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PRINCIPAL INVESTIGATOR: Tracy Nolen

CONTRACTING ORGANIZATION: Research Triangle Institute
3040 Cornwallis Road
Research Triangle Park, NC 27709

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14. ABSTRACT

The PASA objective is to identify promising potential treatments for ASUD particularly in the presence of other psychological disorders including PTSD. The importance of this objective is that the military population is more likely to see ASUD in combination with PTSD and other similar psychological disorders including suicidality. Of particular concern is opioid use disorder (OUD) in Veterans. Veterans with PTSD appear to be more susceptible to develop OUD, and the prevalence of this is increasing. Therefore, the PASA Core solicits, funds, and participates in the conduct and analysis of non-clinical basic science studies, preclinical animal research studies, and early-phase clinical trials of potential treatment compounds. The intention of these studies is to provide early-stage evidence that these compounds are safe and potentially effective in treating ASUD, particularly in the presence of PTSD and other psychological disorders.

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1. Introduction

The Pharmacotherapies for Alcohol and Substance Use Disorder Alliance (PASA) is funded by the Congressionally Directed Medical Research Programs (CDMRP) as part of its Alcohol and Substance Use Disorder Research Program (ASUDRP). The goal of the PASA is to fund research for developing new medications that can improve treatment outcomes for alcohol and substance use disorders (ASUD), especially as related to post-traumatic stress disorder (PTSD) and other psychological disorders. Research proposed can be for an alcohol use disorder (AUD), substance use disorder (SUD), or both an AUD and SUD.

Clinical trials that include military service member and Veteran populations are highly desirable because these comorbidities are common in these populations. Alcohol use disorder (AUD) is the most common ASUD in the military, but opiate use disorder (OUD) also has developed significant clinical importance due to prolonged pain treatments with opiates. FDA approved pharmacotherapies are available for AUD, OUD, and PTSD. While traumatic brain injury (TBI) is of interest, it has no FDA approved specific pharmacotherapies, and none of these combined disorders have FDA approved pharmacotherapies.

Under a Cooperative Agreement, RTI International is partnering with the CDMRP to solicit, select, and operationalize research studies that support the goals of PASA.

PASA has three aims under the primary objective to develop medications to treat ASUD in the context of the reciprocal relationship between ASUD, the physiological state of stress, and the subjective state of anxiety

- ***Aim 1:*** *Discover: Test new chemical entities and repurpose existing medications in strictly pre-clinical and non-clinical models of ASUD with comorbid PTSD and other psychological disorders.*
- ***Aim 2:*** *Phase 1 First-in-Human Safety: Conduct clinical trials of potential medications that include assessment of medical safety and doses for potential efficacy in subjects with ASUD and comorbid PTSD and other psychological disorders.*
- ***Aim 3:*** *Phase 2 Efficacy: Conduct multiple site clinical trials to test preliminary efficacy and safety of potential medications or medication combinations in humans with ASUD and comorbid PTSD and other psychological disorders, and to also explore precision medicine tools for matching patients to these medications.*

2. Keywords

- alcohol and substance use disorder (ASUD)
- alcohol use disorder (AUD)
- substance use disorder (SUD)
- opiate use disorder (OUD)
- post-traumatic stress disorder (PTSD)
- request for applications (RFA)
- pharmacotherapy

3. Accomplishments

In addition to routine monitoring and supporting ongoing studies, our overarching accomplishments for the fifth year were:

- Increased awareness of PASA prior to RFA release via:
    - Arranging and holding a PASA focused workshop at the 2023 ASCP conference.
    - Developing and launching a new PASA website with enhanced capabilities and global improvements in format and functionality.
- Supported communication and collaboration among PASA investigators via hosting a PASA-wide investigator meeting (06/02/2023).
- Expanding future clinical trial pipeline for PASA via:
    - Contract executed and protocol and clinical development planning underway and will be ready for funding evaluation by the Programmatic Panel in early 2024 for AS170014-A10 Brexanolone Planning Award/ Dr. Peltier
- Launched new PASA-funded studies via:
    - Contract executed and study launched for AS170014-A11 NOP/mu partial agonist PPL-138 study/Dr. Cippitelli
    - Contract executed and study launched for AS170014-A12 Pre-clinical HNK study/ Dr. Lucki
- Enhanced operationalization of ongoing PASA-funded studies via:
    - Launching second site with improvement in enrollment rates observed for AS170014-A6: Lofexidine/Dr. Verrico.
    - Developing the Study 2-outpatient protocol for funding evaluation by the Programmatic Panel next quarter AS170014-A7: BXCL501-Alcohol interaction Study/Dr. Petrakis.
    - Continuing work on pre-clinical testing and vaccine manufacturer for AS170014-A8: Anti-Fentanyl Vaccine (Expansion 1)/Dr. Haile.
- Supported reporting out of completed PASA-funded studies via:
    - Completing clinical study report for FDA submission for AS170014-A7: BXCL501-Alcohol interaction Study/Dr. Petrakis.
    - Completing study conduct and analyses and preparing primary manuscript for AS170014-A7: BXCL501-Alcohol interaction Study/Dr. Petrakis.
    - Completing study conduct and analyses and preparing primary manuscript for AS170014-A9: In silico/Dr. Webb.

3.0 PASA Activities

The PASA research program continued in year 5 with oversight of ongoing PASA work as well as launching studies and planning awards funded from the RFA6 cycle.

3.0.a Primary objectives and milestones for the fifth year were:

A PASA objective is to efficiently manage and monitor studies that lead to accurate, quality data for publication and dissemination. This is achieved through PASA management responsibilities such as regularly scheduled check-ins, follow-ups, data accountability, statistical analysis, quality control and assurance, and other oversight activities. Another objective of PASA is to ensure the PASA website remains a living entity with a complete website redesign that was completed this

year. As always, there are ongoing updates in order to provide up to date, useful resources, and tools.

Consistent with the 3 Aims of this program as detailed in the Introduction, the overall focus of the PASA project is in (i) aiding in establishing priorities and endpoints for each project; (ii) providing scientific guidance in achieving project goals; and (iii) facilitating the navigation of challenges incurred in study conduct toward successful and timely completion of objectives. PASA ensured close communication with all research sites and tracked status through shared internal documentation.

At the start of year 5, PASA Leadership began implementing and developing the studies and planning awards approved at the end of Year 4.

3.0.b Accomplishments under the goals include:

- Updated and maintained the PASA website.
- Monitored and supported ongoing studies.
- Launched 3 new projects (1 planning award and 2 non-clinical studies).

3.0.c Training and professional development provided:

The PASA data coordinating center (DCC) staff performing study related activities on PASA are responsible for complying with training requirements set forth by RTI and federally mandated regulations. All PASA DCC staff performing study related activities train on the PASA and RTI standard operating procedures (SOPs). Exceptions to this requirement are for staff who solely manage either the PASA website or manage the financial/subcontracting processes. Individual staff are responsible for providing documentation of current training for central PASA files.

For study site staff, PASA monitors that personnel are adequately trained on all relevant study documents, as warranted per their study role, including but not limited to the study protocol, manual of procedures (MOP), electronic data capture system (EDC), and other applicable study procedures, materials and tools.

3.0.d Dissemination to communities of interest:

PASA hosts a public and private website. The private side of the website is password protected and has role-based access for unaffiliated individuals, PASA-affiliated individuals, and funded researchers. An expertise directory, study specific templates, tools, dashboards, and trackers are accessed via the private side of the portal. The public side of the website allows dissemination of public information on PASA, including updates on completed research and information on funding opportunities.

PASA personnel disseminate study results and data through regulatory submissions to the FDA, study specific manuscripts, conference workshops and presentations, and other modes of public dissemination (e.g., the website).

3.0.e Plans for next reporting period to accomplish (goals and objectives):

Over the next reporting period, an ongoing focus will be providing support for our funded studies and planning awards. PASA plans to continue collaboration on presentations and manuscripts across all PASA studies.

3.1 AS170014-A1 Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-Fentanyl Vaccine and Buprenorphine Combination Therapy (closed)

Primary manuscript: *Haile CN, Baker M, Sanchez S, Lopez-Arteaga C .. Kosten TR, et al. An immunoconjugate vaccine alters distribution and reduces the antinociceptive, behavioral and physiological effects of fentanyl in male and female rats. Biologics and Biosimilars, Oct 26, 2022; 14(11):2290. PMID: 36365109, PMCID: PMC9694531. doi: 10.3390/pharmaceutics14112290.*

3.2 AS170014-A5 Pre-clinical testing of FKBP5 Inhibitors for alcohol use disorder-PTSD comorbidity (closed)

Primary manuscript: *Cruz B, Vozella V, Carper BA, Xu JC, Kirson D, Hirsch S, Nolen T, Bradley L, Fain K, Crawford M, Kosten TR, Zorrilla EP, Roberto M. FKBP5 inhibitors modulate alcohol drinking and trauma-related behaviors in a model of comorbid post-traumatic stress and alcohol use disorder. Neuropsychopharmacology. 2022 Nov 18. doi: 10.1038/s41386-022-01497-w. Epub ahead of print. PMID: 36396784. https://pubmed.ncbi.nlm.nih.gov/36396784/*

3.3 AS170014-A6 Lofexidine Combined with Buprenorphine for Reducing Symptoms of Post-Traumatic Stress Disorder and Opioid Use Relapse in Veterans

The primary purpose of this Phase II, single center clinical trial is to evaluate the efficacy of Lucemyra™ (Lofexidine; LFX), an alpha-2-adrenergic receptor (α2-AR) agonist, as a medication for the prevention of opiate relapse and the alleviation of post-traumatic stress disorder (PTSD) symptoms in opiate-dependent veterans. The present trial was designed as an efficacy trial and utilizes a placebo-controlled, double-blind, single-site design. Currently, there is no non-opiate medication approved by the Food and Drug Administration (FDA) for this indication in the United States. If this trial demonstrates clinical safety and efficacy of LFX for opiate relapse prevention and/or PTSD symptoms, then the first clinical development accomplishment will be made paving the way for regulatory approval. Contingent upon the review and approval by the FDA, this will then permit the clinical development of LFX and depending upon the success of these other clinical trials may lead to a New Drug Application (NDA) for LFX for the indication of opiate relapse prevention and/or PTSD symptom alleviation. Therefore, the current trial has the potential of facilitating the regulatory approval of the first non-opiate medication for the prevention of opiate use relapse and/or alleviation of PTSD symptoms.

During the Screening Phase, potential participants will be reviewed for eligibility against inclusion and exclusion criteria, and eligible participants will be enrolled into the trial. Eligible participants will be randomized (1:1) to study agent (either LFX or PLB) using an adaptive randomization schema that will be implemented via an electronic data capture system. The dose of LFX will escalate over the first 3 study visits, at which point a flexible dosing regimen will be employed. Titration will occur in a blinded fashion such that individuals assigned to PLB will go through a similar perceived titration process as those in the LFX group. During the Treatment Phase, participants will receive medications and complete study procedures/assessments. During the Follow-Up Phase, participants will complete study procedures/assessments. The expected maximum duration of participation is up to 18 weeks, consisting of up to 30 days of screening, a 12-week treatment period, and a 2-week follow-up period. Notably, the

goal is to determine whether there is enough evidence of efficacy and safety for this medication combination to support development of later phase clinical trials.

3.3.a Primary objectives and milestones for the fourth year were:
The overall objective of the proposed study is to determine if LFX as an adjunct to BUP treatment improves symptoms of both OUD and PTSD. The specific aims are two-fold: 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 1.44mg/d) as adjuncts to BUP; and 2) To determine change from baseline scores on the PTSD Checklist (PCL-5) at the end of study. 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 specific hypotheses are: 1) compared to adjunct PLB, a greater proportion of veterans randomized (1:1) to adjunct LFX will submit opioid-negative urine drug screens (UDS) and self-report no opioid use across treatment weeks 5 to 12; and 2) veterans randomized to adjunct LFX will achieve a greater decrease on the PCL-5 at week 12. The research team's hypotheses are based on the distinct yet complementary mechanisms by which each medication reduces symptoms of both disorders.

Administrative goals for the year include the addition of Ben Taub General Hospital (SA site) as an active recruitment site. Currently, the site refers eligible candidates to the research team for recruitment. With the approval of the latest amendments, the research team will have the ability to perform onsite recruitment. The research group is working to reach the anticipated monthly recruitment goal of enrolling 3 at the BCM site and 3 at the SA site PTSD and OUD veterans and non-veterans quarterly.

3.3.b Accomplishments under the goals include:
Administrative goals accomplished during the past year include receiving IRB approval at both sites following the submission of multiple amendments, including:

- Expanding the inclusion criteria to allow alcohol use disorder (AUD) as a permissible condition.
- Expanding the list of permissible medications.
- Increasing the permissible age from 65 to 70.
- Removing ECG procedures from visits 2-11.
- Reducing the required number of CAROMA visits prior to beginning study medication.

On 04/11/2023, the San Antonio (SA) site was added to the study. After a slow start, the SA site screened 5, consented 5, and enrolled 5 participants from June to September. The first SA participant completed all study-related visits at the end of this reporting period. A similar slowdown was seen in May and June at BCM with 48 telephone screenings resulting in only one in-person screening and no enrollments; however, BCM had 9 enrollments in July through September.

On 08/02/2023, IRB continuing approval was granted. Twenty-seven (27) subjects have completed the preliminary screening visit during the last annual period. Of the 27 study subjects consented, 18 have been randomized to the study. Seven subjects have completed all study-related visits since the last annual review.

On 08/25/2023, A routine audit by the MEDVAMC Compliance Office was conducted and yielded no findings.

On an ongoing basis, Dr. Kosten continues frequent contact with study personnel (at both sites) as screenings and admissions continue to occur into the LFX study.

3.3.c Training and professional development provided:
Baylor College of Medicine (BCM) and the Michael E. DeBakey VA Medical Center (MEDVAMC) regularly provides training courses for research personnel. Trainings seminars at BCM are conducted by the Office of Research and Sponsored Programs Office and are Society of Clinical Research Associates (SOCRA) approved training programs. In addition, two psychiatry residents (Olusegun Adebisi Popoola, MD, Alexandra Sibley, MD) were involved in the study, providing research training opportunities. All study personnel also utilized Medidata, an electronic data capture platform, which provided an opportunity to gain proficiency in a research database platform. The SA study physician is being mentored, as this is his first time in this role.

3.3.d Dissemination to communities of interest:
The study is in active recruitment and data collection stage, with plans to disseminate information on hold until database lock.

3.3.e Plans for next reporting period to accomplish (goals and objectives):
The research team and respective sites continues to collaborate with MEDVAMC, BTGH suboxone prescribers, and the SA site in efforts to increase recruitment rates. The research team at BCM plans to expand the number of suboxone providers currently listed as co-investigators. BCM and SA expect to finish randomization of study participants (n = 60 at each site) during the next fiscal year.

3.4 AS170014-A7 Effect of Sublingual Formulation of Dexmedetomidine HCl (BXCL501) on Ethanol in Heavy Drinkers with PTSD- Alcohol Interaction Study
The overall objective of the proposed study is to determine if Dexmedetomidine HCl (BXCL501) is safe for treatment of alcohol use disorder (AUD) with comorbid posttraumatic stress disorder (PTSD) and also shows potential signals of efficacy thereby supporting the conduct of later phase clinical trials. Safety endpoints will be compared following an alcohol challenge without and concurrent with BXCL501 treatment.

This laboratory study is a phase 1, double-blind, placebo-controlled, within subjects' study. This study will consist of 3 laboratory test sessions following pretreatment with BXCL501/placebo for 10 heavy drinker participants with comorbid PTSD. Study participants will participate in a laboratory study with 3 test days (minimum of 2 days, but no longer than 2 weeks between each test day). Each test day the participant will be assigned to receive sublingual BXCL501 40µg, 80µg and placebo in a randomized fashion. Test days will be conducted to evaluate stress (PTSD) reactivity and alcohol cue reactivity. Participants will also receive IV ethanol administered via "clamp methodology" to assess for the effects of BXCL501 in combination with ethanol.

3.4a Primary objectives and milestones for the third year were:
The overall objectives for the third year were to recruit subjects, have 10 subject completers for Study 1, make changes to Study 2 protocol, complete and submit the clinical study report t

the FDA for Study 1, submit the Study 2 protocol to DSMB, Programmatic Panel, and FDA, and lastly, start the primary manuscript for Study 1.

3.4.b Accomplishments under the goals include:

In September and October 2022, the research team continued recruitment and screenings efforts, amended the Study 1's exclusion criteria to exclude participants with heart rate <55. Also, allowed participants to receive the study medication if their heart rate is greater than or equal to 55. In November 2022, the research team worked to complete the protocol for Study 2 as well as continued recruitment and screening efforts. In December 2022, the research team had 6 subjects complete the study and submitted their Continuing Review to the VA IRB. In January 2023, the research team had 1 additional subject complete the study and the Continuing Review was approved by the VA IRB. In February 2023, the research team continued efforts to finalize Study 2's protocol. March and April 2023, the research team focused on completing all data entry and queries for Study 1 in preparation for database lock in June 2023. In July through September 2023, the research team closed out Study 1 at the VA pharmacy. Medication was destroyed at the VA pharmacy. Additionally, they reviewed results of Study 1 clinical study report and submitted to the FDA while working on and finalizing the primary manuscript for Study 1. They continued planning for Study 2 included developing and finalizing the budget, protocol, and CRF packet. The Study 2 protocol was submitted to the DSMB, Programmatic Panel, and FDA for review.

3.4.c Training and professional development provided:

Site study staff have all been trained and signed-off on study specific trainings. No further trainings were conducted during this reporting period.

3.4.d Dissemination to communities of interest:

The Study 1 clinical study report was completed and submitted to the FDA. Currently the manuscript is being completed and will be reviewed by the co-authors. The research team plans to submit to the *American Journal of Psychiatry* by the end of the October.

3.4.e Plans for next reporting period to accomplish (goals and objectives):

The research team is finalizing the results of Study 1 and preparing the primary manuscript for submission. They are also anticipating the approval of Study 1 safety data from the FDA . Once approved, the team will prepare to move forward with Study 2. In parallel, the research team is awaiting approval from the Programmatic Panel for the Study 2 budget. Both approvals expected in the upcoming quarter.

3.5 AS170014-A8 Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-Fentanyl Vaccine and Buprenorphine Combination Therapy

Illicit synthetic opioid use has led to increases in opioid use disorder (OUD) and accidental opioid-related overdose deaths creating a significant public health crisis. Over a period of 12 months, (2019-2020) overdose deaths in the United States increased to the highest ever recorded (81,000) and alarmingly, the latest statistics are even higher with nearly 70% of all overdose deaths (96,700) involving opioids. Also concerning is that overdose deaths have dramatically increased in young people (age 10-24 years). Most recent estimates indicate the years of life lost secondary to unintentional drug overdose over a 5-year period (2015-2019) is greater than 1.25 million years. Fentanyl (FEN) is a highly potent μ opioid receptor agonist indicated for the treatment of moderate to severe pain. FEN and FEN-analogs (e.g.,

carfentanil, sufentanil, alfentanil, lofentanil) are highly lipophilic and rapidly penetrate the CNS which can be lethal. The standard medication to reverse FEN's effects is the mu antagonist naloxone. Because of FEN's potency however, high doses of naloxone are needed in a timely manner to avert overdose.

The research team's primary goal in PASA2 was to develop an anti-FEN vaccine to address the opioid epidemic. Overall, the research team completed the primary goals of these PASA2 studies: 1) the team generated an anti-FEN vaccine, 2) showed that vaccinated animals produced appreciable anti-FEN antibody levels, 3) demonstrated near complete blockade of FEN's analgesic effects in vaccinated animals, 4) discovered promising interactions of buprenorphine with the vaccine and, 5) determined that the vaccine prevented FEN from entering the brain in both male and female rats. The expansion studies are allowing the research team to determine whether our anti-FEN vaccine will attenuate FEN's reinforcing effects and importantly, assess whether it will block FEN-induced effects on physiology, such as hypoxia and bradycardia linked to drug overdose and death. Other support is for the manufacture of clinical grade vaccine for toxicology studies and eventual FDA approval for a Phase 1 clinical trial.

3.5.a Primary objectives and milestones for the second year were:
There were three aims for the second year which are the following:

Aim 1. *Rats vaccinated with FEN-CRM+dmLT will generate significant anti-FEN antibodies that will block FEN-induced reinstatement of drug-seeking behavior.*

Aim 2. *Rats vaccinated with FEN-CRM+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 a high dose of FEN.*

Aim 3. *The research team will contract to manufacture clinical grade (cGMP) components of the vaccine for conjugation under appropriate subcontracts.*

3.5.b Accomplishments under the goals include:

Aim 1. First, the research team replicated and further characterized the efficacy of our vaccine to generate anti-FEN antibodies and determined whether these antibodies cross-react with other FEN-derivatives. Ongoing studies include assessing the ability of the vaccine to block drug-induced reinstatement of drug seeking behavior in an animal model of relapse.

Aim 2. The research team have completed the overdose study and definitively show that our FEN-CRM vaccine in combination with the adjuvant dmLT blocks FEN's effects on multiple physiological measures in both male and female rats. They also discovered that FEN has greater effects on the physiology of male rats compared to female rats.

Aim 3. Vici Health Sciences (Elkridge, MD) has established the protocol at their facility and has synthesize hapten without impurities. Walter Reed Army Institute of Research (WRAIR) (Silver Spring, MD) is in the process of establishing a protocol at their facility to synthesize the conjugate vaccine. The research team have also designed experiments for toxicology testing that will be executed by Charles River Laboratory.

The following activities were completed during this period of performance:

- FEN-induced reinstatement in male and female rats.

- Vici Health Sciences synthesized hapten free of impurities.
- Walter Reed Army Institute for Research (WRAIR; Bethesda, MD) was previously established to do the hapten conjugation between the synthesized material from Vici and the CRM197 protein from Fina Biosolutions, LLC. Since the research team is closer to synthesis completion, they have now assigned a project manager specifically to this project as of last month (09/2023). The team's monthly meetings continue and include a host of specialties from WRAIR as well as the UH and BCM teams. They are currently on track to have the non-GMP run included in WRAIR's November 2023 production cycle, which is the last cycle for the 2023 calendar year.
- BCM and research team are in the process of contracting with Charles River Laboratories, Inc (Waltham, MA) for the animal toxicology studies. The scope of work was expanded substantially with additional arms and dosing schedules added which has increased overall costs. They are now working with an industry partner to help with cost-sharing in covering expenses over and above funds provided by primary funding through the DoD grant. They remain on track for this work to commence by no later than the Spring of 2024.
- The FEN-vaccine has been licensed from the University of Houston to an investment group that will help facilitate the approval and execution of a Phase I clinical trial.
- Andrew Lees PhD, principal and owner of Fina Biosolutions, LLC (Rockville, MD), was formally contracted with BCM in June 2023 for supplying the CRM197, a non-toxic mutant form of the 58-kd diphtheria toxin that will serve as the carrier protein for the conjugate anti-fentanyl vaccine. An updated quote for the CRM197 purchase was received and costs have now doubled because the research team are needing to create a bigger batch than previously anticipated in order to ensure enough material for the non-GMP and GMP runs with sufficient materials left over for problem-solving anomalies as has become evident during the non-GMP run, and potentially through the GMP run because of different testing standards.
- The research team is in the process of contracting with the Program for Appropriate Technology in Health (PATH), a non-profit agency working to advance health equity through the UH-BCM partnership with Tulane University. PATH will be providing the adjuvant, dmLT (double-mutant labile toxin) which has proven successful across multiple trials to boost the immune response of both oral and injected vaccines safely and effectively. They will be providing the dmLT at no cost for development of the anti-fentanyl vaccine.

Methods are described below:

FEN-CRM Conjugate Vaccine

FEN-CRM was synthesized using a FEN derivative with a carboxylic acid linker coupled to lysine residues on CRM197 (Fina Biosolutions). The FEN hapten was created in a series of four chemical reactions. The product of each step was characterized and validated by 1H and 13C NMR spectrum and purity of the FEN hapten was validated by HPLC. The final product was then conjugated to CRM197. Prior to immunization, the conjugate was dialyzed in PBS (Slide-A-Lyzer, Thermo Scien-tific), sterilized by passing it through a 0.2um filter (Acrodisk, Life Sciences) and quanti-fied using a protein assay kit (BCA, Pierce). GLP–grade dmLT was produced

according to cyclic GMP (cGMP) specification by IDT in sodium phosphate buffer supplemented with 5% lactose in vials containing 400-µg of lyophilized product in a 3-mL sterile, multidose, Wheaton serum vial and stored at 4°C. dmLT was re-suspended prior to use with IXPBS. Each batch of FEN-CRM is quality controlled using SDSPage/Western Blot.

Immunization and Sample Collection

The vaccine formulation (5µg FEN-CRM + 1µg dmLT) was prepared immediately before administration by admixing antigen and adjuvant in sterile PBS in a 100-µL volume per vaccination per animal. Animals were injected with a 0.5cc insulin syringe into the right and left caudal thigh muscle (50-µL per hind limb) Immunizations occurred at 0, 3 and 6 weeks. Blood samples were collected from the saphenous vein on weeks 4, 6, 8 and 10 post-initial vaccination. Following administration of the high dose of FEN (0.1 mg/kg) a final collection occurred at week 20 whereby rats were placed under isoflurane anesthesia and the heart exposed via bilateral thoracotomy and blood obtained by cardiac venipuncture. The brain was also removed at this time.

Anti-FEN Antibody Quantification

Anti-FEN antibodies were quantified by using corning 96 well flat bottom plates (Costar 9018) that were coated with 0.2-µg FEN-BSA and detected using AKP-conjugated anti-rat IgG (Sigma). ELISAs were quantified using dilutions of purified rat standard IgG (Sigma) to generate a standard curve that were used to calculate IgG anti-FEN antibody concentrations in samples. Results are ex-pressed as ELISA units/mL.

FEN-induced Reinstatement of Drug-Seeking Behavior

Male and female Sprague Dawley rats were first trained to lever press for 25 ug of oral FEN solution (FR 3) in two-hour sessions. Following dose-response testing FEN was replaced with water and extinction sessions started. Saline was injected (SC) following extinction sessions. Once rats met testing criteria (below 10% active lever presses during maintenance), they were administered one of two doses of fentanyl (10ug/kg, 30ug/kg, SC) and number of lever presses recorded.

Results are described below.

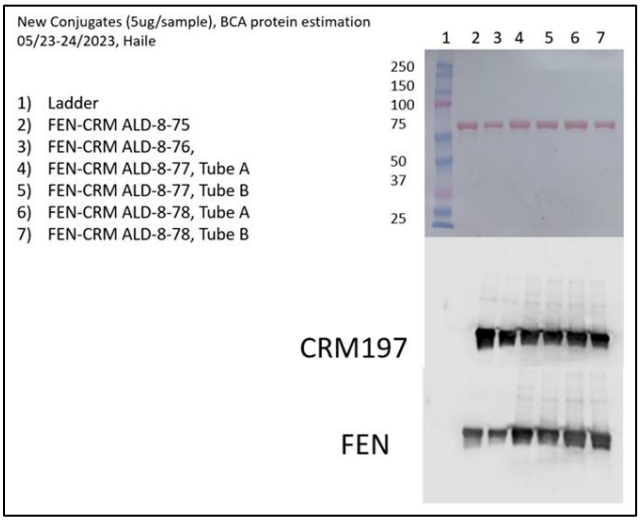


Figure 1 FEN-CRM Conjugate Vaccine. Results show successful conjugation of FEN-CRM using sodium phosphate buffer during synthesis resulting in significantly lower amounts of oligomer/aggregates.

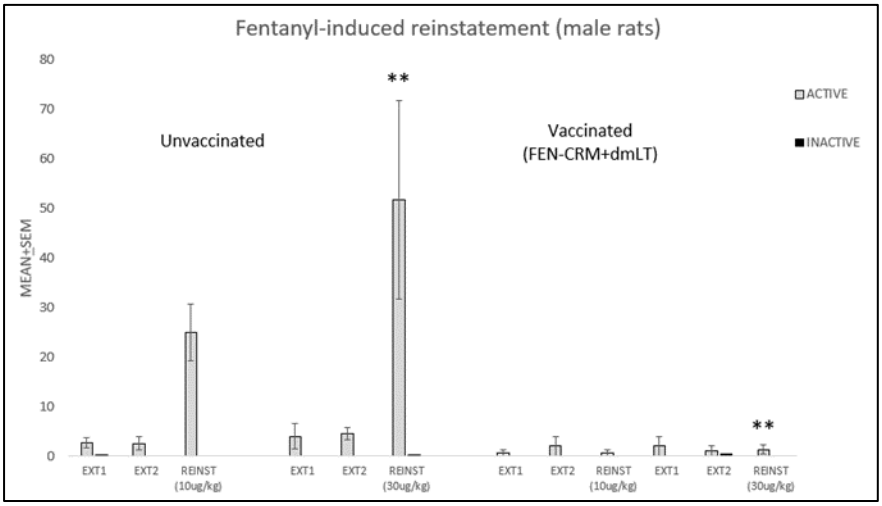


Figure 2 Extinction and reinstatement tests pre- and post-vaccination (male rats). Active (gray bars) and inactive lever (black bars) lever presses following extinction (EXT1 and EXT2) and reinstatement (REINST) tests in male Sprague Dawley rats (N=3) before vaccination with FEN-CRM (5ug)+dmLT (1ug) at 0, 3 and 6 weeks. Rats were first trained to lever press for 25 ug of oral fentanyl solution (FR 3) in two-hour sessions. Following dose-response testing fentanyl was replaced with water and extinction sessions started. Saline was injected (SC) following extinction sessions. Once rats met testing criteria, they were administered one of two doses of fentanyl (10ug/kg, 30ug/kg, SC) and number of lever presses recorded. As shown, the highest dose of fentanyl produced significantly greater lever presses on the active lever thereby reinstating drug-seeking behavior. Fentanyl-induced reinstatement of drug-seeking behavior was completely blocked following vaccination. **P<0.01

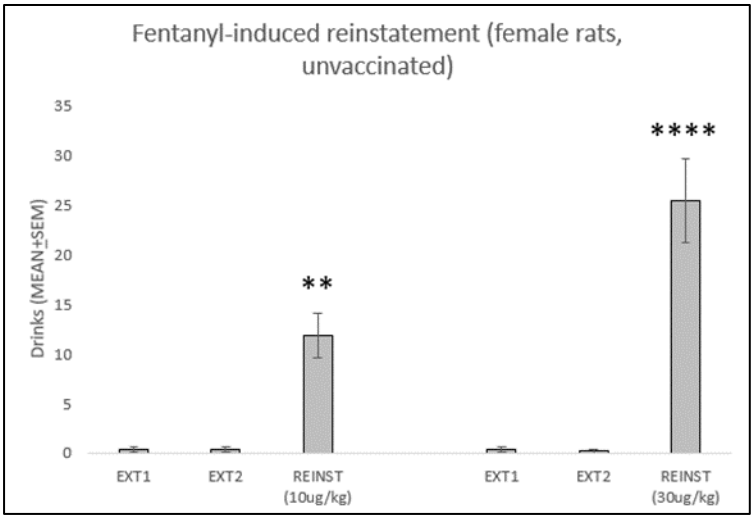


Figure 3 Extinction and reinstatement tests pre- and post-vaccination (female rats). Active (gray bars) and inactive lever (black bars) lever presses following extinction (EXT1 and EXT2) and reinstatement (REINST) tests in female Sprague Dawley rats (N=5) before vaccination with FEN-CRM (5ug)+dmLT (1ug) at 0, 3 and 6 weeks. Rats were first trained to lever press for 25 ug of oral fentanyl solution (FR 3) in two-hour sessions. Following dose-response testing fentanyl was replaced with water and extinction sessions started. Saline was injected (SC) following extinction sessions. Once rats met testing criteria, they were administered one of two doses of fentanyl (10ug/kg, 30ug/kg, SC) and number of lever presses recorded. As shown, both doses of fentanyl produced significantly greater drinks thereby robustly reinstating drug-seeking behavior. **P<0.01, ****P<0.0001, extinction vs. FEN dose.

Conclusions are detailed below:

- Improved synthesis method significantly improved the FEN-CRM conjugate profile. Quality control tests indicated decreased oligomers/aggregates increasing conjugate purity.
- The research team demonstrated that oral FEN could act as a reinforcer using the self-administration procedure in both male and female rats. Reinstatement of drug-seeking behavior was achieved in both male and female rats. Vaccination with FEN-CRM completely blocked FEN-induced reinstatement in male rats.
- VICI has successfully synthesized hapten under GMP conditions. They have scaled up to synthesize bulk hapten for residual solvent analysis and provide material to Walter Reed for non-GMP conjugation for toxicology testing.

3.5.c Training and professional development provided:
Nothing to report for this period.

3.5.d Dissemination to communities of interest:

- One published manuscript.
- Interview published:

- *Brazil Pins Hope on Vaccine to Fight Crack Epidemic: The world's second-largest consumer of cocaine bets on a long-shot solution. Interview by Samantha Pearson, The Wall Street Journal. August 17, 2023.*

- Press interviews and appearances during this reporting period:
  - 11/14/2022:
    - Fast Company
    - TIME Magazine
  - 11/15/2022:
    - News Nation
    - Daily Mail
    - KUHF Radio
  - 11/16/2022:
    - KHOU Channel 11
    - Scripps Research
    - FOX 13 TV Seattle
    - KHOU 11 News Live
    - ABC Channel 13 News
  - 11/17/2022:
    - KPRC TV
    - Bell Media Canada
    - FOX Channel 26
    - Telemundo Houston
    - KPRC TV Chanel 2
    - 1010 WINS Radio
    - NewsRadio 1080 KRLD
    - Texas State Networks
  - 11/18/2022:
    - Houston Chronicle
    - ABC10 San Diego
    - Freethink.com
    - Estrella TV
  - 11/21/2022:
    - KPRC
    - VeryWellHealth
    - FOX26
    - KPRC TV NBC
    - UH Student Paper, "The Cougar"
    - Newt's World, Newt Gingrich
  - 11/23/2022:
    - KCBS Ratio, CBS, San Francisco,
    - SciFoundation, Google Meet,
    - CBS News Radio
  - 11/26/2022:
    - News Nation, Prime live
  - 12/01/2022:

        - Texas Governor Greg Abbott lab visit, closed round table discussion and press conference; Governor Abbott, Chairman Tilman Fertitta, UH President Renu Kahator, Dr. Colin Haile, and Dr Therese Kosten
    - 12/02/2022:
        - NPR KUHF Radio, Houston Matters
        - Financial Times
        - Televisa Univision, Inc
        - FOX7 Austin KTBC
        - KTVU FOX, San Francisco
    - 12/05/2022:
        - KRGV Radio
    - 12/06/2022:
        - Texas Public Radio, San Antonio,
    - 12/07/2022:
        - EP Talk/Emergency Physicians Monthly
    - 12/14/2022:
        - NBC LX, Sara Sanchez, Producer
    - 12/27/2022:
        - WBOY West Virginia, Makayla Schindler
    - 01/17/2023:
        - WSB-TV Atlanta Channel 2; https://www.wsbtv.com/news/local/atlanta/doctors-reveal-game-changer-help-stop-fentanyl-overdose-crisis-across-metro-country/ANMIXLQL5VAWPN3DYVY2IHAO3I/
    - 01/20/2023:
        - Dallas Morning News
    - 01/24/2023:
        - Fox.com; https://www.foxnews.com/video/6319355881112
    - 01/27/2023:
        - CBS Las Vegas
    - 02/01/2023:
        - Fox.com; https://www.fox32chicago.com/news/fentanyl-vaccine-to-start-human-clinical-trials
    - 02/02/2023:
        - The Dallas Morning News, Mike Wyke,
    - 02/03/2023:
        - News Nation Prime Ryan Bass; https://www.newsnationnow.com/health/fentanyl-vaccine-opioid-addiction/
    - 03/06/2023:
        - CBS San Antonio
    - 03/29/2023:
        - Discover Magazine; https://www.discovermagazine.com/health/could-a-fentanyl-vaccine-breakthrough-save-people-from-overdoses
    - 04/12/2023:
        - UH Spring Magazine
    - 04/13/2023:
        - Investigate TV

- 06/05/2023:
  - KJZZ, NPR Interview

3.5.e Plans for next reporting period to accomplish (goals and objectives):

The research team plans to complete the self-administration study, submit a second manuscript characterizing the anti-fentanyl vaccine, complete hapten synthesis, complete residual solvent analysis, manufacture a clinical grade vaccine for toxicology studies, and, outside of existing PASA funding, initiate phase 1 clinical trial testing the anti-FEN vaccine.

3.6 AS170014-A9 Leveraging multi-omic data integration for in silico compound prioritization

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 studies 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 pre-clinical 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, the project has three aims including: 1) Identifying genetic loci influencing common versus specific risk to PTSD, AUD, and OUD 2) Identifying gene expression modules enriched for ASUD and PTSD liability, and) Prioritizing therapeutic compounds targeting risk genes and modules.

3.6.a Primary objectives and milestones for the fourth year were:

Aim 1: *Identify genetic loci influencing common versus specific risk to PTSD, AUD, and OUD:*

1a) Collect genome-wide association studies (GWAS) meta-analyses of ASUDs, PTSD, and comorbid psychiatric disorders.

1b) Leverage cross-disorder genetic correlations to disentangle pleiotropy to identify gene targets common and specific to PTSD, AUD, and OUD risk.

Aim 2: *Identify gene expression modules enriched for ASUD and PTSD liability:*

2a) Collect gene expression studies of post-mortem human brains.

2b) Perform cross-omic network analysis to detect expression sub-modules enriched for genes both common and specific for influencing risk to PTSD, AUD, and OUD.

Aim 3*: Identify and prioritize therapeutic compounds:*

3a) Rank all genes in human genome using the combined evidence from a) individual risk loci (Aim 1) and b) network analysis (Aim 2).

3b) Identify compounds targeting high ranked genes and networks by searching multiple compound identification and drug repurposing resources.

3c) Produce a catalog of genes and compounds to inform repurposing studies of ASUD

and PTSD.

3.6.b Accomplishments under the goals include:

- Collected additional GWAS results (Aim 1). In year two, the research team shifted focus to OUD and Alcohol Use Disorder (AUD) and away from GWAS-by-subtraction and PTSD.
- Completed the pilot analysis pipeline (Phase 1) which focused on OUD.
- Delivered OUD repurposing candidate compound list from Phase 1 analysis.
- Evaluated the results of Phase 1 including a) genes with multiple lines of evidence and b) the approved compounds targeting these genes.
- Completed data processing for AUD related datasets.
- Performed dmGWAS network analyses using AUD related GWAS results.
- Performed WGCNA gene expression network analyses using data from post-mortem human brains AUD cases and controls.
- Developed a method for replicating WGCNA gene expression network results across independent datasets.
- Tested strategies for incorporating expression network results into gene ranking.
- Completed Phase 2 evidence integration and compound identification pipeline which focused on OUD and AUD.
- Disseminated project results at four national scientific meetings including describing the pipeline and app for data integration, gene ranking, and identification of candidate repurposing compounds.
- Improved data integration and compound identification pipeline.
- Developed and delivered a R Shiny app based on the prototype pipeline/dashboard for gene & drug prioritization from year 1. Features include allowing:
    - Users to upload and integrate multiple sources of biological evidence to include in prioritization and ranking.
    - Flexible selection of inputs including drug approval level, drug database, ranking strategy, and number of gene targets with evidence.
    - Export of results and a detailed summary of inputs, filters, and parameters used to document analysis steps and to facilitate reproducibility.
- Produced documentation for the R Shiny app including a User Guide and FAQ to facilitate future use by PASA associated investigators.
- Delivered final OUD candidate repurposing compound list.
- Completed draft manuscript summarizing Phase 1 results.

3.6.c Training and professional development provided:

No formal training or professional development activities were included in the scope of work. However, various members of the analysis team gained new or deeper experience in analytic domains used in the project and presented a scientific meetings. Bryan Quach (Yr. 1), Caryn Willis (Yrs. 1-2), Melyssa Minto (Yr. 2) gained experience in network analyses by applying dmGWAS and WGCNA under the direction of the PI. Dr. Minto explored novel methods to replicate gene expression networks analyses across datasets with the goal of producing an additional robust source of evidence for repurposing compound candidates. They plan on summarizing this work in a publication with Dr. Minto as lead author which will increase her scientific stature, a key part of ongoing professional development. Jeran Stratford and Logain Elnimeiry gained experience in incorporating gene ranking and drug candidate scripts based in R into an interaction Shiny app. This professional development including learning to improve

efficiency and robustness of Shiny apps. Jeran Stratford, Melyssa Minto, and Caryn Willis each presented posters at different annual scientific meetings including the American Society of Human Genetics (ASHG), NIDA Genetics and Epigenetics Cross Cutting Research Team (GECCRT) meeting, and Military Health System Research Symposium (MHSRS). These experiences will help to raise their scientific stature.

3.6.d Dissemination to communities of interest:
Over the course of the project, results were presented internally to other members of omics research community at RTI, both formally and informally. Project results were also presented at five different external meetings including:

- Posters:
    - American Society of Human Genetics (ASHG)
    - NIDA Genetics and Epigenetics Cross Cutting Research Team (GECCRT) meeting
    - Military Health System Research Symposium (MHSRS)
- Oral Presentations:
    - American Society of Clinical Psychopharmacology
    - PASA annual investigators meeting

3.6.e Plans for next reporting period to accomplish (goals and objectives):
The period of performance has ended as of 09/14/2023. Although not supported, the research team plans on the following:

- Publishing the primary OUD drug repurposing manuscript. This is currently being reviewed and edited by co-authors and will soon be released as preprint in coordination with submission to a peer reviewed scientific journal.
- Writing and submitting for publication a manuscript summarizing the cross-dataset network replication work.
- Working with PASA to incorporate the candidate compound list into their website and demonstrating the R Shiny app.

3.7 AS170014-A10 Brexanolone to target concurrent Posttraumatic Stress Disorder (PTSD) and stress induced alcohol use in Veterans: A dose finding study
Posttraumatic Stress Disorder (PTSD) and alcohol use frequently co-occur in Veteran populations and are associated with overall poorer treatment outcomes. Brexanolone may target stress, an underlying mechanism of PTSD and alcohol use disorder (AUD), and thus prove to be a novel treatment. The overall objective of the proposed study is to evaluate the safety of various doses of Brexanolone (90, 60, and 30mcg/kg/h) and investigate the efficacy of each dose to reduce PTSD symptoms, alcohol consumption, and stress reactivity, via a mood-induction paradigm among US Veterans with concurrent AUD and PTSD. The proposed study is a Phase 2, double-masked, randomized, 4-arm parallel design, dose-finding laboratory study. All participants will complete the intake session and a drug administration session, which is followed by daily telephone contact for two days. Participants then complete two laboratory sessions 3-5 days apart (which include a stress-induced ad-libitum drinking paradigm) with daily phone contact between laboratory sessions. This is followed with weekly telephone contact for 4 weeks, as well as a 30-day follow-up session.

3.7.a Primary objectives and milestones for the year were:
To develop a Phase 2, dose-finding laboratory study to evaluate the safety of various doses of Brexanolone and investigate the efficacy of each dose to reduce trauma-related symptoms, alcohol use, and stress reactivity. This included:

- Development of Clinical Development Plan and study protocol
- Identification of unique case report forms
- Development of budget for proposed study
- Submission and subsequent approval of amendment to FDA IND Application (IND#211371, Sponsor: Peltier) to include the proposed study protocol

3.7.b Accomplishments under the goals include:

- Throughout reporting period:
    - Research team worked to develop the study protocol.
    - Unique Case Report Forms were identified for proposed study.
    - Study budgets developed.
- On 12/12/2022, the Clinical Development Plan was drafted.
- On 03/14/2023, a 3-way CDA between Yale School of Medicine, Sage Therapeutics, and PASA was completed.
- On 05/11/2023 and throughout this reporting period, the research team worked to develop the study protocol.
- On 05/23/2023, Sage Therapeutics approved the protocol.
- On 07/19/2023, the PASA DSMB review comments and requested changes completed, and protocol approved.
- On 08/29/2023, the study protocol, Informed Consent Forms, and other regulatory documents were submitted to the FDA, as part of an FDA IND Amendment to add the developed protocol to FDA IND# 211371 (Sponsor: Peltier).
- On 08/31/2023, the CDP revised to final version.
- On 09/22/2203, the research team received requested edits from the FDA.
- On 09/27/2023, the research team submitted a response to FDA, approval is pending.

3.7.c Training and professional development provided:
Nothing to report for this period.

3.7.d Dissemination to communities of interest:
The Principal Investigator presented the proposed study methodology and progress at the PASA investigator meeting. While the study remains under development currently, it may produce a medication which improves the treatment of PTSD/AUD for US Veterans.

3.7.e Plans for next reporting period to accomplish (goals and objectives):
The research team will continue to work with the FDA to obtain approval for the amendment to include the proposed study in FDA IND# 211371.

3.8 AS170014-A11 Evaluation of PPL-138, a NOP/mu partial agonist, for treatment of alcohol abuse in the context of PTSD

The overall objectives of this project will be a quantitative evaluation of the NOP/mu partial agonist PPL-138 in its ability to attenuate relevant symptoms (increased mechanical and thermal hypersensitivity and anxiety-like behaviors) arising from comorbid post-traumatic stress disorder (PTSD) (Aim 1) as well as its ability to attenuate alcohol consumption in the context of PTSD (Aim 2).

3.8.a Primary objectives and milestones for the first year were:

The goal of Aim 1 was to determine if PPL-138 reduced co-morbid PTSD symptoms of allodynia, hyperalgesia and anxiety-like behaviors in male and female rats using the SPS model of PTSD. Two different doses of PPL-138 (0.3 and 1.0 mg/kg) or 3 mg/kg SB612,111 were to be administered 3 times each week, with nociceptive sensitivity tested weekly, and anxiety like behaviors assessed on days 9 and 30. Serum was to be assayed for N/OFQ and CORT by RIA; CSF was to be assayed for N/OFQ. The hypothesis was that PPL-138 would show efficacy in males and females, whereas SB would reduce co-morbid symptoms in males only. The goals of Aim 2 were to define susceptible and resilient populations for SPS-induced alcohol-anxiety comorbidity and examine the effects of PPL-138 on escalated vs non-escalated alcohol self-administration in male and female rats and determine whether PPL-138 is effective in reducing relapse vulnerability associated with the SPS model of PTSD in rats of both sexes.

3.8.b Accomplishments under the goals include:

IACUC protocols were approved and modified, Dr. Cippitelli was trained in the SPS procedure during a visit to OKC, OK, and 95% of the study with male rats was completed (Aim 1). In Aim 2, the research team was able to profiling male rats based on anxiety-like and alcohol self-administration behaviors. They identified 5 different rat populations that developed or not these traits following SPS and tested PPL-138 on alcohol self-administration in all populations and in a population of male control rats that did not undergo SPS. The same behavioral profiling approach was used to identify vulnerable or resilient populations of female rats. Behavioral profiling was successfully achieved. Over the next month, the research team will test the compound on escalated vs not escalated alcohol self-administration in “anxious” and “not anxious” female rats with previous SPS experience and in a population of female control rats that did not undergo SPS. Finally, they carried out a pilot experiment to verify whether rat populations can be successfully identified using alcohol seeking (i.e., relapse-like behavior) rather than alcohol taking as main feature of alcohol vulnerability. This experiment was attempted in 10 male rats and will need to be completed by adding more experimental subjects. The same experiment will be conducted in female rats once the experiment in males is completed.

For results from Aim 1, the animal work included in this annual report was accomplished between 02/03/2023 and 09/15/2023. Rats were run in five cohorts of 8-16 rats/set. Initially the research team proposed to administer all drugs by the i.p. route, but since PPL-138 would not be administered to humans i.p. or orally, they opted to modify the route of administration to s.c. to reflect its likely human administration as sublingual. Absorption from SC delivery was not a problem for PPL-138, they found deposits of SB in the first cohort of rats, at the site of SC administration that had never been absorbed. Since SB was included as a positive control for efficacy in males based upon previous use of a NOP antagonist, and not as a potential

therapeutic, moving forward SB was administered by the i.p. route. Since the rats did not absorb the full dose of SB, the four rats that received SC SB were excluded from the study and a fifth rat had to be euthanized early. Therefore, they did not reach the full complement of male rats by 09/15/23. A new cohort of the last 8 male rats needed has arrived and will begin testing shortly to complete the study with males. While all PPL-138 groups were filled, the remaining rats will complete the two SB and the SPS+veh groups, along with one extra control rat. They are on track based upon the timeline in the SOW. Their results with data from the five cohorts (except for 4 excluded and 1 death) are as follows for nociceptive sensitivity (*Figure 1*) and 9 different anxiety-like behaviors (*Figures 2-4*).

The research team previously demonstrated that SPS induces mechanical allodynia and thermal hyperalgesia as determined by reduced paw withdrawal threshold and reduced paw withdrawal latency, respectively. Enhanced nociceptive sensitivity was found in this study as well (*Figure 1*).

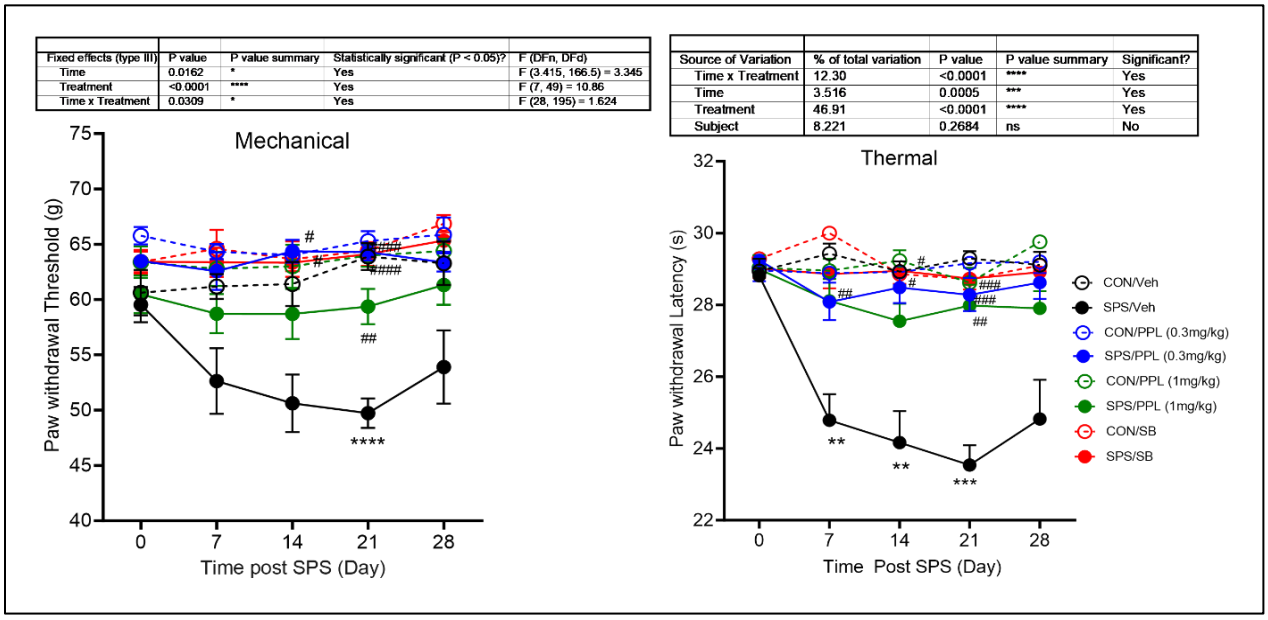
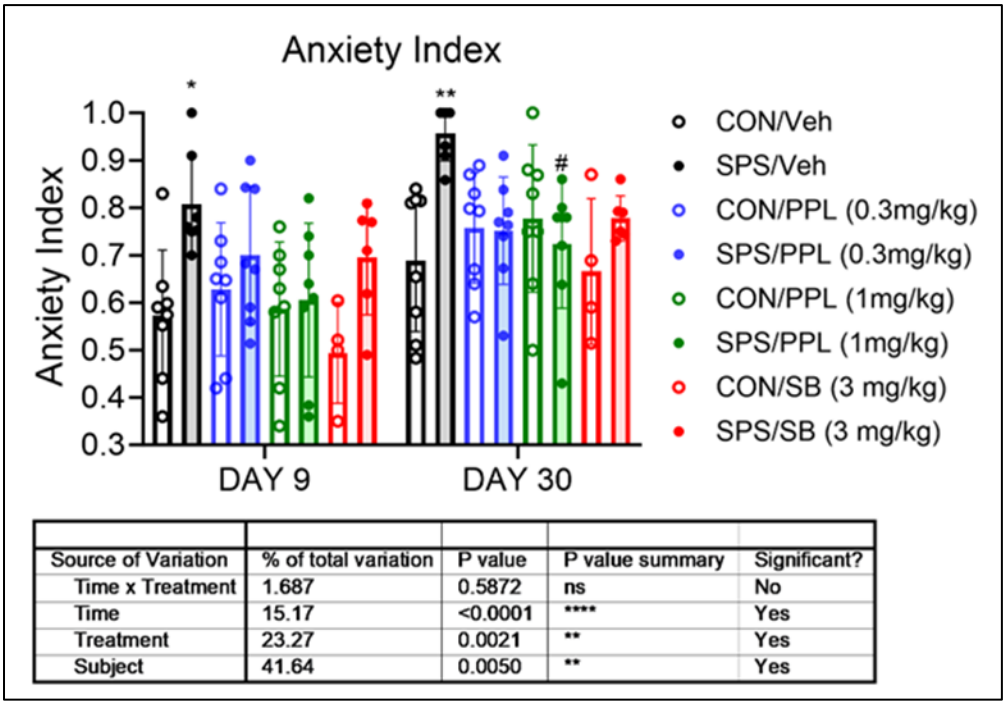


Figure 1 PPL-138 reduces SPS-induced mechanical allodynia (left) and thermal hyperalgesia in male Sprague-Dawley rats (right panel). SPS increased nociceptive sensitivity (solid black circles; N=7) compared to sham control+veh (open black circles, n=8). By day 14, 0.3 PPL-138 (N=8) and SB (N=6) returned SPS paw withdrawal thresholds to baseline levels. By day 21, 1 mg/kg PPL-138 (N=8) significantly alleviated allodynia as well, but the differences were gone by day 28 as SPS effect began to wane. Thermal sensitivity was assessed by paw withdrawal time compared to Sham controls and was reduced by SPS by day 7, lasting until day 21. Both doses of PPL-138 and SB were equally effective at reversing hyperalgesia during that time. Data are presented as the mean of 3 replicate determines/rat/time point ± SEM. Data were analyzed by 2-way ANOVA with repeated measured or mixed effects model as best determined by GraphPad Prism software (v. 9.5). Both studies revealed a significant effect of time and treatment, and a significant interaction between time and treatment.

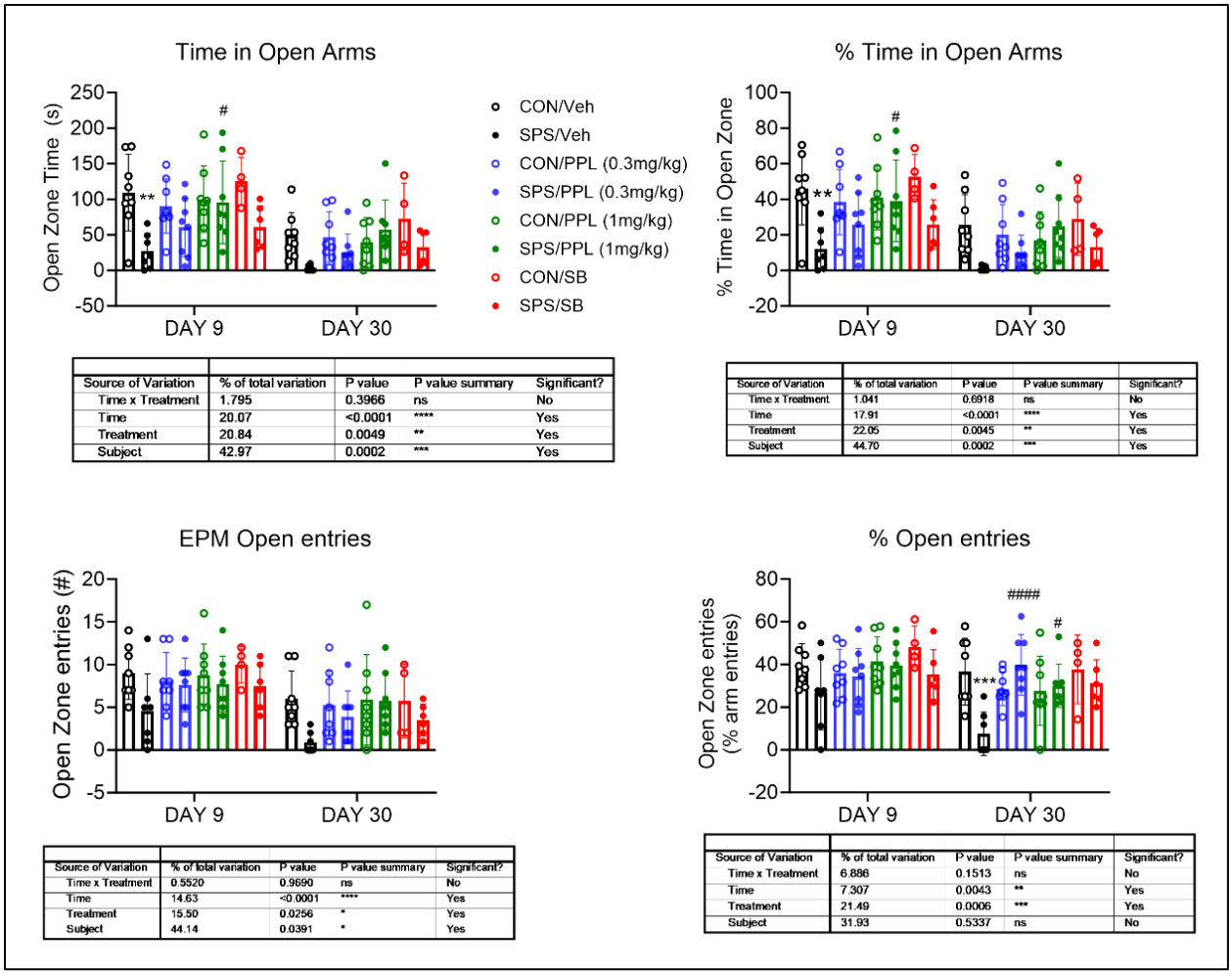
Anxiety-like behaviors were determined using the elevated plus maze. Behaviors were assessed with Any-maze software and were not subject to human interpretation. Parameters assessed

include time in open arms, # entries into open arms, % total arm time that was in open arms, % total arm entries that were into open arms, distance traveled in open and closed arms, time immobile, distance traveled in closed arms, and anxiety index (*Figure 2*). The Anxiety index was calculated as described in the proposal from three of the variables listed above including total time in the maze (300 sec). Anxiety like behaviors were increased by SPS at both 9 and 30 days post-SPS (as indicated by * in *Figure 2*). Significant differences from SPS were noted with PPL-138 (1 mg/kg) at day 30. The SB appears to show a similar effect, but the research team will not know for certain until the next cohort of rats is completed. Differences in other EPM parameters are shown in *Figures 3 and 4*.



Source of Variation	% of total variation	P value	P value summary	Significant?
Time x Treatment	1.687	0.5872	ns	No
Time	15.17	<0.0001	****	Yes
Treatment	23.27	0.0021	**	Yes
Subject	41.64	0.0050	**	Yes

Figure 2 PPL-138 reduces SPS-induced increase in anxiety index at day 30 as determined by 2-way ANOVA with repeated measures. Results are shown as mean ± S.D. of N=4-8 per group).



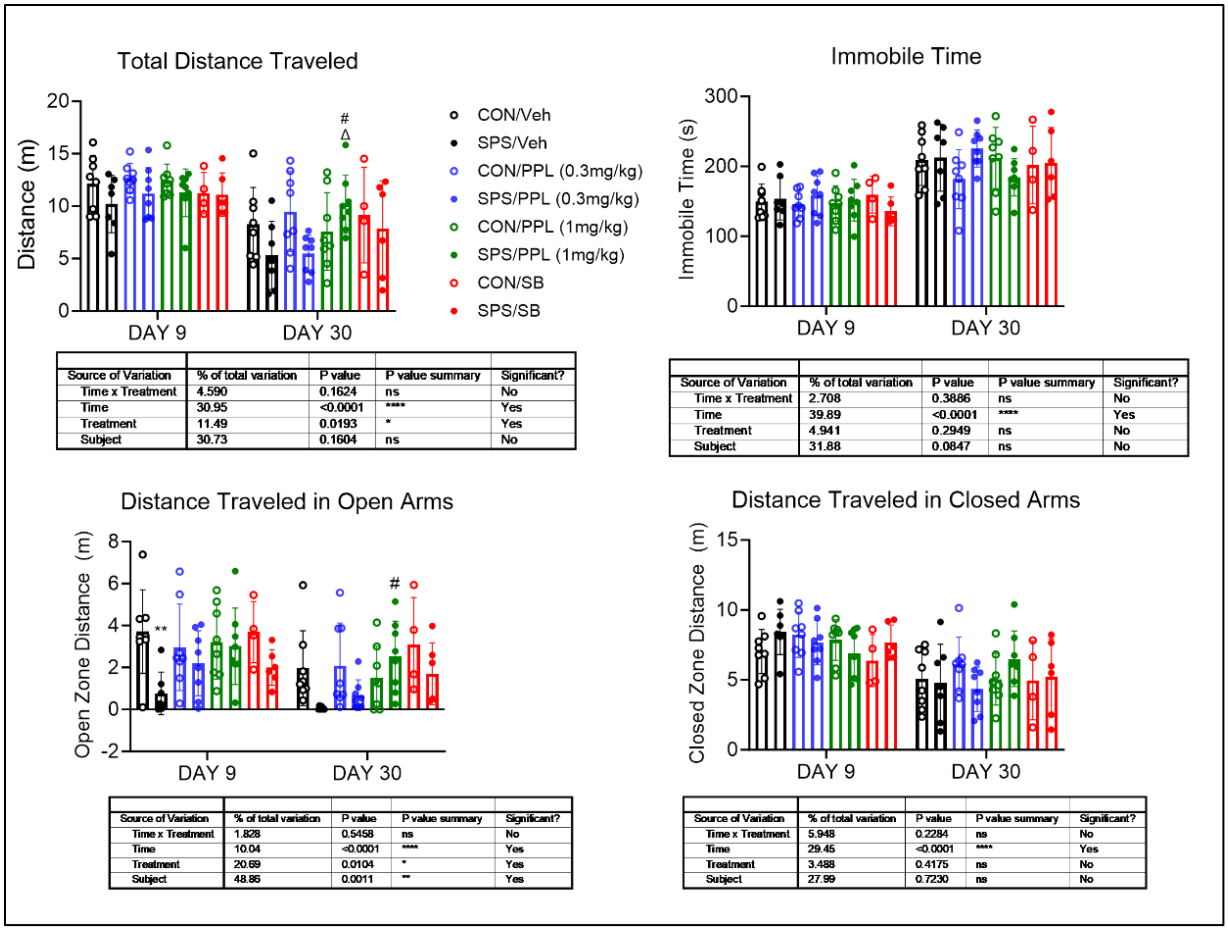
Source of Variation	% of total variation	P value	P value summary	Significant?
Time x Treatment	1.795	0.3966	ns	No
Time	20.07	<0.0001	****	Yes
Treatment	20.84	0.0049	**	Yes
Subject	42.97	0.0002	***	Yes

Source of Variation	% of total variation	P value	P value summary	Significant?
Time x Treatment	1.041	0.6918	ns	No
Time	17.91	<0.0001	****	Yes
Treatment	22.05	0.0045	**	Yes
Subject	44.70	0.0002	***	Yes

Source of Variation	% of total variation	P value	P value summary	Significant?
Time x Treatment	0.5520	0.9690	ns	No
Time	14.63	<0.0001	****	Yes
Treatment	15.50	0.0256	*	Yes
Subject	44.14	0.0391	*	Yes

Source of Variation	% of total variation	P value	P value summary	Significant?
Time x Treatment	6.886	0.1513	ns	No
Time	7.307	0.0043	**	Yes
Treatment	21.49	0.0006	***	Yes
Subject	31.93	0.5337	ns	No

Figure 3 Anxiety like behaviors related to behaviors in open arms are reflected by reductions entries, time and or % of each. SPS increases anxiety-like behaviors (as shown by asterisks) at both 9 and 30 days and alleviated by one or both doses of PPL-138 at each time point (as reflected by hash tag). Data were analyzed by 2-way repeated measures ANOVA. Results are shown as mean ± S.D. of N=4-8 per group).Traveled distance is an indicator of changes in mobility in general. Rodents prefer the closed arms, so if mobility is not affected by the stress, mobility in closed arms will not change. When anxious, animals will spend less time in open arms and may exhibit a lower distance traveled in open arms, as indicated in the bottom left panel of *Figure 4*.



Source of Variation	% of total variation	P value	P value summary	Significant?
Time x Treatment	4.590	0.1624	ns	No
Time	30.95	<0.0001	****	Yes
Treatment	11.49	0.0193	*	Yes
Subject	30.73	0.1604	ns	No

Source of Variation	% of total variation	P value	P value summary	Significant?
Time x Treatment	2.708	0.3886	ns	No
Time	39.89	<0.0001	****	Yes
Treatment	4.941	0.2949	ns	No
Subject	31.88	0.0847	ns	No

Source of Variation	% of total variation	P value	P value summary	Significant?
Time x Treatment	1.828	0.5458	ns	No
Time	10.04	<0.0001	****	Yes
Treatment	20.69	0.0104	*	Yes
Subject	48.86	0.0011	**	Yes

Source of Variation	% of total variation	P value	P value summary	Significant?
Time x Treatment	5.948	0.2284	ns	No
Time	29.45	<0.0001	****	Yes
Treatment	3.488	0.4175	ns	No
Subject	27.99	0.7230	ns	No

Figure 4 SPS reduced the distance traveled in open arms on day 9. Despite the huge reduction in distance traveled in the SPS group at day 30, it did not reach the $p<0.05$ threshold for significance. However, SPS rats receiving PPL-138 (1 mg/kg) were significantly different from SPS rats at day 30. Similar effects were reflected in total distance traveled. Data were analyzed by 2-way repeated measures ANOVA. Results are shown as mean ± S.D. of N=4-8 per group).

Therefore, it appears that the PPL-138 shows efficacy at relieving hypersensitivity and reducing anxiety-like behaviors in male rats. It would be useful for the PASA Core to perform more advanced analysis if the aim 1 data to confirm a drug or dose effect once the last group of male rats is completed. The research team is on track with their SOW time frame to meet the goals of the project.

For Aim 2, the research team generated standard distribution curves for anxiety-like behavior using % time spent on open arms of the EPM and the number of open arm entries and Δ reinforcers (i.e., difference in the number of FR-1 reinforcers earned before and after SPS, and difference in break point measures before and after SPS exposure). Alcohol SA and anxiety variables were z-score normalized to allow comparison of relative performance across variables that do not have the same units and plotted against frequency. Then, they set thresholds to ultimately identify rats with co-morbid anxiety-like behavior and escalated alcohol SA, and rats that were resilient to the effects of traumatic stress, as well as rats that showed anxiety-like behavior but not escalation or showed escalation but not anxiety-like behavior. As shown in

Figure 5 rats below the threshold set at 0.5 standard deviations were considered as “anxious,” whereas rats above the threshold set at 0.0 standard deviations were considered as “non-anxious”. Similarly, rats above the threshold set at 0.5 standard deviations above the population mean were considered as “escalated,” whereas rats below the threshold set at the population mean were considered as “non-escalated”.

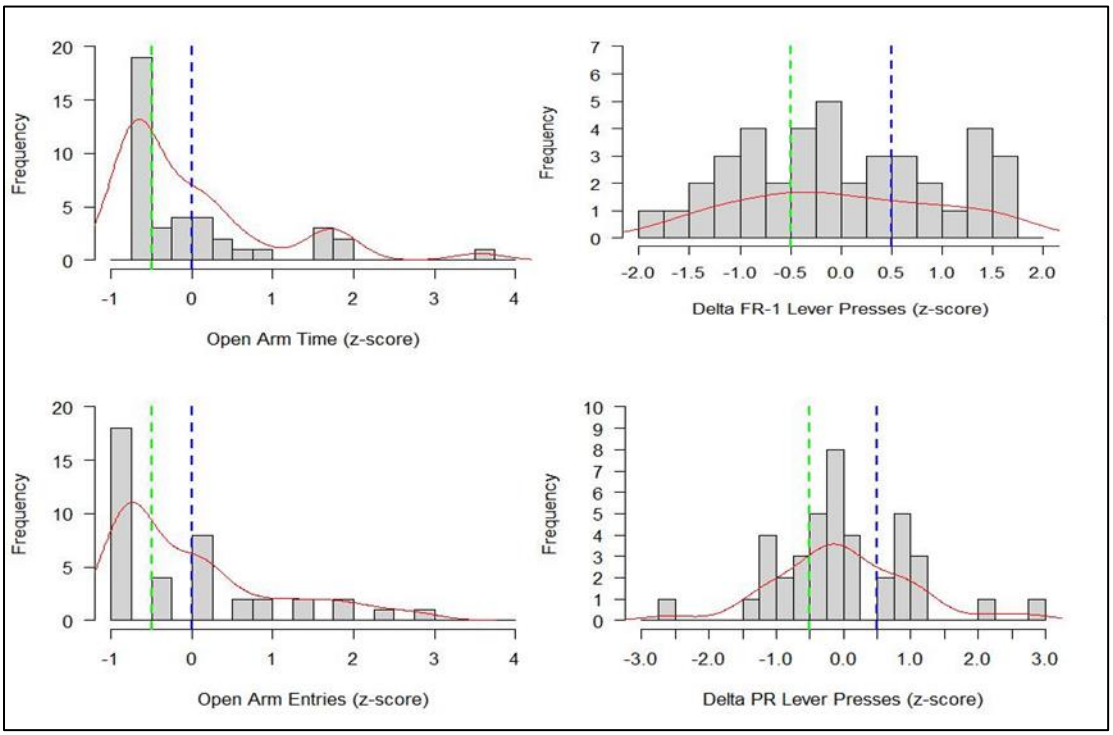


Figure 5 Behavioral Profiling. A two-threshold approach for both anxiety and self-administration parameters was used. Left: Rats below the threshold of -0.5 were identified as subjects that exhibit anxiety-like behavior post-SPS, whereas z-scores over the mean were considered non-anxious subjects. Right: Scores above the threshold of 0.5 were identified as subjects that escalated their lever pressing after trauma, whereas scores below -0.5 were classified as resilient rats.

The research team started with 50 male rats of which 40 rats were SPS exposed and 10 rats were not exposed to trauma. They found 8 rats that shared trait anxiety and escalated drinking under a fixed ratio-1 (FR-1) reinforcement schedule and 9 rats sharing anxiety and escalated motivation to press for alcohol under a progressive ratio (PR) schedule. Some rats escalated under both reinforcement conditions; others under one condition only. They established that if one animal showed increased lever pressing under only one of the two conditions, the rat was still labeled as “Comorbid”. Accordingly, the population of “Comorbid” included n=11 rats in total (see *Figure 6*). Using the same criteria, they found n=9 rats that showed none of the traits, and n=5 showing one of the two traits. N=10 rats were assigned to the category “others”, as their scores fell in between the dotted threshold lines. The remaining rats were used as “non-stressed” controls (no trauma). One rat never learned how to press the lever and was eliminated from the study.

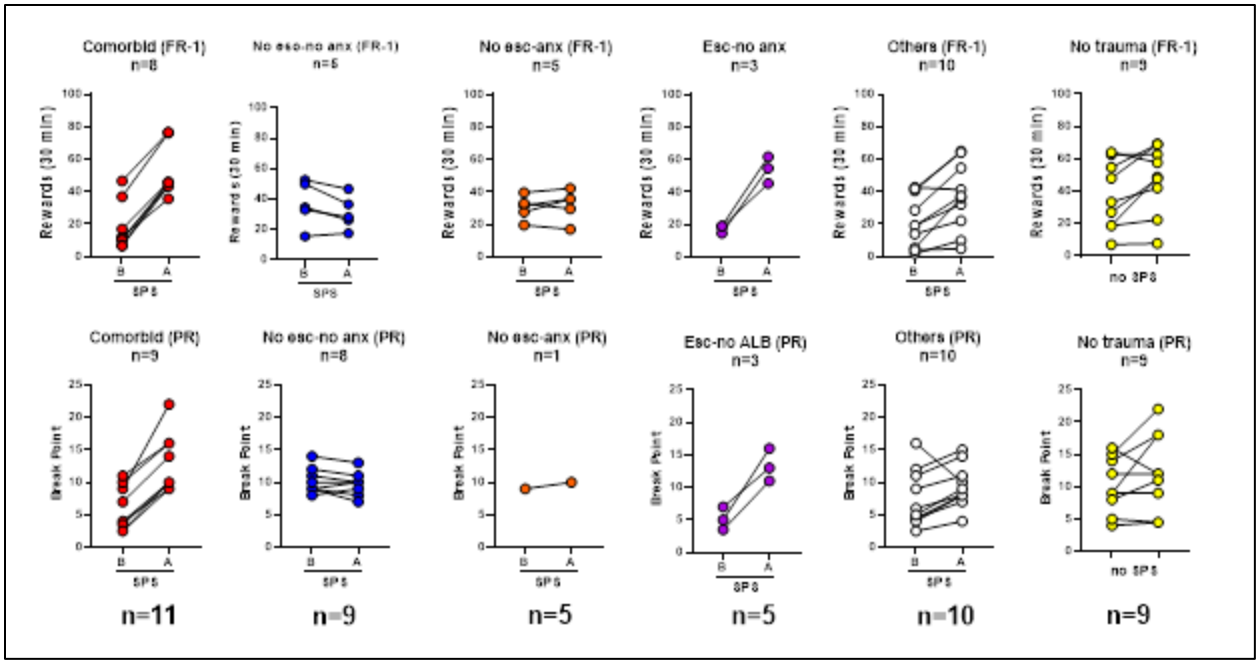


Figure 6 Define Rat Populations. Experimental populations included various combinations of the two traits of interest: presence of anxiety-like behavior versus lack of anxiety-like behavior and escalators versus non-escalators. FR-1= fixed ratio -1; PR= progressive ratio; B=before SPS; A=after SPS.

PPL-138 obtained from Phoenix PharmaLabs was tested subcutaneously according to a within-subject Latin Square design. Testing days occurred every 4th day. PPL-138 was injected 20 min prior to an FR-1 30-min session. After each testing session one day off was allowed for drug wash out. Three doses of the compound plus vehicle (3% DMSO + 97% of 0.5% hydroxypropyl cellulose) were administered in counterbalanced order. A testing session was always preceded by at least 2 regular 20% alcohol sessions.

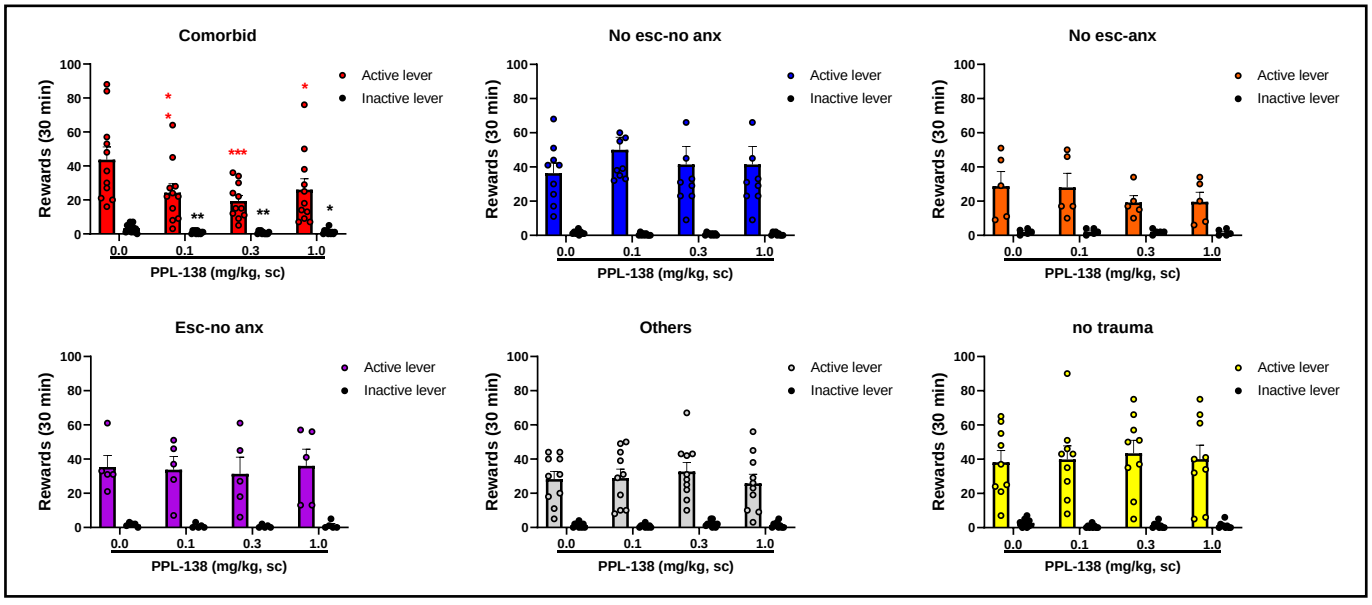


Figure 7 Treatment Testing (above). PPL-138 was administered at doses of 0.0, 0.1, 0.3, and 1.0 mg/kg, s.c. according to a Latin Square counterbalanced design. PPL-138 only had a significant effect within the comorbid population at al 3 doses examined. Asterisks denote significant differences *p<0.05, **p<0.01, ***p<0.001 vs PPL-138 0.0 mg/kg dose.

3.8.c Training and professional development provided:
The research study trained a new graduate student in the model, SC injections and behavioral tests. They also trained a PhD student to the SPS-alcohol self-administration procedure. The two PIs and Co-I, Dr. Toll, attended the PASA Investigator Meeting held 06/02/2023.

3.8.d Dissemination to communities of interest:
An abstract of their preliminary data was presented at the International Narcotics Research Conference in July 2023.

3.8.e Plans for next reporting period to accomplish (goals and objectives):
N/OFQ and CORT levels were assayed from fluids from the 5 completed cohorts of male rats at the end of September. The research team will complete the last set of males and all the females for aim 1 by the end of spring 2024, as well as the RIA assays. They plan to complete Aim 2 by the onset of summer. They will write and submit at least one manuscript.

3.9 AS170014-A12 (2R,6R)hydroxynorketamine, a putative therapeutic for comorbid post-traumatic stress disorder and alcohol use disorder in mice
Treatment of major depressive disorder with low-dose racemic ketamine has been transformative for psychiatry, producing relief from symptoms within hours that are sustained for days for a single infusion. Ketamine has also been shown to robustly alleviate suicidal ideation in hundreds of patients. Reports also support the possible use of ketamine as a treatment for post-traumatic stress disorder (PTSD) and considerable efficacy in treating alcohol use disorder. In those patients with AUD, low dose racemic ketamine decreased the number of heavy drinking days and increased the length of abstinence. Despite the transformative pattern of results for ketamine, its widespread use has been hindered by concerns regarding dissociation, sedation, and potential abuse liability. The research team's work and that of others has demonstrated that the ketamine metabolite (2R,6R)-hydroxynorketamine ((2R,6R)-HNK) produces comparable effects to ketamine on preclinical assays that are sensitive to medications that treat stress related disorders in humans, without the ketamine associated side effect. Therefore, the goal of the proposed studies is to evaluate the ability of (2R,6R)-HNK to reverse stress induced behavioral deficits and attenuate alcohol consumption. These will be the first set of data to evaluate the use of this compound for alcohol consumption and stress potentiated alcohol consumption and withdrawal. If these studies are successful, (2R,6R)-HNK would be an invaluable pharmacotherapy for military medicine, providing a safe and effective medication for service members, veterans, and their dependents suffering from PTSD, AUD, and those with difficult to treat comorbid PTSD and AUD.

3.9.a Primary objectives and milestones for the first year were:
This research study will determine whether (2R,6R)-hydroxynorketamine can 1) effectively counter the behavioral and physiological effects of stress and alcohol consumption in murine

models of post-traumatic stress disorder (PTSD) and alcohol use disorder (AUD) and 2) attenuate stress potentiated alcohol consumption and alcohol seeking behaviors.

Aim 1: *Establish whether (2R,6R)-HNK can reverse the behavioral impairments and physiological traits induced by a model of traumatic stress.*
Aim 2: *Determine the impact of (2R,6R)-HNK on alcohol consumption and compulsive drinking.*
Aim 3: *Confirm that (2R,6R)-HNK can mitigate stress potentiated alcohol consumption and comorbid negative affective behaviors.*

3.9.b Accomplishments under the goals include:

- IACUC/ACURO protocol approvals received.
- Administrative documents (MOP, QAP etc.) finalized.
- Established baseline data for stress studies under Aim 1.
- Optimized electrocardiogram acquisition parameters for all Aims.
- Commenced studies under Aim 2.
- Established ranges for ethanol consumption for male and female mice under this paradigm, with and without stress.
- Established the protocol for ELISA quantification of blood ethanol concentrations.
- Data transfer commenced for Aim 1.

3.9.c Training and professional development provided:
Nothing to report for the period.

3.9.d Dissemination to communities of interest:
The study experiments are active, but the team plans to release a primary manuscript in the future.

3.9.e Plans for next reporting period to accomplish (goals and objectives):
The research team plans to complete Aim 1 cohort 2 and cohort 3 during the next quarter. To do so, the team will have to order all animals in the remaining Aim 1 cohorts in November 2023.

4. Impact

4.0 PASA Management
Results Dissemination:

The work, findings, and specific products of the projects sponsored through PASA are ongoing, but collaboration on required regulatory reports, manuscripts and presentations has provided quality data to push innovations forward. As PASA continues to finalize and publish regulatory reports and additional manuscripts, this strengthens PASA's impact.

Pharmaceutical Partners:

Another important impact during this reporting period has been with the research team's pharmaceutical company partners. Two new pharmaceutical companies have partnered with PASA: Sage Therapeutics (AS170014-A10) and Phoenix PharmaLabs (AS170014-A11). These partners have favorably

noted their major accomplishments, innovations, and successes for identifying promising new medications for substance and opioid use disorders.

Future Projects:

PASA has refined the RFA and project award process to better identify viable projects and to make initial low-funded awards to allow for better determination of clinical trial needs for potential compounds.

Leveraging PASA Output:

PASA continues to build their template library as well as maintain the PASA website to allow for efficiency and consistency across studies. PASA has also established excellent working relationships with several VA Medical Centers across the USA for conducting the PASA clinical studies. PASA has used knowledge across studies conducted within the PASA, as well as knowledge of clinical trials conducted outside of the PASA with the PASA established collaborators, to help inform initial and continued funding decisions for compounds being studied within PASA. To further expand on PASA's ability to select novel compounds efficiently and effectively, PASA now funded two non-clinical studies (i.e., an In-silico project under PASA2). This work will help to generate a formalized catalog of promising compounds that can then be incorporated into clinical or pre-clinical pursuits based on their novelty and fit in the regulatory pathway. Taking this additional step before implementing trials will help identify innovative therapies, ensure resources are utilized efficiently, and achieve the goal of expediting the translation from bench to bedside.

4.1 AS170014-A1 Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-Fentanyl Vaccine and Buprenorphine Combination Therapy

The research team made significant progress in the development of the anti-fentanyl vaccine their contract period to the extent in which the plans include to manufacture of clinical grade vaccine for toxicology testing (funded separately, see AS170014-A8) and a Phase 1 clinical trial. The University of Houston intellectual property committee continues to support and execute the study's provisional patent to full patent status [*Colin N. Haile, Gregory D. Cuny, Elizabeth B. Norton, Therese A. Kosten, Adjuvanted Conjugate Opioid Vaccine (05/27/2020)].*

4.2 AS170014-A5 Pre-clinical testing of FKBP5 Inhibitors for alcohol use disorder-PTSD comorbidity

The research team successfully used for the first time a novel PTSD/AUD comorbidity model recently developed and characterized in their laboratory (*Steinman et al., 2020, in Molecular Psychiatry*). The model has shown efficacy in generating non-associative fear sensitization as well as Pavlovian and operant conditioning and has more translational value for PTSD and drinking behavior. Using this comorbidity model, the team was able to identify effects of benztropine, an FDA-approved drug, and SAFit2, a selective FKBP5 inhibitor, to significantly reduce voluntary ethanol drinking and, in females, acoustic startle responses, a putative indicator of hyperarousal (Benztropine only). This work is informative and was published in the *Neuropsychopharmacology* journal.

4.3 AS170014-A6 Lofexidine Combined with Buprenorphine for Reducing Symptoms of Post-Traumatic Stress Disorder and Opioid Use Relapse in Veterans

This project is actively recruiting; however, the site anticipates a positive impact in the near future. The team is dedicated to enrolling participants and collecting data accurately and efficiently to ensure the study is scientifically significant in studying symptoms of PTSD and opioid use relapse.

4.4 AS170014-A7 Effect of Sublingual Formulation of Dexmedetomidine HCl (BXCL501) on Ethanol in Heavy Drinkers with PTSD- Alcohol Interaction Study

This project was a first step to the development of BXCL501 as a potential treatment for alcohol use disorder and posttraumatic stress disorder (PTSD). Study 1 showed the medication safety -clearing the way for the FDA to approve use in an outpatient setting. In addition, data was promising for its ability to be used as a treatment in this population.

4.5 AS170014-A8 Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-Fentanyl Vaccine and Buprenorphine Combination Therapy

The development of a potential treatment to address OUD and opioid overdose is of extreme importance since deaths from fentanyl and other synthetic opioids is continuing to rise. The research team has made significant progress in the development of their anti-fentanyl vaccine during the reporting period to the extent in which their plans include initiating a Phase I clinical trial.

4.6 AS170014-A9 Leveraging multi-omic data integration for in silico compound prioritization

The following findings have impact on the development of the principal disciplines of the project:

- A meaningful finding resulting from this project is that the use a single drug repurposing database is not sufficient to identify all promising compounds. While the research team expected this to be the case, this can now be demonstrated empirically.
- While drug repurposing databases are widely available to scientists working to discover genes influencing risk (GWAS) or measuring the neurobiological signatures (expression) related to a disorder, the ability to look up and summarize the compounds targeting large numbers of genes was not practicable for most researchers. The results of this project will facilitate the rapid look up of many targets. This has potential utility beyond the target disorders (AUD, OUD, PTSD).
- Using large-scale omic results and the drug identification pipeline, the research team was able to identify a) existing first line and off-label (Ondansetron) medications used to treat OUD as well as promising (Esketamine) and novel targets for repurposing studies.
- The project developed an R Shiny app to facilitate data integration, gene ranking, and candidate compound identification. Originally, this was made to accelerate within project analyses. While still in development, the app can be more used by other investigators including PASA's current collaborators which contributed as yet unpublished results to the current project. Sharing the app more broadly after testing by PASA and/or collaborators is a goal. The research team notes that producing a publicly available tool was not part of the scope of work and further development is not currently supported.

4.7 AS170014-A10 Brexanolone to target concurrent Posttraumatic Stress Disorder (PTSD) and stress induced alcohol use in Veterans: A dose finding study
This project is a planning award to develop a clinical trial protocol, therefore, nothing to report.

4.8 AS170014-A11 Evaluation of PPL-138, a NOP/mu partial agonist, for treatment of alcohol abuse in the context of PTSD
Prior to the research team's award from PASA, a patent disclosure at their Universities was filed and a provisional patent was obtained during this reporting period based upon the preliminary data that went into their proposal.

Additionally, a press release has been delivered during this reporting period (Business Wire Thu, Sept 14, 2023. https://finance.yahoo.com/news/phoenix-pharmalabs-lead-compound-ppl-121000239.html).

4.9 AS170014-A12 (2R,6R)hydroxynorketamine, a putative therapeutic for comorbid post-traumatic stress disorder and alcohol use disorder in mice
This project is actively experimenting; however, the site anticipates a positive impact in the near future.

5. Changes/Problems

5.0 PASA Management
The RTI co-PI for this contract was changed from Dr. Nathan Vandergrift to Dr. Ryan Whitworth.

PASA actively tracked each site's status and routinely assessed for impacted abilities at the site level. Of important note is that regulatory approvals from FDA and DoD advisory boards, as well as local IRB and VA R&D committees, remain on track for successful resolution of projects.

5.1 AS170014-A1 Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-Fentanyl Vaccine and Buprenorphine Combination Therapy
The research team has completed all required activities in this study, publication of manuscript is completed.

5.2 AS170014-A5 Pre-clinical testing of FKBP5 Inhibitors for alcohol use disorder-PTSD comorbidity
The research team has completed all required activities in this study, publication of manuscript is completed.

5.3 AS170014-A6 Lofexidine Combined with Buprenorphine for Reducing Symptoms of Post-Traumatic Stress Disorder and Opioid Use Relapse in Veterans
The research team modified the BCM protocol at the request of The Office of Research Oversight and Research Compliance Services. This modification addressed parameters regarding the Certificate of (CoC) and the inclusion of research data into the participant's medical record. Amendments regarding the CoC were approved on 06/30/2023.

Subjects are recruited from the Michael E. DeBakey Opioid Treatment Program (OTP) roster. Most of these patients have pre-existing comorbidities, some of which require the prescription of prohibited medications. The research team has evaluated a list of frequently prescribed medications and dosages and configured a list of allowable medications. This modification to the protocol will allow recruitment for a subset of veterans who were previously excluded.

Subject 01G022 reported increased lethargy during day 4 of enrollment. Following the reporting of the AE, the participant's dosage was decreased to 0.8mg daily. On day 7 of enrollment, the participant requested to withdraw from the study due to continued fatigue and lethargy. The study clinician was notified of the AE and agreed the participant should be withdrawn. The participant withdrawal was reported to the sponsor and no further action was taken by the research team. During the annual renewal period, the IRB requested additional verbiage be added to the ICF detailing the potential for lethargy while enrolled in the study. This change was approved by the IRB on 08/02/2023.

The research team encountered several protocol deviations relating to research visits occurring out of the permissible window.

Additionally, the research team has had several protocol amendments to accommodate the San Antonio site and its local procedures as well as to simplify the protocol to make it more feasible. The current version of the protocol is 3.7 dated August 18, 2023. The current version of the protocol loosens restrictions on subject recruitment by increasing the maximum age to 65 and permitting enrollment of someone taking 300 mg bupropion. It also reduces the number of required CAROMA visits prior to beginning randomized medication.

5.4 AS170014-A7 Effect of Sublingual Formulation of Dexmedetomidine HCl (BXCL501) on Ethanol in Heavy Drinkers with PTSD- Alcohol Interaction Study

The research team initially struggled with slow enrollment, but through protocol amendments altering inclusion criteria in an effort to broaden the patient population to pull from, managed to recruit 10 participants who all completed this alcohol interaction study. The research team was able to submit the clinicals study report to the FDA and they have continued preparing for the second part of this originally proposed study scope of work (Study 2: Outpatient Study) .

5.5 AS170014-A8 Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-Fentanyl Vaccine and Buprenorphine Combination Therapy

As mentioned in a previous report, the research team switched from IV to oral self-administration to enable them to achieve the goals of Aim 1. Results are promising.

5.6 AS170014-A9 Leveraging multi-omic data integration for in silico compound prioritization

The research team had to deprioritize PTSD, in order to have a robust list of candidate compounds, the team focused on OUD and AUD. Given the PoP has ended, the team does not anticipate future changes or problems.

5.7 AS170014-A10 Brexanolone to target concurrent Posttraumatic Stress Disorder (PTSD) and stress induced alcohol use in Veterans: A dose finding study

As with the development of clinical studies, there may be a delay in the administrative paperwork, including amendments to the current FDA IND. Such potential delays will be mitigated by submitting the requirement documents prior to anticipated deadlines. For instance, the FDA IND amendment paperwork was submitted ahead of Programmatic Panel Review for the proposed project.

5.8 AS170014-A11 Evaluation of PPL-138, a NOP/mu partial agonist, for treatment of alcohol abuse in the context of PTSD

The research team does not anticipate problems or delays.

5.9 AS170014-A12 (2R,6R)hydroxynorketamine, a putative therapeutic for comorbid post-traumatic stress disorder and alcohol use disorder in mice

A minor modification was taken to adjust the treatment dates across all Aims, to bring all experiments in line with current treatment schedules used in the laboratory. All test sessions for primary endpoints will be conducted ~24 h after the final administration of drug. A minor modification to the IACUC procedure was sought and approved in July 2023.

6. Products

6.0 PASA Management

Specific products that have resulted from these projects during the reporting period include conference presentations and publications.

Presentations

Presentations are as noted below.

Publications

Publications are as noted below.

6.1 AS170014-A1 Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-Fentanyl Vaccine and Buprenorphine Combination Therapy

The research team published the primary manuscript:

> *Haile CN, Baker M, Sanchez S, Lopez-Arteaga C .. Kosten TR, et al. An immunoconjugate vaccine alters distribution and reduces the antinociceptive, behavioral and physiological effects of fentanyl in male and female rats. Biologics and Biosimilars, Oct 26, 2022; 14(11):2290. PMID: 36365109, PMCID: PMC9694531. doi: 10.3390/pharmaceutics14112290.*

6.2 AS170014-A5 Pre-clinical testing of FKBP5 Inhibitors for alcohol use disorder-PTSD comorbidity

The research team published the primary manuscript:

> *Cruz B, Vozella V, Carper BA, Xu JC, Kirson D, Hirsch S, Nolen T, Bradley L, Fain K, Crawford M, Kosten TR, Zorrilla EP, Roberto M. FKBP5 inhibitors modulate alcohol drinking and trauma-related behaviors in a model of comorbid post-traumatic stress and alcohol use disorder. Neuropsychopharmacology. 2022 Nov 18. doi: 10.1038/s41386-022-01497-w. Epub ahead of print. PMID: 36396784. https://pubmed.ncbi.nlm.nih.gov/36396784/*

6.3 AS170014-A6 Lofexidine Combined with Buprenorphine for Reducing Symptoms of Post-Traumatic Stress Disorder and Opioid Use Relapse in Veterans

The project is still actively recruiting participants, and no products have been reported.

6.4 AS170014-A7 Effect of Sublingual Formulation of Dexmedetomidine HCl (BXCL501) on Ethanol in Heavy Drinkers with PTSD- Alcohol Interaction Study

The research team has concluded recruitment and data collection. The manuscript for Study 1 of the study is being finalized.

6.5 AS170014-A8 Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-Fentanyl Vaccine and Buprenorphine Combination Therapy

The following manuscripts were published:

Haile CN, Baker MD, Sanchez SA, Lopez Arteaga CA, Duddupudi AL, Cuny GD, Norton EB, Kosten TR, Kosten TA. An Immunconjugate Vaccine Alters Distribution and Reduces the Antinociceptive, Behavioral and Physiological Effects of Fentanyl in Male and Female Rats. Pharmaceutics. 2022 Oct 26;14(11):2290. doi: 10.3390/pharmaceutics14112290.

Colin N. Haile, Miah D. Baker, Sergio A. Sanchez, Anantha L. Duddupudi, Gregory D. Cuny, Elizabeth B. Norton, Thomas R. Kosten, Therese A. Kosten, An anti-fentanyl conjugate vaccine alone and in combination with buprenorphine attenuates fentanyl-induced antinociception and distribution. ***(preparing to re-submit)***

Working under the current patent application:

Haile CN, Cuny GD, Norton EB, Kosten TA. Adjuvanted conjugate opioid vaccine, (05/27/2020).

6.6 AS170014-A9 Leveraging multi-omic data integration for in silico compound prioritization

Summaries of the project and results were presented at four different meetings listed below in addition to the PASA annual investigators meeting.

Stratford J, Carnes MU, Schu M, Quach BC, Willis C, Mathur R, Johnson EO, Carter J, Nolen TL, Vandergrift N, Kosten T, Webb BT. Identifying compounds to treat opiate use disorder by leveraging multi-omic data integration and multiple drug repurposing databases. American Society of Human Genetics (ASHG) annual meeting in October 2022.

Willis C, Minto MS, Quach BC, Carter JK, Stratford JK, Carnes MU, Schu M, Mathur R, Nolen TL, Vandergrift N, Johnson EO, Kosten T, Webb BT. Gene Correlation Networks Replication Analysis Across Studies to Identify Top Candidate Gene Targets in Opioid Use Disorder. NIDA Genetics and Epigenetics Cross Cutting Research Team (GECCRT) meeting, Bethesda, MD, May 2023.

Stratford J, Carnes MU, Schu M, Quach BC, Willis C, Mathur R, Johnson EO, Carter J, Nolen TL, Vandergrift N, Kosten T, Webb BT. In silico identification of candidate compounds for substance use disorder (SUD) repurposing studies by leveraging multi-omic data and resource integration. Oral presentation as part of workshop "Facilitating Rapid Development of Medications for Substance Use Disorders and PTSD: The DoD-Pharmacotherapies for Alcohol and Substance use disorders Alliance (PASA)". American Society of Clinical Psychopharmacology, June 2023.

Willis C, Minto MS, Quach BC, Stratford JK, Carnes MU, Schu M, Mathur R, Johnson EO, Carter JK, Nolen TL, Vandergrift N, Kosten T, Webb BT. Identifying Candidate Pharmacotherapies for Opioid Use Disorder by Leveraging Multi-omic Data Integration and Multiple Drug Repurposing Databases. 2023 Military Health System Research Symposium (MHSRS), August 2023.

The research team has also produced a pilot R Shiny app as a proof of principle with the goal of making the final catalog of results easily accessible and searchable. Currently, this is only being tested internally and is not ready for public access.

6.7 AS170014-A10 Brexanolone to target concurrent Posttraumatic Stress Disorder (PTSD) and stress induced alcohol use in Veterans: A dose finding study

The research team presented at the PASA Annual Investigator meeting.

> *Annual Meeting of PASA Investigators, Miami, Florida. "Brexanolone to target concurrent PTSD and stress induced alcohol use in Veterans: A dose finding study," June 2023.*

6.8 AS170014-A11 Evaluation of PPL-138, a NOP/mu partial agonist, for treatment of alcohol abuse in the context of PTSD

One abstract was presented as a poster at a scientific meeting:

> *Kealoha K, Idriss A, Zhang Y, Patankar P*, Toll L, Standifer K, Cippitelli A. Effects of the non-selective opioid ligand PPL-138 on an animal model of comorbid PTSD-AUD. International Narcotics Research Conference, Atlanta, GA (July 2023).*

One press release:

> *Business Wire Thu, Sept 14, 2023. https://finance.yahoo.com/news/phoenix-pharmalabs-lead-compound-ppl-121000239.html.*

Working under the current patent application:

> *Provisional Patent Provisional Patent Application (63/502,950) 'Methods of Treating Post-Traumatic Stress Disorder and Alcohol Use Disorder' (05/22/2023), Larry Toll, Andrea Cippitelli, and Kelly Standifer.*

6.9 AS170014-A12 (2R,6R)hydroxynorketamine, a putative therapeutic for comorbid post-traumatic stress disorder and alcohol use disorder in mice

Project is still actively experimenting, and no products have been reported.

7. Participants and Other Collaborating Organizations

PASA DCC and Management (out of RTI International)

Nolen, Tracy	Principal Investigator	18%
Whitworth, Ryan	Co-Principal Investigator	34%
Kendrick, Amy	Lead Project Manager	45%
Abella, Julie	Subawards Manager	18%
Beverly, Jennifer	Research Coordinator	10%
Carper, Ben	Statistician	3%
Chang, Samantha	Statistician	6%
Fain, Katie	Research Coordinator	5%
Gatto, Gregory	Regulatory Specialist	2%
Gianci, Christel	Administrative Staff	1%
Gieck, Chelsea	Administrative Staff	2%
Gizlice, Selen	Statistician	2%
Ham, Michael	Website Programmer	1%
Hanlon, Sean	Website Programmer	3%
Hirsch, Shawn	Statistician	16%
Hoellerich, Joey	Website Programmer	2%

Hudspeth, Julie	Financial Analyst	3%
Nowak, Kayla	Statistician	12%
Oakland, Ashleigh	Administrative Staff	3%
Okam, Ukoha	Financial Analyst	3%
Pickett, James	Programmer/Analyst	1%
Richards, Brian	Website Programmer	13%
Schwarze, Lori	Administrative Staff	3%
Smith, Emily	System Analyst	6%
Talbert, Jennifer	Research Coordinator	24%
Tang, Yan	Programmer/Analyst	4%
Thomas, Brittany	System Analyst	1%
Tillman, Stefanee	Statistician	6%
Turner, Gene	Clinical Data Manager	30%
Vandergrift, Nathan	Senior Statistician	10%
Williams, Alexis	Research Coordinator	10%
Williams, Kristi	Research Coordinator	16%

Baylor College of Medicine (PASA Management)

Kosten, Thomas	PI/PD	25%
Domingo, Coreen	Key Personnel	75%

RTI International

Leveraging Multi-omic Data Integration for In-Silico Compound Prioritization

Webb, Todd	Principal Investigator	23%
Mathur, Ravi	Co-Investigator	9%
Quach, Bryan	Co-Investigator	4%
Carnes, Megan	Co-Investigator	3%
Schu, Matthew	Co-Investigator	0%
Johnson, Eric	Co-Investigator	1%
Changar, Cynthia	Project Specialist	0%
Hill, Christine	Project Specialist	0%
Stratford, Jeran	Staff Scientist	15%
Willis, Caryn, MS	Bioinformatics Analyst	18%
Elnimeiry, Logain	Bioinformatics Analyst	10%

University of Houston

Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-Fentanyl Vaccine and Buprenorphine Combination Therapy

Haile, Colin	Principal Investigator	75%
Kosten, Therese	Co-Principal Investigator	25%
Cuny, Greg	Co-Investigator	25%
Arteaga, Carlos	Research Technician	75%
Kostecki, Gabrielle	Research Technician	75%
Duddupudi, Anantha	Post Doc	16%

The Scripps Research Institute*

Pre-clinical testing of FKBP5 inhibitors for alcohol use disorder-PTSD comorbidity

Roberto, Marisa	Principal Investigator	5%

Zorrilla, Eric	Co-Investigator	5%
Cruz, Bryan	Study Coordinator	100% (no cost)
Vozella, Valentina	Post doc	50%

*Subcontract ended on 03/31/2022. However, the above numbers are included because it was the effort reported when the contract was still in effect (which is still within this reporting period for this FY.

Baylor College of Medicine

Assessing Lofexidine combined with buprenorphine for reducing symptoms of Post-Traumatic Stress Disorder and Opioid Use Relapse in Veterans (LFX)

Verrico, Christopher	Principal Investigator	33%
Kosten, Thomas	Co-Principal Investigator	20%
Fermo, John	Co-Investigator	0%
Sibley, Alexandra	Co-Investigator	0%
Asif Khan, Mohammad	Co-Investigator	0%
Vaughan, Adetola	Study Coordinator	32%
Chii, Philip	Study Clinician	0%
Moukaddam, Nidal	Co-Investigator	0%

University of Texas Health Science Center - San Antonio

Assessing Lofexidine combined with buprenorphine for reducing symptoms of Post-Traumatic Stress Disorder and Opioid Use Relapse in Veterans (LFX)

Roache, John	Principal Investigator	10%
Murff, Bill	Study Coordinator	35%
Aviles, Lizette	Research Assistant	45%
Diaz, Daniel	Study Clinician	10%

Yale School of Medicine

Effect of Sublingual formulation of Dexmedetomidine HCl (BXCL501) on Ethanol in Heavy Drinkers with PTSD – Alcohol Interaction Study

Petrakis, Ismene	Co-Principal Investigator	20%
Krystal, John	Co-Principal Investigator	1.5%
Emily Pisani	Coordinator/RA	25%
Jane, Jane Serrita	Psychologist	50%

Yale School of Medicine

Brexanolone to target concurrent Posttraumatic Stress Disorder (PTSD) and stress induced alcohol use in Veterans: A dose finding study

Peltier, Mackenzie	Principal Investigator	0%
McKee, Sherry	Co-Principal Investigator	0%
Petrakis, Ismene	Co-Principal Investigator	0%
DeNegre, Diana	Project Coordinators	50%

Florida Atlantic University

Evaluation of PPL-138, a NOP/mu partial agonist, for treatment of alcohol abuse in the context of PTSD

Cippitelli, Andrea	Principal Investigator	30%
Toll, Lawrence	Co-Investigator	5%
Idriss, Ali	Lab Technician	100%

Oklahoma University Health Sciences Center (OUHSC)

Evaluation of PPL-138, a NOP/mu partial agonist, for treatment of alcohol abuse in the context of PTSD

Standifer, Kelly	Co-Principal Investigator	20%
Zhang, Yong	Co-Investigator	30%
Patankar, Panini	Research Assistant	10%
Al Yacoub, Omar	Research Assistant	10%

Uniformed Services University of the Health Sciences (USU)

(2R,6R)hydroxynorketamine, a putative therapeutic for comorbid post-traumatic stress disorder and alcohol use disorder in mice

Lucki, Irwin	Principal Investigator	15%
Browne, Caroline	Co-Principal Investigator	8%
Pampalone, Justin	Research Assistant	26%
Campanile, Maria	Research Assistant	23%
Castell, Kaitlin	Research Associate	24%
Tsuda, Mumeko	Consultant	5%
Johnson, Kari	Consultant	5%
Thomas, Craig	Consultant	5%

7.1 AS17004-A1 Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-Fentanyl Vaccine and Buprenorphine Combination Therapy

7.1.a. Individuals who have worked on the project include:

Dr. Haile and other staff efforts have ended for this study as all deliverables have been delivered.

7.1.b. Other organizations that have been involved as partners:

Dr. Norton, Tulane University School of Medicine, provided expert guidance and provided the adjuvant for the study (dmLT).

7.2 AS170014-A5 Pre-clinical testing of FKBP5 Inhibitors for alcohol use disorder-PTSD comorbidity

7.2.a. Individuals who have worked on the project include:

Dr. Marissa Roberto and other staff efforts have ended for this study as all deliverables have been delivered.

7.3 AS170014-A6 Lofexidine Combined with Buprenorphine for Reducing Symptoms of Post-Traumatic Stress Disorder and Opioid Use Relapse in Veterans

7.3.a. Individuals who have worked on the project include:

Dr. Christopher Verrico (PI): Responsible for all trial activates conducted at the Michael E. DeBakey VA Hospital and Baylor College of Medicine; responsible for reviewing and confirming participant eligibility.

Dr. Thomas Kosten (Co-PI): Responsible for performing and overseeing study related procedures; responsible for making important study related decisions in compliance with the ethical conduct of the study.

Ms. Adetola Vaughan (Study Coordinator): Responsible for managing day-to-day conduct of the study; responsible for ensuring that the research is conducted in compliance with the study protocol as well as federal, state, and institutional guidelines and regulations; responsible for making changes to the IRB and communicating changes to the IRB.

Dr. Philip Chii (Study Clinician): Responsible for obtaining medical history; conducting screening and post study physical exams; evaluates study related test results; responsible for assessing adverse events.

Dr. John Fermo (Co-I): Responsible for making study related medical decisions; responsible to assessing adverse events and serious adverse events.

Dr. Fang Yang (Co-I): Responsible for making study related medical decisions; responsible to assessing adverse events and serious adverse events.

Dr. Mohammad Asif Khan (Co-I): Responsible for making study related medical decisions; responsible to assessing adverse events and serious adverse events.

Dr. Alexandra Sibley (Study Clinician): Responsible for obtaining medical history; conducting screening and post study physical exams; evaluates study related test results; responsible for assessing adverse events.

Dr. Nidal Moukaddam (Co-I): Responsible for making study related medical decisions; referring suboxone patients for recruitment; responsible to assessing adverse events and Serious adverse events.

Dr. Olusegun Adebisi Popoola (Study Clinician): Responsible for obtaining medical history; conducting screening and post study physical exams; evaluating study related test results; assessing adverse events.

Ms. Destiny Moore (Study Coordinator): Responsible for managing day-to-day conduct of the study; ensuring that the research is conducted in compliance with the protocol and other guidelines and regulations.

Ms. Emmy Vazquez (Study Coordinator): Responsible for managing day-to-day conduct of the study; ensuring that the research is conducted in compliance with the protocol and other guidelines and regulations.

Dr. John Roache (PI): Responsible for all trial activates conducted at the University of Texas Health at San Antonio and the South Texas Veterans Health Care System; responsible for reviewing and confirming participant eligibility.

Mr. Bill Murff (Study Coordinator): Responsible for managing day-to-day conduct of the study; ensuring that the research is conducted in compliance with the protocol and other guidelines and regulations.

Ms. Lizette Aviles (Research Assistant): Assist the research staff with various activities for day-to-day conduct of the study.
Dr. Daniel Daz (Study Clinician): Responsible for obtaining medical history; conducting screening and post study physical exams; evaluating study related test results; assessing adverse events.

7.5.b. Change in other active support of PI/PD or senior/key personnel since last reporting period:
Dr. Rajendra Badgaiyan, MD left the South Texas Veterans Health administration, and has been removed as site PI. That role is now played by Co-PI John Roache, PhD. No other changes have occurred.

7.5.c. Other organizations that have been involved as partners:
BTGH, USWorldmeds, The South Texas Veterans Health Care System

7.4 AS170014-A7 Effect of Sublingual Formulation of Dexmedetomidine HCl (BXCL501) on Ethanol in Heavy Drinkers with PTSD- Alcohol Interaction Study

7.4.a. Individuals who have worked on the project include:
Dr. Ismene Petrakis (Co-PI): no change
Dr. John Krystal (Co-PI): no change
Ms. Lucienne Levy (Research Assistant): Removed from the study
Ms. Emily Pisani (Coordinator/RA): no change
Dr. Jane Serrita Jane (Study Clinician): no change

7.4.b. Other organizations that have been involved as partners:
BioXcel Therapeutics, Inc remains partners in this study.

7.5 AS170014-A8 Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-Fentanyl Vaccine and Buprenorphine Combination Therapy

7.5.a. Individuals who have worked on the project include:
Dr. Colin Haile (PI): Direct all phases of the study including but not limited to conducting experiments, generating and interpreting data, animal protocol approval, providing updates, and disseminating results.
Dr. Thomas Kosten (Co-I): Provides input into the design of the studies and manuscript preparation.
Dr. Greg Cuny (Co-I): Synthesizes the conjugate vaccine.
Dr. Anantha Duddupudi (Postdoc): Assists with synthesis of the conjugate vaccine.
Mr. Carlos Arteaga (Research Technician): Conducts behavioral experiments and utilized numerous types of ELISA assays for fentanyl and anti- fentanyl antibody quantification.

7.5.b. Other organizations that have been involved as partners:
Dr. Norton, Tulane University College of Medicine remains to provide expert guidance and the adjuvant for the studies (dmLT).

7.6 AS170014-A9 Leveraging multi-omic data integration for in silico compound prioritization

7.6.a. Individuals who have worked on the project include:

Dr. B. Todd Webb (PI): Responsible for supervising overall project activities including study design, monitoring progress of small group aim specific working groups, and adjusting goals based on interim results.

Dr. Ravi Mathur (Co-I): Responsible for performing aim 1 related objectives including GWAS data collection and GenomicSEM analyses.

Dr. Bryan Quach (Co-I): Responsible for overseeing and performing aim 2 related objectives and analyses including gene expression meta and integrated network analyses.

Dr. Jeran Stratford (Co-I): Responsible for performing aim 3 related objectives and analyses including drug database querying, data integration, R shiny app development, and disseminating results.

Dr. Matt Schu (Co-I): Responsible for supporting aim 3 objectives including developing semi-automated querying of drug repurposing databases. Also assisting in managing and planning staff effort across project aims.

Dr. Megan Carnes (Co-I): Responsible for supporting aim 3 objectives including drug database querying, data integration design, and gene ranking algorithm development.

Ms. Caryn Willis (Bioinformatics Analyst): Supports aim 2 related objectives and analyses including performing gene expression meta and integrated network analyses.

Ms. Logain Elnimeiry (Bioinformatics Analyst): Responsible for performing aim 3 related objectives and analyses including drug database querying, data integration, and R shiny app development. Joined team in year 2.

7.6.b. Change in other active support of PI/PD or senior/key personnel since last reporting period:

The planned reallocation in effort for Drs. Schu, Quach, Carnes, and Stratford was implemented as planned. A new bioinformatic analyst, Logain Elnimeiry, joined the project as non-key personnel.

7.7 AS170014-A10 Brexanolone to target concurrent Posttraumatic Stress Disorder (PTSD) and stress induced alcohol use in Veterans: A dose finding study

7.7.a. Individuals who have worked on the project include:

Drs. Peltier, McKee and Petrakis, as well as the proposed study Coordinator (Diana DeNegre) have been refining the protocol and clinical development plan (CDP) per the expectation/SOW of a planning award.

7.7.b. Other organizations that have been involved as partners:

Organization Name: Sage Therapeutics

Location of Organization: Cambridge, MA

Partner's Contribution to the project:

Sage Therapeutics is the pharmaceutical collaborator for this project. Sage Therapeutics collaborated with Drs. Peltier, McKee and Petrakis regarding the FDA IND amendment submission (IND# 211371) to include the proposed PASA protocol. In the event that this project receives funding, Sage will provide Brexanolone medication for the study.

7.8 AS170014-A11 Evaluation of PPL-138, a NOP/mu partial agonist, for treatment of alcohol abuse in the context of PTSD

7.8.a. Individuals who have worked on the project include:

Dr. Andrea Cippitelli (PI): Oversees the project, actively conducting research when SPS is ongoing and meets with PASA to report on progress. He analyses data with PASA staff and drafts reports.
Dr. Kelly Standifer (Co-PI): Oversees the project, meets at least weekly with the lab for updates and presents progress during bi-weekly (now monthly) PASA group meetings. She assists with data analysis and presentation, drafts the quarterly/annual reports from OUHSC site and uploads data into the portal each month.
Dr. Lawrence Toll (Co-I): Facilitates relationship with Phoenix PharmaLabs and meets with PASA biweekly or monthly, as required.
Dr. Yong Zhang (Co-I): Performed the SPS and most of the behavioral assessments and injections, and trained Panini in those areas throughout the spring of 2023.
Panini Patankar (Graduate Research Assistant): Administers injections, assists with euthanasia and sample collection, and performed behavioral assessments for cohort #4.
Omar Al Yacoub (Graduate Research Assistant): devoted a total of 16 hour during this reporting period from February to June 11, 2023 by naming rats with a code so that both Yong and Panini were blind to the treatment and stress groups until after rats were euthanized.
Ali Idriss (Lab technician): Preforms self-administration sessions and other experimental aspects of the projects with the support of Kylie Kealoha, a PhD student in the lab.

7.6.b. Change in other active support of PI/PD or senior/key personnel since last reporting period:
Dr. Zhang reduced from 80 hour effort/month to 40 hour/month as of 07/16/2023 to fulfill obligations on newly funded awards. Additionally, Mr. Idriss replaced Bianca Fakhoury as dedicated lab technician for this project.

Both Dr. Standifer and Dr. Zhang received two additional sources of support since this project was initiated:

Standifer, KM (PI; 20% effort), Zhang Y (Co-I; 50% effort). 'NOP receptor modulator treatment optimizes cognitive, locomotor and sensory outcomes of mild concussive TBI with and without PTSD', HT9425-23-1-0340. Department of Defense CDMRP Investigator-initiated TBIPHRP-IIA, $725,000 total costs. (Apr 15, 2023 – Apr 14, 2026).

Standifer, KM (PI, 30% effort), Zhang Y (Co-I; 25% effort). 'Neuropeptide modulation of cerebral blood flow to improve neurological and psychological outcomes following TBI in the presence and absence of traumatic stress', HT9425-23-1-0517, Department of Defense PRMRP Investigator-initiated, $2,273,306 total costs. (7/1/23 – 6/30/2027).

Drs. Cippitelli and Toll received an additional source of support since the project was initiated.
R41DA057430 "Identification of Mixed NOP/mu partial agonists as lead compounds for treatment of methamphetamine use disorder" Cippitelli, Andrea PI, 25% effort for 1 year; Toll, Lawrence (mPI), 10% effort for 1 year.

7.7.c. Other organizations that have been involved as partners:
Phoenix PharmaLabs provided the PPL-138 for these studies.

7.9 AS170014-A12 (2R,6R)hydroxynorketamine, a putative therapeutic for comorbid post-traumatic stress disorder and alcohol use disorder in mice

7.9.a. Individuals who have worked on the project include:

Drs. Lucki and Browne, as well as supporting staff, have been refining the protocol and commencing experiments per the expectation/SOW of this research project.