Project Log for PASA 1 & 2 Awards – Apr2022 – Meds Under Study

Log Number	Proposal Title
AS140026-A1	Preclinical Analysis of Combined Zonisamide and Doxazosin Treatments in Stress--Alcohol Drinking Models
AS140026-A2	Assessing pharmacotherapies in animal models of post-traumatic stress disorder and alcohol use disorder
AS140026-A3a	Efficacy and Safety Study of PT150 (formally ORG34517) in Veterans with Co-morbid PTSD/AUD
AS140026-A3b	Efficacy and Safety Study of PT150 (formally ORG34517) in Veterans with Co-morbid PTSD/AUD - Alcohol InPatient Study
AS140026-A3c	Effects of Ethanol on the Pharmacokinetics of PT-150 (Formerly ORG34517)
AS140026-A4	Zonisamide as a New Treatment for PTSD & Co-Occurring AUD
AS140026-A5	Kappa Opioid Receptor Antagonist for the Treatment of Alcohol Use Disorder and Comorbid PTSD
AS140026-A6	Lofexidine Combined with Buprenorphine for Reducing Symptoms of Post-Traumatic Stress Disorder and Opioid Use Relapse in Veterans
AS140026-A7	N-acetylcysteine Treatment to Reduce Alcohol Use, Cognitive Impairment and PTSD Symptom Severity in Veterans with Traumatic Brain Injury and Alcohol Use Disorder: A Confirmatory Study
AS170014-A1	Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-Fentanyl Vaccine and Buprenorphine Combination Therapy
AS170014-A2	Preclinical assessment of PT-150 for opioid use disorder and PTSD
AS170014-A3	Developing a Proof of Concept Clinical Trial to Evaluate the Use of a Safe and Highly Selective α2a Adrenergic Receptor Agonist, BXCL 501, for the Treatment of ASUD Comorbid with PTSD and/or TBI
AS170014-A4	An Aldehyde Dehydrogenase 2 (ALDH2) Inhibitor for Treatment of PTSD and AUD
AS170014-A5	Preclinical Testing of FKBP5 Inhibitors for Alcohol Use Disorder-PTSD Comorbidity
AS170014-A6	Lofexidine Combined with Buprenorphine for Reducing Symptoms of Post-Traumatic Stress Disorder and Opioid Use Relapse in Veterans
AS170014-A7	Effect of Sublingual formulation of Dexmedetomidine HCl (BXCL501) on Ethanol in Heavy Drinkers with PTSD
AS170014-A8	Medication Development of an Anti-Fentanyl Vaccine for Opioid Use Disorder
AS170014-A9	Leveraging Multi-Omic Data Integration for In-Silico Compound Prioritization

The following medications/compounds are being studied by PASA for their effects on alcohol use disorders (AUD) and opioid use disorders (OUD) on patients with comorbid PTSD:

1) a kappa opiate receptor (KOR) antagonist (CERC-501)
2) alpha adrenergic blockers (doxazosin, lofexidine and BXCL501 (dexmedetomidine))
3) a cortisol blocker (PT-150)
4) a gamma amino butyric acid type B($GABA_B$) receptor allosteric modulator (ASP8062)
5) a GABA receptor agonist (baclofen)
6) a complex anti-seizure medication (zonisamide)
7) a partial opioid agonist at the mu-opioid receptor and an antagonist at the kappa-opioid receptor (buprenorphine)
8) an opioid antagonist (naltrexone)
9) a FKBP5 inhibitor (benztropine mesylate)
10) vaccine formulation of CRM-GFEN +dmLT (CRM197-glutaryl fentanyl plus adjuvant dmLT)

Nine medications are undergoing or have completed *pre-clinical* investigation (baclofen, doxazosin, zonisamide, ASP8062, CERC-501, PT150, FKBP5 inhibitor, buprenorphine, and a CRM197-GFEN vaccine) and six are undergoing or being considered for *clinical* evaluations (PT150, buprenorphine, naltrexone, lofexidine, BXCL 501). In order to broadly explore treatment of AUD and post-traumatic stress disorder (PTSD), we have selected compounds with a range of non-overlapping mechanisms of action (MOA) that have been shown relevant in these diseases.

Some medications in this list are positive controls such as ***baclofen*** for its action in reducing alcohol use in animal models and humans, doxazosin for its efficacy reducing alcohol use and PTSD symptoms in animal models and humans, and ***naltrexone*** for its efficacy in reducing alcohol use in animals and humans.

Three medications deserving a special explanation are ***doxazosin***, ***lofexidine and BXCL501 (dexmedetomidine-DEX on a sublingual film)***. DEX is a selective and potent alpha2-adrenergic receptor agonist, other alpha2 adrenergic agonists have been developed for clinical use including clonidine, lofexidine and guanfacine, however DEX is more potent than these other alpha2 adrenergic agonists and achieves high free brain levels after dosing suggesting it may have superior pharmacological and pharmacokinetic properties than other drugs in this class. BioXcel is currently developing BXCL501 (dexmedetomidine on a sublingual film) for the treatment of acute agitation in patients with schizophrenia and bipolar disorders. The properties of BXCL501 indicate it may be an effective therapeutic for the treatment of patients with PTSD as well, especially those that are undergoing treatment for ASUD. DEX exerts its effects by preventing release of the neurotransmitter norepinephrine from neurons in the locus coeruleus (LC). LC neurons and norepinephrine are responsible for stress-related agitation as a result of hyper-arousal of the sympathetic nervous. Because PTSD is associated with hyper-arousal and high sympathetic nervous system activity, BXCL501 has the potential to alleviate agitation that occurs in PTSD. Doxazosin is a direct blocker (antagonist) of post-synaptic alpha 1 adrenoreceptors, while lofexidine and BXCL501 are agonists at pre-synaptic or auto- alpha 2

adrenoreceptors. The MOA for lofexidine and BXCL501 reducing adrenergic activity in the brain is through feedback inhibition of norepinephrine release from these pre-synaptic neurons rather than directly blocking the effects of norepinephrine on the post-synaptic neurons. Thus, lofexidine and BXCL501 have a much broader effect than doxazosin in reducing brain adrenergic activity because lofexidine and BXCL501 not only reduce activity at alpha-1 receptors, but also reduce activity at all the four types of both alpha and beta adrenroreceptors.

Endogenous opioid systems in the brain are involved in regulation of mood, stress modulation, and cravings. Kappa opioid receptors are densely localized in limbic and cortical areas comprising the brain reward system, which play a role in modulating stress and in promoting addictive behaviors. The MOA for KOR antagonists like ***CERC-501*** involve blocking the increased dynorphin A (endogenous ligand for KOR) and increased KOR signaling in the amygdala with AUD. This excessive signaling in the dynorphin/KOR system during the stress response contributes to the anxiogenic and dysphoric responses to stress which directly produce negative emotional/mood states that accompany alcohol withdrawal and contribute to excessive alcohol consumption to avoid withdrawal. KOR antagonists block the actions of endogenous dynorphins and alleviate the negative mood states from AUD. Similar actions are postulated for reduction in PTSD symptoms. CERC-501 is currently unavailable for additional studies due to its being purchased by another pharmaceutical company that is pursuing its use in major depression as the first approved indication.

Alpha adrenergic blockers like doxazosin have an MOA that reduces the excessive adrenergic activity characteristic of PTSD and AUD and withdrawal. Doxazosin, an alpha-1 specific blocker is FDA approved for prostate hyperplasia and hypertension, and has been shown to reduce PTSD in clinical studies and reduce AUD in preclinical and clinical studies. It has a longer half-life than prazosin which is another alpha adrenergic blocker that has been shown to also reduce PTSD symptoms (nightmares). Lofexidine is in contrast an alpha2-adrenergic receptor agonist. Like doxazosin it can be used as an anti-hypertensive, but is mostly used to help relieve symptoms of heroin or opiate withdrawal in opiate dependency. It also has broader efficacy in reducing all types of adrenergic activity through feedback inhibition of adrenergic neurons, as explained above.

Cortisol blockers like ***PT150*** *h*ave an MOA that are relevant due to the significant dysregulation of the Hypothalamic-Pituitary-Adrenal axis in both AUD and PTSD leading to elevated cortisol activity.

GABA systems have been implicated as targets for ethanol at the cellular, molecular and behavioral level. The MOA relevant to modulating the $GABA_B$ receptor to increase its activity, which decreases alcohol responding and like the prototypic agonist, baclofen, reduces the self-administration of alcohol. The $GABA_B$ receptor allosteric modulator, ***ASP8062*** reduced voluntary responding for 10% ethanol in a dose-related manner in initial animal studies. Increased GABA activity can increase depleted dopamine levels and possibly reduce drug cravings. A reduction in $GABA_B$ activity is also characteristic of PTSD and augmentation of this activity should reduce PTSD symptoms. Astellas has now begun enrolling subjects in an

outpatient Phase II study of ASP8062 for alcohol use disorder and comorbid PTSD, another indication of the success of the PASA in moving new medications into treatments for substance use disorders.

Zonisamide is an anticonvulsant that has a broad combination of complementary MOA as an antiepileptic agent. Chemically it is a sulfonamide. By altering the fast inactivation threshold of voltage dependent sodium channels, zonisamide reduces the sustained high-frequency repetitive firing of action potentials. Zonisamide also inhibits low-threshold T-type calcium channels in neurons, which may prevent the spread of seizure discharge across cells. Both alcohol and zonisamide regulate/modulate the activity of the same neuro transmitter systems. Zonisamide in repeated doses decreases the sensitivity of the hippocampus to ethanol. Its MOA for reducing PTSD symptom is less clear and was tested in a rodent study, where it showed little efficacy, thereby leading to cancellation of a planned human study using it for alcoholism and PTSD.

Buprenorphine itself is an opioid, but the maximal effects are less than other more dangerous opioid agonists, like methadone and heroin, and is FDA-indicated for the treatment of opioid dependence, because it is a partial rather than full agonist of the mu receptors. More importantly for the proposed human studies, buprenorphine is the only clinically available medication that acts as an antagonist of the kappa opioid receptors. Kappa opioid antagonists in animal models and buprenorphine in preliminary human studies have reversed PTSD symptoms more effectively than the currently FDA approved selective serotonin reuptake inhibitors (SSRIs) used for treating PTSD (sertraline and paroxetine). These antagonists can also reduce alcohol use, which is being tested in an ongoing clinical trial currently.

Naltrexone reverses the effects of opioid analgesics by binding to the various opioid receptors in the central nervous system, including the mu, kappa and delta-opioid receptors. This leads to an inhibition of the typical actions of opioid analgesics, including analgesia, euphoria, sedation, respiratory depression, miosis, bradycardia, and physical dependence. Naltrexone is longer-acting and more potent compared to naloxone (Narcan). Naltrexone is used to block cravings for both opioids and alcohol (FDA-indicated for the treatment of alcohol dependence) but naloxone is not. Both drugs block the analgesic and respiratory depressant actions of exogenous opioids and the pleasure response of endogenous endorphins, which are released when drinking alcohol. However, to treat an overdose naltrexone does not act quickly enough, while naloxone is both quick and short acting for opioid overdose treatment. While naltrexone cannot be used to rescue someone from an opioid overdose, it can help people who are addicted to opioids have less craving for them (FDA-indicated for the prevention of relapse to opioid dependence following detox).

Aldehyde dehydrogenases (ALDHs) are a family of enzymes known primarily for their critical role in ethanol (EtOH) metabolism; ALDH2-induced metabolism of acetaldehyde (ACD) is the rate-limiting step in EtOH metabolism. Inherited human ALDH2 deficiency causes an accumulation of ACD when alcohol is consumed, leading to a highly aversive reaction (ethanol reaction, ER), which reduces the risk for an AUD. The ER in human ALDH2 deficiency also

underlies the mechanism by which the FDA-approved treatment for AUD, disulfiram, deters alcohol consumption. Disulfiram irreversibly and non-selectively inhibits both ALDH1 and ALDH2, resulting in ACD accumulation and the corresponding aversive ER. In contrast to disulfiram, selective and reversible ALDH2 inhibitors reduce alcohol consumption and prevent alcohol-induced dopamine release in the brain independent from increasing ACD levels. Selective ALDH2 inhibitors also decrease norepinephrine (NE) release, which likely underlies the anxiolytic properties of ALDH2 inhibition in animal models of anxiety. Not only is elevated NE associated with alcohol withdrawal and a critical treatment component for preventing alcohol use relapse, it is also implicated in the pathophysiology of PTSD. ***ANS-6637*** is being developed by Amygdala Neurosciences as an aid for substance use disorders based on its mechanism of action in the brain to prevent pathophysiologic dopamine surge without changes to basal dopamine. Unfortunately, an early Phase 2 clinical trial conducted by NIAAA recently uncovered liver toxicity that halted PASA plans to start a similar clinical trial examining it for alcohol use disorder and PTSD.

FKBP5 inhibitors, SAFit2 (a highly specific small molecule that crosses the blood brain barrier) and benztropine mesylate (more broad acting and FDA-approved for Parkinson's disease) are compounds that potentially target stress related molecular pathways. FKBP5 is a co-chaperone that interacts with the glucocorticoid receptor (GR)/heat shock protein 90 complex and thus interferes with glucocorticoid (GC) binding to its receptor. Therefore, FKBP5 regulates GC signaling, including hypothalamic-pituitary-adrenal (HPA)-axis negative feedback. Dysregulation of the HPA-axis and extrahypothalamic GR are well established in PTSD and AUD. FKBP5 inhibitors could be repurposed for the treatment of PTSD/AUD and a meta-analysis of 14 studies with >15,000 volunteers found that FKBP5 gene variants predict PTSD rates.

Anti-fentanyl vaccine (CRM-GFEN+Alum+dmLT (CRM197-glutaryl fentanyl plus the adjuvant dmLT)) is composed of fentanyl attached to the carrier protein CRM197, which is derived from pertussis toxoid, and then added to a new vaccine adjuvant dmLT, which is currently in human studies world-wide for increasing antibody responses to various vaccines. This vaccine produces antibodies in the blood that attach to fentanyl, if it is ingested, and this large antibody-fentanyl complex cannot get out of the bloodstream to enter the brain, heart or other vulnerable organs to produce psychological effects, analgesia or respiratory depression. Thus, these antibodies prevent both abuse of fentanyl and overdose. These blocking effects are critical because our existing FDA approved treatments for opioid relapse prevention and overdose – methadone, buprenorphine and naltrexone – are not able to block fentanyl's effects. Overall, preventing fentanyl overdose has two critical roles for the DoD. First, it can prevent the high rate of overdoses and deaths occurring among veterans with opioid use disorders. Second, it can prevent terrorist or related combat attacks using aerosolized fentanyl. This vaccine is moving towards human use in FDA testing during the next couple of years with this DoD and NIH support. The dmLT adjuvant is being provided at no cost for animal and later human studies from the WHO and PATH foundation through NIH. Human studies are now actively being pursued using PASA funds for early manufacturing, stability and toxicology testing in preparation for IND filing to do a Phase 1 human clinical trial in about 2 years.

Pre-Clinical

AS140026-A1 *"Preclinical Analysis of Combined Zonisamide and Doxazosin Treatments in Stress-Alcohol Drinking Models"* - Howard Becker, Medical University of South Carolina

Objective and Study Design: The overall objective of this study is to employ two well-established mouse models of alcohol dependence and stress/PTSD exposure to evaluate the ability of medications to reduce excessive alcohol consumption and, in particular, the exacerbating effects of stress that perpetuate hazardous and harmful drinking associated with dependence. Based on clinical and preclinical evidence, two promising medications, a GABA B PAM modulator and doxazosin, will be evaluated alone and in combination to assess the effectiveness of the drugs on stress facilitation of drinking in alcohol dependent vs. nondependent male and female mice and on drinking in a PTSD-alcohol dependence model in male and female mice. Carisbamate was originally planned for this study, but it was suddenly withdrawn by the supplying pharma company prior to study start. Thus, we determined that zonisamide would be an appropriate replacement compound worthy of study.

Compounds Under Study: Two medications, zonisamide (anticonvulsant) and doxazosin (noradrenergic alpha-1 receptor antagonist), will be evaluated alone and in combination to assess the effectiveness of the drugs on stress facilitation of drinking in alcohol-dependent vs. nondependent male and female mice and on drinking in a PTSD-alcohol dependence model in male and female mice.

Hypothesis: Doxazosin and Zonisamide treatment, alone or in combination, will decrease voluntary alcohol drinking in dependent mice with a history of stress exposure

Aim 1 (Alcohol Disorder Model). Examine the effects of:
- zonisamide alone
- doxazosin alone (positive control for the PTSD and alcohol models)
- zonisamide and doxazosin in combination on stress facilitation of drinking in alcohol-dependent vs. nondependent male and female mice.

Primary outcome: alcohol drinking during Forced Swim Stress procedure using the Chronic Intermittent Ethanol-Forced Swim Stress (CIE-FSS) Drinking Model

Aim 2 (PTSD and Alcohol Disorder Model). Examine the effects of:
- zonisamide alone
- doxazosin alone (positive control for the PTSD and alcohol models)
- zonisamide and doxazosin in combination on drinking in a PTSD-alcohol dependence model in male and female mice.

Primary outcome: alcohol drinking using the PTSD-CIE Drinking Model Chronic Intermittent Ethanol-Forced Swim Stress (CIE-FSS Drinking Model)

At a Glance Info:
Population: Transgenic male and female mice
Number of mice: 256 mice

This study has closed. Manuscript accepted for publication. Evaluation of the effect of doxasozin and zonisamide on voluntary ethanol intake in mice that experienced chronic intermittent ethanol exposure and stress Marcelo F. Lopez, Sarah E. Reasons, Benjamin A. Carper, Tracy L. Nolen, Rick L. Williams, Howard C. Becker, Alcohol, 89 (2020)

AS140026-A2 - *"Assessing pharmacotherapies in animal models of post-traumatic stress disorder and alcohol use disorder"* - Colin Haile, University of Houston

Background: Recent evidence shows the endogenous opioid dynorphin and its receptor (kappa opioid receptor, KOR) play a significant role in stress reactivity and alcohol reinforcement. In particular, KORs modulate noradrenergic (NE) in brain stress circuits important in animal relapse models. KOR antagonists block the reinforcing effects of alcohol, and stress-induced increases in alcohol consumption. KOR antagonists have been shown to reduce stress in preclinical studies. KOR antagonists also possess potent antidepressant effects. Thus, for this application we are collaborating with Cerecor Inc. to assess CERC-501, a centrally penetrant, orally active, high-affinity and selective KOR antagonist in animal models of PTSD and AUD.

Doxazosin, another off-patent generic drug, is already being used effectively for reducing adrenergic activity in both alcoholism and PTSD as supported by several outpatient clinical trials, and is acting as a positive control in this study examining kappa opioid antagonism and now GABA-b agonism as other MOAs for reducing PTSD and AUD. Moreover, pursuing a different mechanism of action (MOA) as a treatment option makes better scientific and commercial sense for a pre-clinical discovery study, and the MOA of Astellas' GABA-B PAM (ASP8062) provides this option, as well as the BIG pharma support to move this medication forward into Phase 2 and 3 clinical trials for an NDA filing under these two indications. Its MOA as an allosteric modulator of the gamma amino butyric acid type B (GABA-B) receptor is unique and different from all the other agents being examined for AUD and PTSD. This particular compound is being commercially developed by Astellas, a large Japanese pharmaceutical company through its USA branches, for use in affective (mood) disorders. Because of this parallel investment by Astellas, a successful FDA Phase 2 clinical study in our DoD PASA consortium pipeline following a successful preclinical discovery study would lead Astellas to develop this GABA-B PAM through a New Drug Application (NDA) filing with the FDA. Thus, this GABA-B PAM offers both a unique MOA for discovery and commercial potential for being marketed and made available for AUD and PTSD.

PTSD and AUD are linked to dysregulated noradrenergic (NE) function, altered Hypothalamic Pituitary-Adrenal axis stress reactivity, and underactivity of the GABA-b system. The endogenous opioid dynorphin and its receptor (kappa opioid receptor, KOR) as well as the GABA-b system play a significant role in stress reactivity and alcohol reinforcement. Thus, the primary objective of this study is to test the ability of FDA investigational medications that target these systems on their ability to reduce PTSD-induced alcohol intake in a rodent model of PTSD/AUD comorbidity

Objectives and Study Design: The experiments to be conducted in this preclinical trial will provide essential information for identifying the most efficacious medications to be translated

to Phase 1 human clinical trials. Specifically, within the context of this preclinical trial, the study team will 1) evaluate whether the study drugs will alter PTSD-like symptoms in a rodent model of PTSD; 2) evaluate whether the study drugs will alter alcohol self-administration; and 3) determine whether the study drugs will alter PTSD-induced increases on alcohol self-administration when administered alone and in combination.

Compounds Under Study:
(1) doxazosin (α1-selective alpha antagonist) (positive control)
(2) ASP8062 (allosteric modulator of the GABA-B PAM receptor)

- GABAB Positive Allosteric Modulator (PAM)
- Other GABAB PAMs have been shown to reduce alcohol self-administration in rodents (Loi et al. 2013, Maccioni et al, 2007, 2009, 2010, deMiguel et al. 2018).
- A recent Phase 2 clinical trial conducted in the USA assessed the effects of ASP8062 in patients with fibromyalgia.

(3) Baclofen (derivative of the neurotransmitter (GABA). Its MOA is by activating (or agonizing) GABA receptors) (positive control)

- GABAB Orthosteric Agonist
- Consistently reduces alcohol self-administration in rodents (Anstrom et al. 2003, Janak & Gill 2003, Walker & Koob 2007).
- Shows some efficacy in decreasing alcohol consumption in humans however studies are inconsistent.
- Associated with significant toxicity (Jamshidi et al. 2019).

Aim 1 will evaluate whether medications (i.e. CERC-501, doxazosin, ASP8062) will alter PTSD-like symptoms in a rodent model of PTSD. The hypothesis is that all the drugs will decrease PTSD symptoms.

Aim 2 will evaluate whether medications (i.e., ASP8062 or baclofen) will alter alcohol self-administration. The hypothesis is that the drugs will reduce drinking.

Aim 3 will determine whether medications will alter PTSD-induced increases on alcohol self-administration. This aim is dependent on whether efficacy of ASP8062 is observed in at least one (and ideally both) Experiments 1 and 2. This experiment will include the most efficacious dose of ASP8062 alone (found in AIM 1) and the most efficacious dose of Doxazosin alone (found in AIM 1) and a vehicle control resulting in a total of 3 drug groups.

At a Glance Info:
Population: Sprague-Dawley rats
Number of rats*:* 20 rats

This study has closed and is in data analysis and manuscript writing. Based on Dr. Haile's preliminary findings, Astellas moved the compound ASP8062 onto their fast track for development in substance use disorder

Based on the present results a Phase 1 randomized, placebo-controlled, crossover clinical trial was initiated to assess potential interaction between ASP8062 and alcohol in healthy adult subjects (N=20) by Astellas (Clinicaltrials.gov indentifier: NCT04003402).
The study was completed October 16, 2019. Results are pending.

AS170014-A1 – "Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-Fentanyl Vaccine and Buprenorphine Combination Therapy" – PI: Colin Haile; Univ of Houston

Background: Fentanyl is a highly potent μ opioid receptor agonist indicated for the treatment of moderate to severe pain. Fentanyl and fentanyl-analogs (e.g. carfentanil, sufentanil, alfentanil, and lofentanil) are highly lipophilic and rapidly penetrate the CNS which can be lethal. The standard medication to reverse fentanyl's effects is the mu antagonist naloxone. Because of fentanyl's potency however, very high doses of naloxone are needed in a timely manner to avert overdose. A novel treatment strategy that could potentially avert overdose and prevent relapse in individuals with OUD trying to remain abstinent is by vaccination with an anti-fentanyl vaccine. The vaccine being developed produces antibodies in the blood that attach to fentanyl. When fentanyl is ingested, this large antibody-fentanyl complex cannot get out of the bloodstream to enter the brain, heart or other vulnerable organs to produce psychological effects, analgesia or respiratory depression. Thus, these antibodies prevent both abuse of fentanyl and overdose. Buprenorphine is an approved medication for treating opioid use disorder, and blocks all of the available opioid analgesics except the fentanyl drugs. Thus, the vaccine is essential. Buprenorphine (BUP) as a prototypical kappa opioid antagonist, has also shown efficacy in some human studies for reducing PTSD symptoms more effectively than the currently approved SSRIs for treating PTSD. Thus, will also be examined in the animal models for reducing PTSD, which the fentanyl vaccine is not expected to impact.

The original carrier protein (tetanus toxoid) and adjuvant (entolimod) for the vaccine were changed before this study began for several reasons. First, the tetanus became unavailable and increased in price by almost 10 fold making the human vaccine too expensive for realistic manufacturing and clinical use. The protein was therefore changed to CRM197, which is much easier to manufacture, far lower in cost, and produced antibody responses equivalent to the tetanus carrier protein. Second, the entolimod became unavailable for using in our vaccine human studies because the manufacturer changed this protein's commercial target from vaccines to cancer immunotherapy. We therefore shifted to dmLT, which is specifically for vaccine use and freely available to us for this fentanyl vaccine development.

Objective: The overall objective of this study is to provide essential information for the development of an anti-fentanyl vaccine and buprenorphine combination to be translated to phase 1 human clinical trials.

Aim 1. Assess anti-FEN antibody levels in combination with 3 doses of BUP administered chronically via osmotic mini-pumps.

Aim 2. Determine whether the functional effects of fentanyl are blocked in rats vaccinated with CRM-FEN alone and in combination with BUP.

Aim 3. Ascertain whether BUP will attenuate predator-odor induced place aversion in an animal model of PTSD.

At a Glance Info:
Population: Sprague-Dawley rats
Number of rats: 240 rats

AS170014-A2 – "Preclinical assessment of PT-150 for opioid use disorder and PTSD" – PI: Michael Bardo; Univ of Kentucky

Background: Selective blockade of glucocorticoid receptors (GRs) in brain with PT-150 will serve as an effective pharmacotherapy for opioid use disorder (OUD) and co-morbid PTSD.

Objective: The goal of this preclinical project is to provide initial proof-of-principle evidence that PT-150 ameliorates stress-induced escalation of fentanyl self-administration and stress-induced reinstatement, thus offering a potential new avenue for treating co-morbid OUD and PTSD.

Compound Under Study:
(1) PT-150

- Glucocorticoid receptor (GR) antagonist
- Analog of mifepristone (Bachman et al., 2003) with higher selectivity for GR over progesterone receptors (Peeters et al., 2004)

Hypothesis: PT-150 will reduce stress-induced reinstatement of fentanyl seeking.

Aim 1: Determine if PT-150 reduces stress-induced reinstatement of fentanyl seeking: This aim will use a reinstatement model of relapse. Rats will first be trained to self-inject escalating doses of the potent opioid fentanyl using a standard 2-lever operant conditioning procedure. Following this, rats will be treated daily with either PT-150 or placebo while undergoing response extinction (abstinence). Stress will then be applied either environmentally (mild footshock) or pharmacologically (yohimbine) and reinstatement of fentanyl seeking will be measured. This procedure is intended to model the use of PT-150 as a pharmacotherapeutic to prevent relapse.
Endpoint: The primary endpoint for this experiment will be the number of responses on the previously active following stress. This measure will be compared to the number of responses on the last day of extinction. Positive results from this experiment will form the scientific premise for determining if PT-150 blocks fentanyl craving in human participants with OUD who are abstinent.

Hypothesis: PT-150 will reduce the stress-induced increase in fentanyl self-administration.

Aim 2: Determine if PT-150 reduces fentanyl self-administration in individuals with co-morbid PTSD: This aim will use two different models of stress: (1) chronic social isolation and (2) acute stress induced by restraint/swim, which have been used to model PTSD. Previous work has shown that these stressful manipulations increase drug self-administration behavior. This aim will determine if oral PT- 150 reduces the effects of chronic social isolation and acute stress, either alone or in combination, on fentanyl self-administration. Rats will be raised in either social isolation or in group housing and then will receive acute restraint/swim stress or control treatment. Plasma corticosterone will be measured immediately before and after the acute stress. On the day after the acute stress treatment, rats will be treated daily with either PT-150 or placebo and then will be trained to voluntarily self-administer i.v. fentanyl using a standard 2-lever operant conditioning procedure.
Endpoint: The primary endpoint for this experiment will be the number of fentanyl infusions across sessions. A secondary measure will be plasma corticosterone levels. We expect that chronic social stress and acute stress will both increase plasma corticosterone and fentanyl self-administration. If this latter result is obtained, it will form the scientific premise for conducting a clinical trial with PT-150 in patients with co-morbid OUD and PTSD.

At a Glance Info:
Population: Sprague-Dawley rats
Number of rats: 168 rats

Aims 1 & 2 completed, manuscript in preparation

AS170014-A5 – "Preclinical Testing of FKBP5 Inhibitors for Alcohol Use Disorder-PTSD Comorbidity" – Marisa Roberto, Scripps Research Institute

Background: FK506-binding protein 51 (FKBP5) is a compelling target for treatment of both comorbid and separate AUD or PTSD. It negatively regulates glucocorticoid (GC) transcriptional activity by reducing GC affinity for the glucocorticoid receptor (GR)/heat shock protein 90 complex. Binding of GC to this complex results in nuclear translocation and gene expression. FKBP5 thereby regulates the hypothalamic-pituitary-adrenal (HPA)-axis by fine-tuning the negative feedback from GC binding to GR. This is critical as dysregulation of the HPA-axis is emblematic of both PTSD and AUD**.** We adapted a two-shock traumatic memory model ("2-hit") to study PTSD/AUD comorbidity. This translationally focused model involves sensitization and both Pavlovian and operant conditioning leading to excess ethanol intake, fear overgeneralization, increased acoustic startle reactivity and sleep disturbance.

Compounds Under Study:
(1) SAFit2: A selective FKBP5 inhibitor, has anxiolytic-like effects and reduces voluntary alcohol intake in unstressed mice.
(2) Benztropine mesylate (brand name Cogentin):

- Is more broad acting but FDA-approved, safe in wide clinical use, and could be repurposed rapidly.
- Cogentin modulates choline, histamine and dopamine activity.

- Cogentin, like SAFit2, also disrupts the association between FKBP5 and GR.

Objective:
Our central hypothesis is that PTSD/AUD comorbid like phenotypes can be reversed using a highly specific FKBP5 inhibitor (SAFit2) or a more broad-acting, but FDA--approved FKBP5 inhibitor (benztropine).

Aim 1: Determine whether acute administration of FKBP5 inhibitors can restore normal behavior in rats exhibiting a PTSD/AUD---like phenotype. We will assess effects of acute systemic injection of SAFit2 or benztropine in Control and Stress rats prior to 2---bottle choice (2BC) testing as well as prior to acoustic startle, fear overgeneralization testing and sleep---cycle analysis. We hypothesize that both drugs will, in a dose---dependent manner, reduce the following endpoints: 1) 2BC ethanol intake and preference in both sexes, 2) fear overgeneralization, especially in males, and 3) hyperarousal (defined by disrupted sleep bout maintenance and increased acoustic startle), especially in females. Relative drug potency and efficacy for each measure will be additional endpoints.

At a Glance Info:
Population: Wistar rats
Number of rats: 292

Aim 1 completed analysis is underway

AS170014-A8 – "Medication Development of an Anti-Fentanyl Vaccine for Opioid Use Disorder" – PI: Colin Haile; Univ of Houston

Background: The focus of our initial studies (AS170014-A1) was to characterize our anti-FEN vaccine, confirm anti-FEN antibody production then assess functional effects using FEN-induced analgesia models. A final experiment demonstrated our vaccine produced antibodies that prevented FEN from entering the brain. Although we have achieved these AIMS, we also must show that our vaccine will attenuate FEN's reinforcing effects and importantly, respiratory depression, which leads to overdose deaths. These data will be essential to move on to the manufacture of clinical grade vaccine for toxicology studies.

Objective: The proposed expansion studies include two behavior and physiology AIMs followed by one subcontracting AIM for manufacturing clinical grade vaccine for FDA-required toxicology studies. Completion of these AIMs will build upon our previous research developing our anti-FEN vaccine. Collected data will also enable us to file an IND with the FDA to initiate a Phase 1 clinical trial using separate funding after this proposed PASA2 extension.
Consistent with our initial studies, the expansion studies will employ rats of both sexes. AIM1 will utilize an animal model of human relapse and drug craving. AIM2 will assess the effects of high dose FEN on physiological measures. AIM3 relates to vaccine manufacturing.

Study Aims:
Aim 1. Rats vaccinated with CRM-FEN+dmLT will generate significant anti-FEN antibodies

that will block FEN-induced reinstatement of drug-seeking behavior
Aim 2. Rats vaccinated with CRM-FEN+dmLT will generate significant anti-FEN antibodies that will block FEN-induced decreases in heart rate, respiratory rate and oxygen saturation and thus increase survival following lethal doses of FEN.
Aim 3. Contract to manufacture clinical grade (cGMP) components of the vaccine for conjugation under appropriate subcontracts.

At a Glance Info:
Population: Sprague-Dawley rats
Number of rats: 580 rats

Study underway

Clinical
AS140026-A3a - "Efficacy and Safety Study of PT150 (formally ORG34517) in Veterans with Co-morbid PTSD/AUD" - Dewleen Baker, UCSD

Background: Proven safe, effective treatments for PTSD alone, AUD alone or co-occurring illness are severely limited; the FDA has given approval to two drugs for the treatment of PTSD, and three for treatment of AUD, but in neither of these disorders are these pharmacotherapies fully effective. Recently, an emerging awareness of the overlap in PTSD/AUD phenomenology, as well as genetics, neurobiology and neural circuitry has driven efforts to exploit these mechanistic overlaps for pharmaceutical development for treatment. The overlapping neural circuitry offers a number of potential molecular targets, in particular stress-signaling pathways converging at glucocorticoid receptor (GR) signaling. There is substantial pre-clinical and clinical evidence for the dysregulation of pathways involving corticotropin releasing factor (CRF) and GR signaling in AUD development and maintenance as well as in fear learning (FL) and PTSD. Antagonism of GR, which modulates brain CRF, has been shown to be a potentially effective pharmaceutical in both alcohol dependent rodents and humans. In alcohol-dependent rats, GR antagonists were shown to be effective in reducing compulsive-like alcohol intake, diminishing withdrawal behaviors (e.g. aggression), and preventing elevations in blood cortisol levels

PT150 is a Glucocorticoid receptor antagonist that blocks the effects of cortisol, an endogenous stress hormone. The studies being conducted examine the efficacy, safety, and tolerability of this drug for AUD dual diagnosis treatment. We have successfully completed a phase I, single center, alcohol interaction study and are now conducting a phase 1, drug-drug interaction study to assess pharmacokinetic interactions between ethanol and PT150. Successful completion of the PK study will enable us to conduct the proof of concept study.

Compound Under Study:
(1) PT150

- Glucocorticoid receptor antagonist that targets modulation of HPA-axis function
- Like mifepristone (RU486), PT150 blocks the effects of cortisol, an endogenous stress hormone
- Shown to reduce AUD in preclinical & clinical studies There is substantial pre-clinical and clinical evidence for the dysregulation of pathways involving corticotropin releasing factor (CRF) and GR signaling in AUD development and maintenance as well as in fear learning (FL) and PTSD. Antagonism of GR, which modulates brain CRF, has been shown to be a potentially effective pharmaceutical in both alcohol dependent rodents and humans. In alcohol-dependent rats, GR antagonists were shown to be effective in reducing compulsive-like alcohol intake, diminishing withdrawal behaviors (e.g. aggression), and preventing elevations in blood cortisol levels
- IND held by PoP test oncology

Objective and Study Design: This proof of concept study aims to examine a novel drug compound (PT150), a Glucocorticoid Receptor (GR) antagonist, in Veterans with co-occurring PTSD and AUD to test the efficacy, safety, and tolerability of this drug for PTSD and AUD dual diagnosis treatment. The three main objectives are to:

- To test the effectiveness of PT150 to significantly enhance extinction recall tested as previously described, and alcohol consumption, measured by blood phosphatidylethanol levels
- To test the effectiveness to improve PTSD symptoms, measured by the Clinician Administered PTSD Scale (CAPS), and AUD symptoms, i.e. alcohol craving, measured by the Yale Craving Questionnaire (YCQ)
- To determine PT150 safety, as measured across safety endpoints, adverse event (AEs), vital signs and laboratory measures

We hypothesize that veterans taking PT150 compared to placebo will show significantly greater extinction recall, an improvement in PTSD and AUD symptoms over the time course of the study and that PT150 will be safe and well tolerated.

The FDA requires an in-patient alcohol/PT-150 interaction study (10 subjects) and an alcohol/PT-150 PK study prior to the Proof of Concept study

The *primary outcome* measures are potentiated startle to the CS+ during the first block of extinction training (to measure cued fear response 15) and the extinction recall index (to measure 24 hr retention of extinction learning).

At a Glance Info:
Population: This study population (n=40) will be drawn from adult veterans (all sites) of any race or ethnicity; males and females who are post-menopausal, or are infertile females and not hormone cycling. Potential enrollees will be drawn of those seeking treatment or enrolled in VA San Diego Healthcare System (SDVAHS) clinics.
Phase: Phase 2 Clinical Proof of Concept Trial
Site: VASDHS main hospital (1 site)
Participant Duration: This outpatient clinical trial consists of 14±4 days of treatment followed by a 14-day follow-up drug-free observation period, so approximately 28 days total duration.

This study is on hold pending completion of the PK study (AS140026-A3c).

AS140026-A3b - "PT150 (Formerly ORG34517) as a Potential Treatment for Alcohol Use Disorder – Alcohol Interaction Study" - PI: Christopher Verrico; Baylor College of Medicine and Dewleen Baker, UCSD

Study Type: Phase I, single center, alcohol interaction study. This within-subjects experimental procedure will assess the effects of PT150 (900 mg qd) on the subjective effects of alcohol in non-treatment-seeking alcohol-experienced volunteers (to include military service members, veterans and/or civilians).

Objective: The objective of this study is to compare safety endpoints following an alcohol challenge prior to and concurrent with PT150 treatment in 10 non-treatment seeking healthy participants by evaluating pharmacodynamic and safety endpoints during alcohol challenge prior to, and after 5 days of PT150 treatment, when PT150 has reached steady state.

- Safety will be assessed via a multitude of assessments including mean changes from both post alcohol challenge pre-treatment (day 1) to post-alcohol challenge with concurrent treatment (day 5) and pretreatment (baseline) to post-treatment (days 5 and 6)
- After completion of the screening period, the remaining study duration for each participant will include a 7-day in-patient stay. For females, a follow-up visit will be scheduled to occur at least 14 days after the final dose of PT150 is administered to ensure pregnancy does not occur.

At a Glance Info:
*Populatio*n: Veterans with co-occurring PTSD and AUD
*Number of Participant*s: 10 veterans and/or civilians
Participating Site: Michael E. DeBakey VAMC, Houston, TX
*Principal Investigato*r: Christopher Verrico PhD, and Dewleen Baker, M.D.
Duration: 7 day in-patient

This study is complete. Manuscript accepted for publication
Morice C, Baker DG, Patel MM, Nolen TL, Nowak K, Hirsch S, Kosten TR, Verrico CD. A randomized trial of safety and pharmacodynamic interactions between a selective glucocorticoid receptor antagonist, PT150, and ethanol in healthy volunteers. Sci Rep. 2021 May 10;11(1):9876. doi: 10.1038/s41598-021-88609-6. PMID: 33972573; PMCID: PMC8111026.

AS140026-A3c - "Effects of Ethanol on the Pharmacokinetics of PT-150" - Dewleen Baker, UCSD

Study Type: A phase 1, double center, and drug-drug interaction (DDI) study. The within-subjects' experimental procedures will assess pharmacokinetic interactions between ethanol (EtOH) and PT150 (900 mg qd) in non-treatment-seeking alcohol-experienced volunteers.

Objective: To determine PK properties of alcohol alone, PT150 alone, and alcohol and PT150 combined.

Design: single site, within-subject, open-label, inpatient human laboratory study in 10 alcohol-experienced, healthy adult volunteers.

Dosing: a single, fixed, daily dose of 900 mg PT150, for 6 days.

Outcome Measures: Cmax, Tmax, etc

Participant Follow-up: After completion of the screening period, the remaining study duration will proceed as follows for each participant:
- Admission for an initial 1-day in-patient stay.
- Discharge followed by 4 days of outpatient visits.
- Readmission for a 4-day inpatient stay.
- For females, a follow-up visit will be scheduled to occur at least 14 days after the final dose of PT150 is administered to ensure pregnancy does not occur.

At a Glance Info:
*Populatio*n: Veterans not seeking treatment for AUD
*Number of Participant*s: 10
Participating Site: Michael E. DeBakey VAMC, Houston, TX and UCSD
*Principal Investigato*r: Christopher Verrico PhD, and Dewleen Baker, M.D.
Duration: 10 days

This study is actively recruiting and enrolling

AS140026-A4 - "Zonisamide as a New Treatment for PTSD & Co-Occurring AUD" - Christopher Verrico, Baylor College of Medicine

Background: The study proposes to test the safety and effectiveness of zonisamide for comorbid PTSD and AUD in veterans. Zonisamide has undergone considerable clinical studies to investigate its efficacy and safety in the adjunctive treatment of epilepsy. Zonisamide is FDA-approved as an adjunct treatment for partial seizures. Zonisamide enhances GABA function, blocks voltage-sensitive sodium channels and T-type calcium channels, and inhibits carbonic anhydrase. The most common side effects of zonisamide are somnolence, anorexia, dizziness, headache, nausea, and agitation/irritability. Zonisamide is similar to topiramate (which recommended first line therapy for AUD in DoD/VA CPG), and has been shown to reduce AUD in preclinical studies. Zonisamide has promising clinical data in alcoholism and there is no need for further alcohol interaction studies in order to obtain an IND for this study. Additionally there is a study that indicates its potential use for anxiety disorders such as PTSD.

Objective and Study Design: The objective of this study is to determine if, compared to placebo, zonisamide (up to 400mg/day, PO) is a safe and efficacious treatment for PTSD and AUD in veterans with PTSD and co-occurring AUD. Researchers hope zonisamide will be a better drug for treating patients with AUD and reducing the symptoms of PTSD. This is a short term clinical trial with outcomes including PTSD severity and alcohol use. There is ample safety data for zonisamide including its interactions with alcohol, and it has a similar therapeutic profile to FDA-approved topiramate, but with a superior safety profile and longer half-life. The outcome measures are well justified and well validated (if not gold standard) with the populations (PTSD, AUD), and the inclusion of many PhenX toolkit measures is a methodological strength.

The study will specifically examine the use of zonisamide for treating PTSD and AUD symptoms in 60 Veterans with co-occurring PTSD and AUD. Veterans will be divided into two groups, one which will get the study drug and another which will get a placebo. Both groups will be treated with either the drug or placebo for 4 weeks and then tested to see if the drug had any effect on the symptoms of PTSD and AUD. The primary outcomes are a change from the baseline in scores on the Clinician-Administered PTSD Scale V (CAPS-V) and fear-potentiated startle (FPS) response as well as percentage of heavy drinking days (%HDD). The secondary outcomes include treatment retention, medication compliance, severity and number of adverse events (AEs), physiological measures including vital signs and ECG abnormalities, breadth alcohol content (BAC), alcohol craving, days of abstinence, drinks per drinking day, and biochemical tests for alcohol use.

At a Glance:
*Number of Participants***:** 60 male and female Veterans with PTSD and AUD

*Study Type***:** Phase 2 randomized controlled trial

Statement of Compliance: This was exempted from IND requirements on 31 Oct 2017.

Design: A randomized, double-blind, placebo-controlled study to examine the ability of 4-weeks treatment with zonisamide, up to 400 mg/day, per os (PO, by mouth).
Objective: The objective of this study is to determine if, compared to a placebo, zonisamide (400mg/day) is a safe and efficacious treatment for PTSD and AUD in Veterans with PTSD and co-occurring AUD.

The study originally was designed to use a novel compound, carisbamate, but drug was withdrawn by the manufacturer. Zonisamide was substituted because it has a similar mechanism of action it, however it is off-patent. The study not only had recruitment issues but preliminary data from pre-clinical PASA study using zonisamide (AS140026-A1) indicated weak evidence to demonstrate efficacy. The study was terminated and funds redirected to other research studies.

AS140026-A5 - "Kappa Opioid Receptor Antagonist for the Treatment of Alcohol Use Disorder and Comorbid PTSD" – PI: Lori Davis; UAB School of Medicine

Background: The use of medication that result in kappa opioid receptor (KOR) antagonism represents a novel potential treatment for veterans and Service members with comorbid AUD and PTSD. KOR antagonists may be beneficial in the treatment of addictions, PTSD, and major depressive disorders. Given their general ability to mitigate the effects of stress, there is substantial interest in the development of Kappa Opioid Receptor (KOR) antagonists for indications such as AUD and PTSD. The combination of buprenorphine, which acts as an antagonist at kappa and partial agonist of the mu receptors, and naltrexone, which blocks the mu receptor, yields a pharmacological net effect of a KOR antagonist, and thus, is an excellent alternative study medication to ALKS-5461 and CERC-501. There is clinical evidence that buprenorphine is effective in attenuating some PTSD symptoms among those with opioid use disorder. The use of buprenorphine in a non-opioid dependent population has ethical implications given its risk of addiction, which has led to the idea to combine it with naltrexone in order mitigate the potential for misuse. Further, preclinical studies suggest KOR antagonism is important for drinking behavior, stress induced reinstatement of drug and alcohol consumption. For these reasons, there is substantial interest in the development of KOR antagonists for indications such as AUD and PTSD and the combination of buprenorphine and naltrexone allows for a proof-of-concept study until a formulated KOR antagonist becomes commercially available.

Objectives and Study Design: A Phase II Randomized, Double-mask, Placebo-Controlled Trial to evaluate the efficacy and physiological effects of sublingual buprenorphine (SL-BUP; Subutex)

combined with extended-release injectable naltrexone (XR-NTX; Vivitrol) which yields a pharmacologically net effect of kappa opioid receptor (KOR) antagonism in the treatment of comorbid AUD and PTSD.

- Aim 1: To evaluate the efficacy of SL-BUP + XR-NTX in the treatment of comorbid moderate-to severe AUD and PTSD based on a response in both AUD and PTSD outcomes.
- Aim 2a: Examine the baseline association between fear extinction and PTSD symptom severity in Veterans/service members with comorbid AUD and PTSD.
- Aim 2b: Examine the baseline association between Psychophysiological Reactivity to a Trauma-Relevant Stimuli and PTSD symptom severity.
- Aim 2c: Examine the baseline association between Psychophysiological Reactivity to Alcohol-Cues Stimuli and measures of alcohol craving.
- Aim 3: Examine the association of baseline fear extinction, stress reactivity, and treatment outcomes.
- Aim 4: Examine whether the degree of change from baseline to 2-week psychophysiological measures are associated with AUD and PTSD outcomes at week 8.

The *primary outcome* is a reduction of both AUD and PTSD symptoms at 8 weeks.

At A Glance:
Study population: 90 male and female treatment-seeking veterans with comorbid AUD and PTSD.
Participant Duration: 12 weeks from the time of randomization
*Number of Sites***:**

1. Tuscaloosa VA Medical Center – Birmingham satellite clinic
2. VA Connecticut Healthcare System, West Haven

This study is actively recruiting and enrolling.

AS140026-A7 – "N-acetylcysteine Treatment to Reduce Alcohol Use, Cognitive Impairment and PTSD Symptom Severity in Veterans with Traumatic Brain Injury and Alcohol Use Disorder: A Confirmatory Study" – PI: Steve Batki; Univ of California San Francisco

Background: To build on the encouraging results from the PI's recently completed pilot clinical trial of N acetylcysteine (NAC) in the treatment of AUD in Veterans with TBI. We propose to conduct a larger confirmatory clinical trial testing the efficacy of NAC to reduce alcohol use, improve neurocognitive functioning, and reduce PTSD symptom severity in Veterans with TBI and AUD. If successful, the proposed study will advance the state of knowledge about the use of pharmacotherapy for AUD in patients with TBI and AUD. This trial will meet the following Primary Aim, with lesser aims in the secondary, tertiary and exploratory positions.

Primary Aim: To conduct a confirmatory test of the efficacy of NAC in reducing alcohol use in Veterans with TBI and AUD.
Secondary Aim: To conduct a confirmatory test of the efficacy of NAC in improving neurocognition in TBI and AUD.

Tertiary Aim: To conduct a confirmatory test of the efficacy of NAC in reducing PTSD symptom severity.
Exploratory Aim: To explore potential mediators and moderators of outcome, including the role of improvement in cognitive function as a mediator of alcohol use reduction

At a Glance:
This was a planning grant for a prospective, parallel groups, randomized, double-blind, placebo-controlled confirmatory clinical trial of NAC treatment of AUD in 80 Veterans with TBI and AUD.

This planning grant was not invited to submit a full application.

AS170014-A6 – "Lofexidine Combined with Buprenorphine for Reducing Symptoms of Post-Traumatic Stress Disorder and Opioid Use Relapse in Veterans" - PI: Christopher Verrico; Baylor College of Medicine

Background: This study was submitted as a planning grant under AS140026-A6 and then awarded a study grant under AS170014-A6. The overall objective of the proposed study is to determine if lofexidine (LFX) as an adjunct to buprenorphine (BUP) treatment improves symptoms of both opioid use disorder (OUD) and PTSD. Lofexidine is an alpha adrenergic blocker while buprenorphine (BUP) is a μ-opioid receptor partial agonist and a kappa-opioid receptor antagonist. Our central hypothesis is that LFX as an adjunct to BUP treatment will reduce opioid use relapse and symptoms of PTSD in Veterans more effectively than treatment with BUP alone. Our hypothesis is based on the distinct yet complimentary mechanisms by which each medication reduces symptoms of both disorders. LFX is an alpha adrenergic blocker while BUP is a μ-opioid receptor (MOR) partial agonist and a K-opioid receptor (KOR) antagonist. Because BUP has a high affinity for, and slowly dissociates from the MOR it also attenuates the surge in noradrenaline (NA) release and thereby suppresses symptoms of opioid withdrawal. On the other hand, BUP-induced blockade of KORs is thought to mediate its observed antidepressant effects in opioid users, anxiolytic effects in healthy adults and therapeutic effects on symptoms of PTSD in Veterans.

This is a randomized, double-blind, placebo-controlled, single-site, parallel groups, 8-week study to compare the safety, tolerability, and efficacy of BUP treatment alone, to BUP treatment with adjunct LFX, on measures of OUD and PTSD in Veterans with both disorders.

Compound Under Study: (1) Lofexidine (LFX) is an FDA-approved alpha-2-adrenergic receptor (α2-AR) agonist treatment for opioid withdrawal.

Primary Objective: To determine if lofexidine (LFX) as an adjunct to buprenorphine (BUP) treatment improves symptoms of PTSD and reduces opioid use lapses and relapse

Study Aims:
Aim 1. To determine the proportion of veterans who achieve 30-days of sustained abstinence from illicit opioid use at the end of treatment with either PLB or LFX (up to 3.2 mg/d) as adjuncts to BUP. Measured by both UDS and self-report using the TLFB.

Aim 2. To determine change from baseline scores on the PTSD Checklist (PCL-5) at the end of study.

Endpoints:
For OUD: Drug-taking behavior (% abstinence from illicit opioid use from Week 5-Week 12).
For PTSD: Mean change scores on the PTSD Checklist for the DSM-5 (PCL-5).

At a Glance info:
Study population: 120 male and female treatment-seeking veterans with comorbid OUD and PTSD.
Participant Duration: 12 weeks from the time of randomization
*Site***:** (Michael E. DeBakey VAMC, Houston, TX

This study is actively recruiting and enrolling

AS170014-A3 – "Developing a Proof of Concept Clinical Trial to Evaluate the Use of a Safe and Highly Selective α2a Adrenergic Receptor Agonist, BXCL 501, for the Treatment of AUD comorbid with PTSD and/or TBI" – PI: John Krystal; VA Connecticut Healthcare System, West Haven

Background: Based on preclinical and clinical data, an alpha-2 receptor agonist with high CNS penetration and high intrinsic activity at the receptor would be a valuable therapeutic option to reduce hyper-sympathetic response during ET. This proof of concept study examines the use of BXCL501 (dexmedetomidine on a sublingual film) as a potentially effective therapeutic for the treatment of patients with PTSD and AUD. DEX exerts its effects by preventing release of the neurotransmitter norepinephrine which is responsible for stress-related agitation and hyper-arousal. Because PTSD is associated with hyper-arousal and high sympathetic nervous system activity, BXCL501 has the potential to alleviate agitation that occurs in PTSD. BXCL501 will provide a minimally invasive rapid-delivery dosage form of dexmedetomidine. Advantages of the sublingual film include bypassing first pass metabolism, rapid onset of action, and flexibility in dosing because of ease of application of films and the possibility of combinations with drugs working through other mechanisms. In addition, because BXCL501 enters the blood stream directly by the sublingual route and bypasses first-pass metabolism in liver, it is less susceptible to variations in hepatic function than orally administered drugs.

Compound Description:
(1) BXCL501

- Sublingual (SL) film containing dexmedetomidine (DX)
- DX is alpha-2 adrenergic receptor agonist
- Higher intrinsic activity; more potent in vitro than either clonidine or lofexidine
- DX does not depress respiratory function
- No abuse potential.
- bypass 1st pass metabolism and therefore helpful in those with compromised liver function

Primary Objective: To determine if BXCL501 is safe for the treatment of AUD & comorbid PTSD and shows potential of efficacy to support later phase clinical trials in individuals with AUD and PTSD recruited from the community.

Aim 1: Does pretreatment with BXCL501 PLA, 40µg and 80µg

- attenuate stress reactivity
- attenuate cue reactivity
- alter subjective effects of ethanol in a laboratory setting

Aim 2: Evaluate the effects of BXCL501 (target 80µg) for 28 days

- Drinking outcomes (drinking, craving)
- Mood, anxiety and other symptoms of PTSD
- Cognitive function, including memory, reaction time and mood

At a Glance Info:

Study Type:

Phase 1: Laboratory study with 3 test days (n=16)

- Randomized order; double blind
- Pretreatment w/ SL BXCL501 40µg, 80µg, or placebo
- IV ethanol administered each day via clamp methodology with target BrAC of 100 mg/dL
- Test day includes stress reactivity and alcohol cue reactivity
- Outcomes:
    - Subjective measures: craving and alcohol effects
    - Subjective measures: mood
    - Cognitive function and reaction time measures

Phase 2: Outpatient study (n=10)

- BXCL501 for 28 days
- Target 80µg
- Medication Schedule: 40µg for 3 days; 80µg for 25 days if tolerated
- Weekly assessments
    - Alcohol use (TLFB) and craving
    - Subjective mood and PTSD symptom report
    - Cognitive function and reaction time measures
    - Adverse events

Number of Participants: 26

This study has completed the planning phase and has been awarded a study grant (AS170014-A7 "Effect of Sublingual formulation of Dexmedetomidine HCl (BXCL501) on Ethanol in Heavy Drinkers with PTSD" – PI: Ismene Petrakis; Yale University)

AS170014-A4 – *"An Aldehyde Dehydrogenase 2 Inhibitor for PTSD and AUD*" - Luba Yammine; University of Texas Houston Cizik School of Nursing

Background: Two completed studies, a Phase 1, randomized, placebo-controlled, double-blind, first-in-human study of ANS-6637 assessed the safety, tolerability, and PK of single and multiple ascending doses in healthy adult subjects; and a Phase 1b, proof of concept, dose-ranging study evaluated the safety of the co-administration of ascending doses of ANS-6637 and alcohol in healthy male alcohol drinkers. Because ANS-6637 has been studied extensively in Phase 1 safety and PK studies it is ready for Phase 2 efficacy studies. NIH/NIAID has started a series of Phase 2 studies of ANS-6637 in patients with opioid use disorder as part of a $12.4M HEAL Initiative award. This includes the recently completed study of the safety, tolerability, and effects of ANS-6637 taken with and without midazolam.

Compound Description:

ANS-6637

- Highly selective and reversible aldehyde dehydrogenase 2 (ALDH2) inhibitor.
- Unique mechanism of action in the brain, preventing pathophysiologic dopamine surges without affecting basal dopamine levels.
- Shows good nonclinical pharmacokinetics with low potential for drug interactions and an acceptable nonclinical safety profile.

Primary Objective: To determine the safety and potential efficacy of ANS-6637 for treating Veterans with PTSD and co-occurring AUD.

Hypothesis: Treatment with ANS-6637 will improve outcomes of PTSD symptoms and the proportion of heavy drinking days, compared to treatment with placebo.

Primary efficacy outcomes

- PTSD Scale for DSM-5 (CAPS-5) scores
- Fear-potentiated startle (FPS) responses
- Percent of heavy drinking days (%HDD)

At a Glance Info:

- Design: Randomized, double-blind, placebo-controlled trial
- Intervention: ANS-6637, 600 mg PO daily or placebo for 8 weeks
- Site: Michael E. DeBakey VA Medical Center (MEDVAMC)
- Sample: n=80 Veterans with comorbid PTSD and AUD

ANS is working with FDA to resolve clinical hold, but were not successful and this study has been discontinued without any enrollments.

AS170014-A7- "Effect of Sublingual formulation of Dexmedetomidine HCl (BXCL501) on Ethanol in Heavy Drinkers with PTSD" – PI: Ismene Petrakis; Yale University

Background: This study grant is a follow-on to planning grant AS170014-A3 – "Developing a Proof of Concept Clinical Trial to Evaluate the Use of a Safe and Highly Selective α2a Adrenergic Receptor Agonist, BXCL 501, for the Treatment of AUD comorbid with PTSD and/or TBI" – PI: Kohn Krystal**.**

The aim of this clinical trial is to determine if Dexmedetomidine HCl (BXCL501) is safe for treatment of alcohol use disorder (AUD) with comorbid posttraumatic stress disorder (PTSD). The present trial was designed as a phase 1, double-blind, placebo-controlled, within subjects study.

The proposed study specifically aims to:
1) Evaluate whether pretreatment with BXCL501 40 μg and 80 μg attenuates stress (PTSD) reactivity in individuals with AUD and PTSD;
2) Evaluate whether pretreatment with BXCL501 40 μg and 80 μg attenuates alcohol cue reactivity in individuals with AUD and PTSD;
3) Evaluate whether pretreatment with BXCL501 40 μg and 80 μg alters subjective effects of ethanol in a laboratory setting; and
4) Evaluate whether pretreatment with BXCL501 40 μg and 80 μg increases side effects associated with ethanol administration including sedation and vital signs.

For details on compound rational, previous studies and current study strategy, please reference AS170014-A3.

This study is actively recruiting and enrolling

AS170014-A9 – *"Leveraging Multi-Omic Data Integration for In Silico Compound Prioritization."* – B. Todd Webb; RTI international

Background: Recent advances in genetics have led to the discovery of hundreds of common genetic variants in the human genome that influence alcohol consumption and major depression, both which are frequently comorbid with PTSD. Progress is also being made in identifying additional loci influencing a) abuse and dependence on alcohol and opiates and, b) other psychiatric outcomes over-represented in SMs and veterans including PTSD. In parallel, there is an increasing wealth of information from other biological domains including gene expression and epigenetics. Integrated multi-omic studies represent opportunities to refine knowledge in one domain and more importantly identify targets for translational studies not obvious via a single domain of investigation.

Primary Objective: This project seeks to leverage large-scale robust evidence across omic domains to produce a catalog of biological targets and candidate compounds for future drug repurposing, aimed at improving the effectiveness and trajectory of treatment for alcohol and substance use disorders (ASUDs) and/or posttraumatic stress disorder (PTSD). This catalog will be made possible by collecting existing multi-omic results, performing integrated analyses, and

systematically searching target-compound databases. The catalog will include summaries of the supporting evidence for the target or compound's inclusion and ranking. This actionable, interpretable, and annotated resource will be made available to the drug repurposing community who are the intended recipients of PASA-funded preclinical and clinical trials. The results will also provide an unbiased distillation of evidence across multiple domains which will aid in the evaluation of future proposals received by PASA. To produce such a resource, we propose the following aims.

Hypothesis: The availability of novel treatments for all three disorders alone or in combination (AUD, OUD, PTSD) will be a major advance since AUD has only three FDA approved treatments and PTSD has only two. While OUD has three effective agents for reducing relapse (methadone, buprenorphine, naltrexone), these agents have not been able to prevent fentanyl overdoses or to provide smooth transitions to drug-free states rather than continued opioid dependence, as occurs with methadone or buprenorphine.

Study Aims:
Aim 1: Identify genetic loci influencing common versus specific risk to PTSD, AUD, and OUD.
Aim 2: Identify gene expression modules enriched for ASUD and PTSD liability***.***
Aim 3: Identify and prioritize therapeutic compounds***.***