CNS. CNS impairment affects a wide range of different capabilities, from motor skills to memory. Cognitive complaints have been particularly troublesome to Veterans with GWI, and studies have suggested that a slowing of CNS response speed is present, affecting function across multiple cognitive domains. CNS

stimulants are substances that work to activate the CNS, increasing attention and focus. Depressants are substances that reduce function of the CNS, increasing feelings of relaxation while lowering levels of awareness in the brain. CNS stimulants or depressants are known to have profound effects on memory, attention, and mood.

V.3.2. Potential Impact

Use of CNS stimulants or depressants to treat neurotoxicant-induced cognitive impairments in ill GW Veterans holds promise. Identification of an effective treatment or a biological target for treatment that has the advantage of direct access to the brain has the potential to be widely applicable. Providing Veterans even moderate relief from these chronic and debilitating symptoms could have a profound impact on improving their overall sense of well-being and QoL.

V.3.3. Completed Clinical Trials

Trial of Naltrexone and Dextromethorphan for Gulf War Veterans' Illness

Dextromethorphan is a CNS depressant while naltrexone blocks opioid receptors and reverses subjective and analgesic effects. A study supported by the GWIRP sought to determine if naltrexone and dextromethorphan were efficacious in relieving cognitive symptoms in ill GW Veterans. A secondary impact was to determine if pro-inflammatory cytokines and markers of neurogenic inflammation are elevated in the Veterans participating in the study, providing evidence for a biomarker of disease and insight into pathophysiology. Study participants taking dextromethorphan did not improve better than those on placebo. On the naltrexone side, the trial had responders (38% of study participants) and non-responders at the doses used; however, no statistical benefit was apparent when averaged over all participants. A nerve growth factor and cytokine panel showed no consistent pattern of variability, and empirical pharmacology demonstrated no benefit relative to those using no medications (Brewer et al., 2018). NCT02206490

Intranasal Insulin: A Novel Treatment for Gulf War Multisymptom Illness

Intranasal delivery of therapeutics offers direct access to the CNS. Treatment advances in other cognitive disorders and in normal subjects suggest that intranasal insulin is a safe, effective, inexpensive, and tolerable treatment that can improve memory, attention, and mood, reduce neuroinflammation, and modulate catecholamines and cortisol levels. Therefore, intranasal insulin has been identified as a treatment strategy with potential to alter leading pathobiological correlates of GWI, including neuroinflammation, synaptic function, and HPA axis dysregulation. This GWIRP-supported pilot trial aimed to assess the efficacy of two different doses of daily intranasal insulin on memory and attention functioning, as well as overall physical health and mood in GW Veterans with chronic multi-symptom illness. Primary outcome measures assessed performance on verbal and list-learning memory and attention. The study has now closed, and publication of findings is pending. If the data demonstrate efficacy, intranasal insulin could be quickly deployed for use since insulin is already FDA-approved (Golier, FY11).

D-Cycloserine: A Novel Treatment for Gulf War Illness

Advances in other neurodegenerative diseases, including Alzheimer's, suggest that d-cycloserine (DCS) is a safe, effective, and tolerable treatment that can improve memory, attention, and mood; reduce neuroinflammation; and modulate glutamate levels. DCS has been identified as a

treatment that alters many of the leading pathobiological correlates of GWI (i.e., neuroinflammation, proinflammatory cytokines, synaptic functions in hippocampus and frontal lobes). Treatment-induced changes could result in significant functional improvements in memory, attention, mood, and fatigue in Veterans with GWI. While DCS treatment generally reduced fatigue, skin problems and feelings of tension, and improved sustained attention, memory was not significantly improved in ill Gulf War Veterans (Toomey et al., 2020).

V.3.4. Ongoing Clinical Trials

Glutamate Neuroexcitotoxicity in GWI

Glutamate is the most ubiquitous neurotransmitter in the human body. Excess glutamate is well known to cause excitotoxicity, resulting in hyperexcitation and death of neurons. Glutamate is not only produced endogenously but is also used as a flavor enhancer in food. Dietary exposure to glutamate may be able to mediate excitatory neurotransmission in the nervous system, leading to a myriad of symptoms throughout the body. A previous pilot trial of this dietary intervention in ill GW Veterans saw profound symptom improvement as well as demonstrable reduction in peripheral inflammation after one month of a low glutamate diet. Improvements were noted in every symptom domain (reduced pain, fatigue, depression, anxiety, etc.) and included significant reductions in overall symptom number (Kirkland et al., 2021). The team will initiate a GWIRP-supported confirmatory, multi-site Phase 3 study to determine whether these findings are applicable to the larger GWI community and ready for transition as an effective treatment for GWI. The potential benefits of the diet include not only improvement in GWI symptoms but improvement in quality of life and potential for improvement in other health markers related to diet, including obesity, diabetes, cardiovascular disease, and high blood pressure.

V.4. Physical CNS or Neural Stimulation

V.4.1. Why This Strategy for GWI

Some GWI researchers are testing therapies using non-invasive direct stimulation of either neural networks in the brain or individual neurons. These treatments use low-level, non-detectable electrical impulses delivered to specific areas of the head to provide plastic changes (improvements) in impaired brain circuitry. The treatments seek to address symptoms like headache, widespread pain, memory loss, and cognitive deficits (e.g., word retrieval).

V.4.2. Potential Impact

These treatments have the potential to benefit ill GW Veterans by improving neural circuit communication and performance. Most of these treatment modalities have been proven effective in other areas such as TBI and neurodegenerative diseases. Some are also FDA-approved for various conditions like depression, and all are non-invasive. These treatments could represent accessible symptom relief for Veterans suffering from some of the biggest components of GWI.

V.4.3. Completed Clinical Trials

Use of a Portable Stimulator to Treat GWI

The VA War Related Illness and Injury Study Center (VA-WRIISC) found that GW Veterans commonly report symptoms of nausea and dizziness, both associated with vestibular (balance system) damage. Dizziness and vertigo can result in poor balance, which contributes to the

threat of falls, significantly reducing QoL. A VA-WRIISC pilot study funded by the GWIRP used a novel stochastic noise stimulator, which delivered an imperceptible signal to the vestibular system, to enhance vestibular function. One hundred percent (100%) of the impaired GWI Veterans using the device improved their mediolateral sway balance, and at an average rate of 42% (Serrador et al., 2018). This study paved the way for more development of this technology and vestibular hypofunction in GW Veterans.

Vagus Nerve Stimulation: A Noninvasive Treatment to Improve the Health of Gulf Veterans with Gulf War Illness

Vagus nerve stimulation (VNS) directly stimulates the vagus nerve and is currently FDA-approved for epilepsy and major depressive disorder. VNS has also been shown to be effective at reducing widespread pain and tenderness in non-Veteran women with FM (Lange et al., 2011). Given this, VNS holds promise to relieve GWI-induced pain, one of the primary complaints of the disease. This proof-of-concept trial aimed to evaluate a non-invasive version of VNS (nVNS) as a putative treatment to relieve GWI-related widespread pain and reduce migraine headaches. GWI Veterans in the study actively treated with nVNS reported no improvement in either widespread pain or migraine frequency or severity relative to Veterans with GWI who received sham nVNS, though there was a slight improvement in pain ratings from baseline to the end of the blinded phase (Natelson et al., 2021). NCT02791893

V.4.4. Ongoing Clinical Trials

Treatment of Memory Disorders in Gulf War Illness with High-Definition Transcranial Direct Cortical Stimulation

Key cognitive symptoms described by Veterans with GWI extend from disruption in the cognitive process of verbal retrieval. This team has evidence to support that a neural circuit of the pre-supplementary motor area (preSMA)-caudate-thalamus is essential for effective retrieval of verbal information. This study uses high-definition transcranial direct current stimulation (HD tDCS) over the preSMA region to strengthen the connections of this retrieval circuit and address this cognitive dysfunction, including improving word retrieval and verbal fluency. The team also aims to formalize procedures for the HD tDCS treatment and generate the training and standard operating procedures to set up and perform the treatment in a clinical setting to quickly transition this treatment to the clinic. The study is currently ongoing. NCT03542383

Long-Term Efficacy of Neuronavigation-Guided rTMS in Alleviating Headache and Pain in GWI and rTMS in Alleviating Pain and Comorbid Symptoms in Gulf War Veterans' Illness

Headaches and muscle and joint pain are some of the most common debilitating symptoms in Veterans suffering from GWI. Transcranial magnetic stimulation (TMS) is an FDA-approved treatment for major depression and migraine headache. Building on a successful pilot trial, these Phase 2 studies of TMS aim to reduce headache pain and general pain in symptomatic GW Veterans. Neuronavigation-guided rTMS non-invasively stimulates the brain, utilizing electromagnetic principles to produce small focal electrical currents in the cortex, directed at a precise location. These clinical trials will provide outcome and preliminary mechanistic evidence to validate rTMS as a low-risk, non-invasive therapy for GWI headache pain and neuropsychological dysfunction. One multi-site trial is funded by the DOD GWIRP and the other, which additionally focuses on Veterans with depression, is funded by the VA. NCT04182659 (DOD Study); NCT04046536 (VA Study)

V.5. Treatments Targeting the Gut-Brain Axis

V.5.1. Why This Strategy for GWI

Since the digestive system is the body's largest interface with foreign agents in the environment, it is profoundly connected with the immune system. The "gut-brain axis" is a complex, bi-directional communication system between the CNS and the enteric (gut) nervous system. This system encompasses the sympathetic and parasympathetic arms of the autonomic nervous system, the endocrine hormonal system (HPA axis), and of course, the immune system. An ecosystem of microbes called the "gut microbiome" or "gut microflora" lives inside the gut. Not surprisingly, the composition and health of this ecosystem has a significant impact on the immune system and, thus, the gut-brain axis and ultimately overall health. Changes in the microbiome have been observed in several diseases, and while in the past these changes have been thought to be a result of disease, it has slowly become clear that therapeutic manipulation of the gut microbiome can alter some diseases and reduce symptoms. Since many manifestations of GWI are related to endocrine/autonomic dysregulation and exaggerated immune/inflammatory responses, researchers felt there was a good possibility that GWI might be abated or at least attenuated by manipulation of the gut microbiome.

V.5.2. Potential Impact

Many symptoms of GWI are thought to directly involve exaggerated immune/neuroinflammatory responses and hormonal imbalance including headache, joint/body pain, GI disruption, and fatigue. One theory holds that individuals suffering such symptoms would most likely benefit from treatments affecting the gut microbiome and gut-brain axis. Other symptoms such as sleep, skin, or respiratory disorders are not so obviously connected to immunity or inflammation; however, an underlying connection to inflammation is not unreasonable, so gut-brain axis treatments may be useful in those cases as well. It is not known whether treatments aimed at the gut microbiome could provide long-term relief or would simply temporarily alleviate symptoms and still require regular treatment.

V.5.3. Completed Clinical Trials

Bacterial Overgrowth Associated with Chronic Multi-Symptom Illness Complex

Rifaximin is a minimally absorbed antibiotic thought to reduce IBS by helping to restore normal gut microflora. A double-blind, placebo-controlled study was carried out to compare the effects of 2 weeks of rifaximin treatment with placebo. Endpoints included various self-reported QoL measures and other measures specifically related to bowel function including an overall Bowel Symptom Score. The study was ended with 44 subjects completing the protocol. Rifaximin was not found to be effective in improving IBS symptoms and QoL in GW Veterans with non-constipated IBS (Tuteja et al., 2019).

V.5.4. Ongoing Clinical Trials

Probiotic (Bifidobacterium infantis) for Gulf War Illness

Altered gut flora may be the etiological factor for IBS and GWI. Probiotics are living organisms that improve health by re-establishing a normal gut flora. Probiotics also have anti-inflammatory properties. Probiotics have been claimed to be of some benefit in non-Veterans with IBS. This study aims to demonstrate the safety and effectiveness of *B. infantis* in the treatment of IBS and

non-intestinal symptoms of IBS that are indistinguishable from GWI. The knowledge gained from this study may also benefit other travelers who develop IBS on return. Endpoints measured include changes in bowel flora and various self-reported QoL measures and other measures specifically related to bowel function including an overall Bowel Symptom Score. For this study, 60 Veterans have been enrolled and 55 have completed to date. Stool samples have been sent for analysis, and the investigators are currently preparing data for analysis (Tuteja, FY09).

Effect of Diet on Gulf War Illness: A Pilot Study

Fermentable oligo-, di- and monosaccharides, and polyols (FODMAPs) are carbohydrates that are poorly absorbed in the small intestine. Undigested FODMAPs are fermented in the colon by microbiota, increase osmotic load, increase delivery of water into the colon, and can produce gas/distension of the colon. FODMAP ingestion does not produce symptoms in most people but can produce GI symptoms in people with IBS. A diet low in FODMAPs has been shown to reduce symptoms and may do so (1) by changing the gut microbiota and/or (2) by production of metabolites, which can indirectly influence host physiology. A low-FODMAP diet has also been shown to improve cognitive function and depression, which are symptoms common in GWI. This study is designed to assess the safety and effectiveness of a low-FODMAP diet in the treatment of GWI. In addition to changes in bowel flora and measures specifically related to bowel function and QoL, endpoints include improvements in a broad assortment of non-bowel-related GWI symptoms. This trial is in progress. NCT02881944

V.6. Treatments Targeting Mitochondria and Reactive Oxygen Species

V.6.1. Why This Strategy for GWI

Mitochondria are the subcellular organelles that produce a large proportion of the cell's energy. Basic research studies have shown that mitochondrial dysfunction and deficiencies in physiological energy availability are associated with GWI. These deficiencies can have two types of effects: One is a direct impact on energy-requiring actions such as muscle contraction, nerve conduction, or chemical processes protein synthesis. The other is a more indirect effect on cellular oxidation state, exposing cells to the detrimental oxidative effects of reactive oxygen species (ROS). Either of these effects can produce fatigue directly and contribute to inflammation, which is thought to give rise to many of the symptoms associated with GWI. Because of this, drugs, vitamins, and dietary supplements that are known to improve energy production and scavenge ROS are being assessed for relief of GWI symptomatology. These substances interact with the cell's energy production machinery to obtain high-energy electrons, which they then transfer to ROS to detoxify them.

V.6.2. Potential Impact

These treatments would be expected to reduce inflammation and affect many symptoms of GWI, but especially chronic fatigue and body pain. Such treatments would provide abatement of symptoms rather than a cure and would be expected to be administered on an ongoing basis.

V.6.3. Completed Clinical Trials

CNDP1 Polymorphisms and Carnosine Therapy in GWI

The dipeptide carnosine (β-alanyl-L-histidine) is a compound found in high concentrations in brain and muscle and has antioxidant and neuromodulatory properties. Studies found that an enzyme called carnosine dipeptidase 1 (CNDP1) that cleaves and destroys carnosine was present in elevated amounts in Veterans with GWI, so investigators undertook this randomized double-blind placebo-controlled 12-week dose escalation study in 25 subjects to assess the effects of carnosine administration on GWI symptoms and try to assess whether subjects possessed mutations in CNDP1 that might affect carnosine metabolism. Outcomes included subjective fatigue, pain, and psychosocial questionnaires, as well as instantaneous fatigue and activity levels recorded by ActiWatch Score devices. Cognitive function was evaluated by Wechsler Adult Intelligence Scale – Revised (WAIS-R) digit symbol substitution test. Significant increases in WAIS-R scores, suggestive of improved cognitive function, and a decrease in diarrhea associated with IBS were observed in ill GW Veterans taking carnosine (Baraniuk et al., 2013).

A Prospective Open-Label Clinical Trial of Methylphenidate plus a GWI-Specific Nutrient Formula in Patients with Gulf War Illness and Concentration Disturbances

The impact of a safe and effective treatment for the fatigue and cognitive issues faced by GWI patients would be substantial for this long-suffering population. This treatment combines a proprietary cocktail, KPAX002, which combined antioxidants with the CNS stimulant methylphenidate. The primary clinical efficacy assessment used was the proprietary GWI Symptoms Assessment Tool (SAT), along with a secondary efficacy assessment, the Checklist Individual Strength (CIS) total score. GWI Veterans taking KPAX002 for 12 weeks had a significant reduction in overall symptom severity as demonstrated by reductions in SAT and CIS total scores, as well as improved Visual Analog Scale scores for concentration disturbance symptoms, fatigue, sleep, and pain. There was also a significant reduction in serum lipid peroxide levels. The study has been published and a patent application is pending (Kaiser, 2016 and Holodniy et al., 2019).

Q10 for Gulf War Veterans

A double-blind placebo-controlled crossover study was used to assess whether administration of Coenzyme Q10 (CoQ10) administration (in the oxidized form called ubiquinone, across two dose levels) would reduce symptoms and improve subjective health in GW Veterans. Endpoints included general self-rated health scores, individual symptom scores (self-rated scores on 20 GWI-associated symptoms), systolic blood pressure, and the summary performance score (SPS). Results in males, which constituted 85% of the cohort, showed that the lower dose of CoQ10 (100mg) significantly benefited general self-rated health when compared to those that received either placebo or the higher dose of CoQ10 (300 mg). The summary performance score, reflecting overall functional performance, was also significantly higher in the 100-mg group versus placebo. Among 20 individual symptoms, each present in half or more of the enrolled Veterans, all but one (sleep problems) trended toward improvement in the 100-mg group versus

placebo (Golomb et al., 2014). These promising results were the basis for an FY21 expanded replication trial currently in progress (see Ongoing Clinical Trials).

Extending Benefits of CoQ10: Mitochondrial Cocktail for Gulf War Illness

Having proven some benefit to Veterans with GWI from CoQ10 treatment in the study described above, the same researchers conceived a further study to attempt combining CoQ10 administration (in the oxidized form called ubiquinone) with a cocktail of other antioxidants and metabolites, which would be adjusted for each subject on an individual basis to achieve optimal metabolic balance and mitochondrial function. In a double-blind, randomized, sham-controlled trial of 6 months duration, researchers will measure balance of amino acids and citric acid cycle metabolites in addition to a large number of validated and non-validated measures of fatigue, cognition and memory, lower extremity function, QoL, pain, and sleep quality. This trial has completed enrollment and is now analyzing results. NCT02804828

Dietary Polyphenol Preparations for Treating Veterans with Gulf War Illness

Polyphenols are a class of antioxidant molecules found in common plants and fruits, which have been shown to improve fatigue and preserve cognitive function in other disorders. Researchers designed a 6-month, randomized, double-blind placebo-controlled study to assess safety, tolerability, feasibility, and efficacy of high-polyphenol dietary supplementation to treat cognitive deficits and chronic fatigue in Veterans with GWI. The treatment was found to be safe and well-tolerated by Veterans with GWI and one measure of cognitive function, the Halstead Category Test–Russell Revised Version, showed statistically significant improvement compared to placebo. Other measures of cognitive functioning did not indicate significant improvement (Helmer et al., 2020). NCT02915237

CoQ10 in Gulf War Illness

The primary objective of this clinical trial is to determine if treatment with ubiquinol, a form of CoQ10, improves the physical function of men and women Veterans suffering from GWI. The primary outcome measure is a change from baseline on the Short Form Health Survey 36-item (SF-36), with respect to physical functioning and symptoms. Secondary outcome measures include changes from baseline levels on GWI-associated biomarkers in peripheral blood and GWI-associated symptoms of chronic pain, fatigue, insomnia, activity level, and cognitive and mental functioning. In trials of CoQ10 for other neurological conditions, the non-oxidized form of CoQ10, called ubiquinol, was found to be more effective than the oxidized form, called ubiquinone, so the researchers are using the non-oxidized form, ubiquinol, in this study. This VA-funded study is completed and results have not yet been published. NCT02865460

A Pilot Randomized Control Trial on the Effect of Resveratrol on Mood, Memory Deficits, Hippocampal Inflammation, and Neurogenesis in Veterans with Gulf War Illness

The polyphenols resveratrol and quercetin are known antioxidant and anti-inflammatory substances found in abundance in grapes and berries and other fruits, nuts, and seeds. Researchers will conduct a three-armed, placebo-controlled, 26-week study of the effects of resveratrol alone or resveratrol plus quercetin in 93 Veterans with GWI. The active study has concluded, and results are being analyzed. NCT03665740

V.6.4. Ongoing Clinical Trials

Replication Phase III Trial of Coenzyme Q10 in Gulf War Illness

Based on the success of the prior small trial of CoQ10 described above, researchers have embarked on a large-scale Phase III trial of CoQ10. This double-blind, placebo-controlled four-site trial has a recruitment target of 225 Veterans with GWI to provide definitive support for this treatment. Endpoints include SF-36 physical function score and fatigue, pain, sleep subscales along with neurocognitive testing. Biomarkers for treatment efficacy and pathophysiological improvement are also included. It is hoped that this replication/validation study for CoQ10 will give definitive support for a widely accessible, proven treatment for those suffering from GWI. The trial is in the introductory stages and will be posted to clinicaltrials.gov when ready for recruitment (Golomb, FY19).

A Randomized Double-Blind Placebo-Controlled Clinical Trial of Nicotinamide Riboside for Restoring Mitochondrial Bioenergetics in Gulf War Illness

Clinical studies have shown that Nicotinamide Riboside (NR) is safe in individuals as an anti-aging supplement. Based on the promising outcomes of these reports and GWI animal model studies, this clinical trial aims to test whether a nutraceutical form of NR can be useful as a future treatment for GWI. The study will examine whether NR treatment can restore nicotinamide adenine dinucleotide (NAD+) levels to normal, restore altered lipid levels associated with abnormal mitochondrial function in GWI, and reduce inflammation. It is anticipated that these metabolic improvements will correspond with reducing fatigue, pain, and memory problems experienced by Veterans with GWI. The trial is currently recruiting. NCT05243290

V.7. Other Treatments

V.7.1. Why This Strategy for GWI

Some GWI research projects fall outside the major focus areas of research. These studies have targeted individual symptoms, like sleep quality, fatigue, and impaired cognitive performance, or explored a different theory of the nature of GWI or applied a known therapy practice to the illness.

V.7.2. Potential Impact

These studies encompass early attempts to address treating GWI at its essence and applying either an established treatment or more recent technology to the disease. Some were shown to be effective while others pointed researchers in a different direction. Study results are being analyzed for one therapy that has yet to realize its potential to help Veterans with GWI but could lead to relief of cognitive problems.

V.7.3. Completed Clinical Trials

Sleep Disordered Breathing in Gulf War Illness and the Effect of Nasal CPAP Treatment

This pilot study investigated sleep-disordered breathing in 17 Veterans with GWI and assessed the effect of treatment with continuous positive airway pressure (CPAP) to alleviate the symptoms of GWI. Participants received 3 weeks of treatment with either therapeutic nasal CPAP or sham nasal CPAP. Beyond improved sleep quality, those receiving therapeutic nasal

CPAP exhibited significant improvement in pain, fatigue, cognitive function, and physical and mental health. The results were published in 2011 (Amin, Belisova, et al., 2011; Amin, Gold, et al., 2011).

Antibiotic Treatment of GW Veterans' Illnesses (ABT)

This study was based on the hypothesis that GWI resulted from systemic *Mycoplasma fermentans* infection. The Antibiotic Treatment Trial of GWVI was a randomized placebo-controlled trial to determine whether a 1-year course of doxycycline treatment in GW Veterans with GWI (and testing positive for *Mycoplasma* species) would improve their overall functional status by a self-reported measure (SF-36V Physical Component subscore). The VA-funded multi-site trial enrolled 491 deployed GW Veterans who took doxycycline or a matching placebo daily for 12 months. The study found no statistically significant difference between the doxycycline and placebo groups for the primary outcome measure, improvement in self-reported health status. The results were published in 2004 (Donta et al., 2004).

Cognitive Behavioral Therapy and Aerobic Exercise for Gulf War Veterans' Illnesses: A Randomized Controlled Trial

Because there was originally no consensus etiology or basis for GWI, CBT and exercise were investigated to relieve symptoms experienced by Veterans. This study compared CBT and exercise, or either activity alone, to usual care to improve self-reported health status measured with the Veteran's SF-36 Physical Component subscore. CBT sessions and exercise sessions were conducted weekly for 12 weeks. After 1 year, 18.4% of the participating Veterans using CBT plus exercise showed improvement in physical function, 18.5% with CBT alone, 11.7% with exercise alone, and 11.5% with usual care. For secondary outcomes, exercise alone or in combination with CBT significantly improved fatigue, distress, cognitive symptoms, and mental health functioning. CBT alone significantly improved cognitive symptoms and mental health functioning. Neither treatment had a significant impact on pain. The study showed that CBT and/or exercise could provide modest relief for some of the symptoms of GWI. The results were published in 2003 (Donta et al., 2003).

Transcranial, Light-Emitting Diode (LED) Therapy to Improve Cognition in GWVI

This study investigated whether application of LED in red (visible) and near-infrared (NIR) wavelengths to the scalp resulted in improvement in cognition in Veterans with GWI. These wavelengths had been shown to improve energy production in cells, especially hypoxic or compromised cells. The treatment increased regional cerebral blood flow, which is associated with improved cognition, in chronic TBI. Impaired cognition is one of the major symptom areas of GWI. The procedure is considered non-invasive by FDA and is painless. The study incorporated a cross-over design where one group received two treatments of active light per week for 7.5 weeks followed by the same regimen with sham treatment. Another group received the sham treatment first for 7.5 weeks, followed by the same regimen with active treatment. The active phase of the study is completed and results are being analyzed. NCT 01782378

VI. Research Resources and Collaborative Efforts

VI.1. Consortia and Biorepositories

In FY12, the GWIRP supported two Consortium Awards (Morris and Klimas, FY12; Sullivan, FY12) that brought together diverse groups of experts with the goal of executing preclinical and clinical studies that would increase understanding of the mechanisms responsible for mediating GWI and lead to the discovery of potential therapeutics for Veterans with GWI. In later fiscal years, these consortium efforts were the recipients of new awards (Klimas, FY17; Sullivan, FY17) aimed at further advancing their efforts toward providing solutions for ill GW Veterans. Since FY12, these teams have also collaborated with other GWIRP-funded research teams through other efforts. The data obtained by these consortia have provided a critical step forward in understanding the pathobiological mechanisms responsible for GWI and have produced key evidence to support the use of specific therapeutics to treat Veterans with GWI.

A summary of each consortium effort is described below.

Gulf War Illness Clinical Trials & Interventions Consortium (GWICTIC)

One FY12 Consortium Award was made to Nova Southeastern University to support efforts led by a team of investigators including Drs. Mariana Morris, Nancy Klimas, and Gordon Broderick. The team focused on identifying candidate treatments by merging preclinical animal model studies comparing cytokine, hormone, and neuropeptide expression profiles with advanced computer simulations of aberrant metabolic activity. Based on these findings, Dr. Klimas and her team selected a combination treatment strategy that targets the inflammation and endocrine dysfunction observed in GWI. The treatment, now being evaluated in a Phase 1 study of ill GW Veterans, consists of Etanercept, a tumor necrosis factor receptor antagonist, and mifepristone, an anti-glucocorticoid.

In FY17, Nova Southeastern University received a GWIRP Clinical Consortium Award to support the consortium led by Dr. Klimas. This consortium, now known as the Gulf War Illness Clinical Trials and Interventions Consortium (GWICTIC), aims to combine their expertise to continue to build upon their earlier results by implementing one Phase 1 and three Phase 2 clinical trials to evaluate candidate therapeutics for GWI. The Phase 1 trial will extend the initial evaluation of the Etanercept plus mifepristone treatment that was performed during the FY12 award and then move forward to a Phase 2 trial of the most promising treatment regimen. Subsequent interventions will target additional pathobiological mechanisms by investigating *Bacopa monnieri* and n-Acetylcysteine (NAC) (Klimas, FY17).

Boston Biorepository, Recruitment, and Integrative Network (BBRAIN)

The second FY12 Consortium Award was granted to Boston University and led by Dr. Kimberly Sullivan. This consortium's central hypothesis identified chronic neuroinflammation as an end result of initial glial activation and subsequent priming of the glial response, giving strong evidence for a neuroinflammatory component in GWI. In support of this effort, they developed an independent GW Veteran biorepository allowing them to complete studies evaluating GW Veteran blood samples. Through these efforts, the team noted significant differences between the immune cells of Veterans with GWI and controls (Steele et al., 2021).

Boston University was also the recipient of a Biorepository Resource Network Award, again led by Dr. Kimberly Sullivan, to create the Boston Biorepository Recruitment and Integrative Network (BBRAIN), which houses the biologic samples collected in the previous effort and will be expanded to include additional biological specimens and clinical data from 300 Veterans with GWI and 200 GW-Era Veteran controls recruited from across the country. Participants will provide blood, urine, and other biospecimens, and a subset of Veterans will complete neuropsychological testing and brain imaging. Blood samples and neuroimaging will be combined with machine learning and evaluated to better characterize GWI, identify potential biomarkers, and facilitate treatment development (Sullivan, FY17).

Notably, the GWICTIC and the BBRAIN teams of investigators have collaborated through their multiple efforts to expand these recruitment networks and researcher resources. Novel treatment strategies and objective markers of GWI are critical needs for the GWI community. The GWICTIC will help meet these needs by establishing a streamlined resource for conducting Phase 1 and 2 clinical trials to evaluate potential GWI therapeutics, while the BBRAIN will provide a key resource for the exploration of mechanisms, identification of biomarkers, and selection of innovative treatment strategies for GWI. With these projects, Drs. Klimas and Sullivan plan to continue fostering and creating a collaborative environment for researchers interested in understanding and tackling the pathology of GWI.

VI.2. Common Data Elements

Through a collaboration of the NIH, CDC, VA, DOD GWIRP, and the GWI community, common data elements (CDE) recommendations for GWI have been developed and published (Cohen et al., 2022). The goals of this effort are to increase the efficiency and effectiveness of clinical research studies and treatment, increase data quality, facilitate data sharing and aggregation of information across studies, and help educate new clinical investigators. Development of CDEs is an iterative process, and updates are expected as research progresses and feedback is received from the community.

VI.3. Deep Phenotyping

Large-scale genotype and phenotype investigative efforts are underway through a collaborative effort by the VA and the National Institute for Neurological Diseases and Stroke (NINDS). The Investigative Deep Phenotyping Study of Gulf War Veteran Health (IN-DEPTH) Study, with lead investigators from each organization, will examine relationships between genetic variations and the physical traits of ill GW Veterans through deep phenotyping. Results from the study will guide the design of future studies to elucidate the biologic mechanisms underlying GWI as well as identify potential mechanisms for intervention. It will also be the basis for a repository of data and specimens to engage the wider VA and non-VA scientific community in GWI research.

VII. Remaining Gaps in Understanding, Identifying, and Treating GWI

Though it has been over 30 years since the GW, there are still several remaining gaps surrounding how Veterans impacted by GWI are diagnosed and treated, and what exposure(s) and mechanisms are behind the development of these clusters of symptoms. Furthermore,

because this is a complex, multi-symptom illness that impacts an aging population, there are also new challenges associated with our ability to diagnose and predict GW progression/symptoms, and effectively treat and improve the QoL those impacted. This final section highlights some of the existing gaps, many of which were highlighted by the DOD GWIRP Programmatic Panel members at the final panel meeting in March 2022.

VII.1. Prognostic Research Needs and the Impact of an Aging Veteran Population

Little is known about the long-term prognosis of those with GWI, the impact of aging on GWI, and the rates at which GW Veterans are affected by other diseases. The 2014 report by the RAC-GWVI summarized investigations addressing health changes related to GWI (RAC-GWVI Research Update and Recommendations, 2014). The report states that Veterans of the 1990-1991 GW generally are in poorer health and present with greater disability than other Veterans of the same era that were not deployed to the Persian Gulf. Research suggests that the GWI symptomatology experienced by Veterans has not improved over the last 30 years, with few experiencing improvement or recovery. Many GW Veterans are currently or will soon begin experiencing comorbidities associated with aging. Indeed, studies by Zundel et al. demonstrate that GW Veterans have significantly greater odds of developing chronic conditions and that, as Veterans age, they will continue to develop these chronic conditions and other diseases associated with aging (Zundel et al., 2019). The effects that aging will have on this unique and vulnerable population remain a matter of significant concern. Population-based, longitudinal studies designed to yield insight into the mortality, morbidity, and symptomatology of GW Veterans would facilitate a greater understanding of the impacts of GWI in an aging Veteran population and hopefully guide treatment/therapeutic options.

VII.2. Etiologic Research Needs

Uncertainty regarding the types and doses of agent exposures, as well as a lack of scientific knowledge about the synergistic effects of exposures to multiple agents, have precluded a consistent theory of GWI etiology. Identification of objective markers of GW-relevant exposures and downstream effects of those exposures, including latent effects that represent the current status of Veterans with GWI, are needed. In addition, comparative studies designed to identify the effects of exposure to multiple toxic agents and stressors remains a priority for the field. Studies investigating genetic predisposition and susceptibility to GW-relevant exposures will improve the field's understanding of why some Veterans have developed GWI while others have not and may also inform individual treatment plans.

VII.3. Pathobiology Research Needs

While some of the underlying pathobiology of GWI has been elucidated, much more still needs to be understood (see Pathobiology of Gulf War Illness). Specifically, which cellular pathways are relevant to GWI, how sex, race, and other subtype differences may impact progression, outcomes, and treatment, and a more thorough understanding of crosstalk between human systems like the gut-brain axis would facilitate better diagnostics and treatment options. Expanded studies focused on molecular target validation and confirmation will strengthen future treatment research. Genotypic and phenotypic analyses and identification of molecular signatures that underlie symptom clusters could improve individualized treatment plans to

support better QoL. Additionally, in the absence of a consensus case definition for GWI, the need continues for applied research aimed at producing a robust, evidence-based definition for both clinical and research applications. These efforts are currently underway at the VA.

VII.4. Treatment Needs

Improving the lives of those impacted by GWI remains a major priority. Treatments focused on addressing the myriad of symptoms experienced by ill GW Veterans aimed at improving their QoL and restoring dysregulated molecular pathways to health are needed. These approaches require validation of promising leads and a clear definition of mechanistic outcomes. Repurposing existing therapeutics and treatments and pursuing those treatments that can quickly be implemented in a clinical setting and be made readily available to Veterans everywhere continue to be important. Treatments that acknowledge aging comorbidities will need to be developed. Personalized medicine approaches tailored to individual Veterans will be of the most benefit, given the heterogeneity of GWI presentation.

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VIII. Acronyms

AChE	Acetylcholinesterase
ACTH	Adrenocorticotropic Hormone, Adrenocorticotrophic Hormone
AD	Alzheimer's Disease
ADEV	Astrocyte-Derived Extracellular Vesicles
AGHD	Adult Growth Hormone Deficiency
APOE	Apolipoprotein E
BBB	Blood–Brain Barrier
BBRAIN	Boston Biorepository, Recruitment, and Integrative Network
BCDT	B-Cell Depletion Therapy
BChE	Butyrylcholinesterase
BDNF	Brain-Derived Neurotrophic Factor
BOLD	Blood Oxygen Level Dependent
BPI	Brief Pain Inventory
CAM	Complementary and Alternative
CARC	Chemical Agent-Resistant Coating
CBT	Cognitive Behavioral Therapy
CBT-I	CBT with Insomnia
CDC	Centers for Disease Control and Prevention
CDE	Common Data Elements
CFS	Chronic Fatigue Syndrome
CIS	Checklist Individual Strength
CMI	Chronic Multi-Symptom Illness
CNDP1	Carnosine Dipeptidase 1
CNS	Central Nervous System
CORT	Corticosterone
CoQ10	Coenzyme Q10
CPAP	Continuous Positive Airway Pressure
CPF	Chlorpyrifos
CpG	Regions of DNA where a guanine nt follows a cysteine nt (5'—C—phosphate—G—3')
CRP	Cell Types and C Reactive Protein
CRS	Chronic Rhinosinusitis
CT	Cognitive Therapy
CT-PRM	Computed Tomography-Parametric Response Mapping

DCS	D-Cycloserine
DEET	N,N-Diethyl-Meta-Toluamide
DFP	Diisopropyl Fluorophosphates
DOD	U.S. Department of Defense
DR	Delayed Release
DR-CB	Deployment-Related Chronic (or Constrictive) Bronchiolitis
DTI	Diffusion Tensor Imaging
DU	Depleted Uranium
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	U.S. Food and Drug Administration
FDC	Ft. Devens Cohort
FGIDs	Functional Gastrointestinal Disorders
FM	Fibromyalgia
fMRI	Functional Magnetic Resonance Imaging
FODMAPs	Fermentable Oligo-, Di and Mono-saccharides And Polyols
GCs	Glucocorticoids
GERD	Gastroesophageal Reflux Disease
GI	Gastrointestinal
GPA	Glycophorin A
GSRH	General Self-Rated Health
GW	Gulf War
GWI	Gulf War Illness
GWICTIC	Gulf War Illness Clinical Trials and Interventions Consortium
HD tDCS	High-Definition Transcranial Direct Current Stimulation
hiPSC	human induced pluripotent stem cell culture
HLA	Human Leukocyte Antigen
HMGB1	High Mobility Group Box Protein 1
HPA	Hypothalamic-Pituitary-Adrenal
HPG	Hypothalamic-Pituitary-Gonad
IBS	Irritable Bowel Syndrome
IL-1β	Interleukin-1β
IL-15	Interleukin-15
IL1R1	Interleukin 1 Receptor Type 1
IN-DEPTH	Investigative Deep Phenotyping Study of Gulf War Veteran Health
IOM	Institute of Medicine

iRest(R)	Integrative Restoration
IV-IG	Intravenous Immunoglobulin
kDa	Kilodalton
LC/MS	Liquid Chromatography / Mass Spectrometry
LED	Light Emitting Diode
LIBS	Laser-Induced Breakdown Spectroscopy
MBB	Mind-Body Bridging
MBIs	Mindfulness-Based Interventions
MBSR	Mindfulness-Based Stress Reduction
MC CBT	Minimal Contact Cognitive Behavioral Therapy
ME/CFS	Myalgic Encephalomyelitis/CFS
miRNA	microRNAs
MOS-SS	Medical Outcomes Study - Sleep Scale
MRI	Magnetic Resonance Imaging
NAC	n-Acetylcysteine
NAD+	Nicotinamide Adenine Dinucleotide
NAM	National Academy of Medicine
NDEV	Neuron-Derived Extracellular Vesicles
NDI	Neurite Density Imaging
NDI	Neurodegeneration Index
NER	Nucleotide Excision Repair
NIH	National Institutes of Health
NINDS	National Institute for Neurological Diseases and Stroke
NIR	Near-Infrared
NR	Nicotinamide Riboside
n-VNS	Non-invasive Version of Vagus Nerve Stimulation
OEA	Oleoylethanolamide
3-PBA	3-Phenoxybenzoic Acid
PBMCs	Peripheral Blood Mononuclear Cells
PER	Pesticides Permethrin
PET	Positron Emission Tomography
piRNA	PIWI-Interacting RNA
PON1	Paraoxonase 1
preSMA	Pre-Supplementary Motor Area
PTSD	Post-Traumatic Stress Disorder

QoL	Quality of Life
RAC-GWVI	Research Advisory Committee on Gulf War Veterans' Illnesses
ROS	Reactive Oxygen Species
rPFS	Revised Piper Fatigue Scale
rTMS	Repetitive Transcranial Magnetic Stimulation
SAT	Symptoms Assessment Tool
SF-36	Short Form Health Survey 36-Item
SMA	Supplementary Motor Area
S-NI	Saline Nasal Irrigation
SNI	Synchronous Neural Interactions
SNOT-20	Sino-Nasal Outcome Test-20
SPS	Summary Performance Score
SR	Sleep Restriction
SSRI	Selective Serotonin Reuptake Inhibitor
START	Stress Test Activated Reversible Tachycardia
STOP	Stress Test Originated Phantom Pain
T1W-MRI	T1-Weighted Magnetic Resonance Imaging
TBI	Traumatic Brain Injury
tDCS	Transcranial Direct Current Stimulation
TMS	Transcranial Magnetic Stimulation
TNF	Tumor Necrosis Factor
TNFR	TNF Receptor
TSPO	Translocator Protein
VA	U.S. Department of Veterans Affairs
VNS	Vagus Nerve Stimulation
WAIS-R	Wechsler Adult Intelligence Scale – Revised
WRIISC	War Related Injury and Illness Study Center
X-NI	Xylitol Nasal Irrigation