AWARD NUMBER: W81XWH-18-2-0044

TITLE: Alcohol and Substance Abuse Disorders Research Program Consortium Award

PRINCIPAL INVESTIGATOR: Tracy Nolen

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

REPORT DATE: October 2021

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
OCTOBER 2021	Annual Report	09/15/2020 – 09/14/2021

4. TITLE AND SUBTITLE

Alcohol and Substance Abuse Disorders Research Program Consortium Award

5a. CONTRACT NUMBER
W81XWH-18-2-0044

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Tracy Nolen

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Research Triangle Institute
3040 Cornwallis Road
Research Triangle Park, NC
27709

8. PERFORMING ORGANIZATION REPORT NUMBER

PASA2_AR_2021

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of the PASA Consortium is to fund research that aims to identify and develop new medications to improve treatment outcomes for alcohol and substance use disorders (ASUD), especially those that occur concurrently with traumatic brain injury (TBI) and post-traumatic stress disorder (PTSD). In the second year, the consortium continued to support two ongoing preclinical studies conducted by Drs. Haile and Bardo and launched a new preclinical study conducted by Dr. Roberto. The consortium funded and supported two planning grants led by Drs. Petrakis and Yammine. Dr. Verrico's Lofexidine study design was finalized and forms and manuals were finalized in anticipation of study launch.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	30	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

1. Introduction

The goal of the PASA Consortium is to fund research that aims to identify and develop new medications to improve treatment outcomes for alcohol and substance use disorders (ASUD), especially those that occur concurrently with traumatic brain injury (TBI) and post-traumatic stress disorder (PTSD). 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 ASUD, OUD, and PTSD. While 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 the PASA Consortium.

The PASA Consortium 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 as manifested in PTSD or TBI. The three broad aims are:

AIM 1. Discover novel medications and combination medications for ASUD

AIM 2. Develop these medications through a rational Phase I proof of concept pipeline

AIM 3. Conduct Phase II preliminary safety and efficacy trials of potential medication combinations in optimal target populations and explore functional genetic polymorphisms for matching patients to these medications.

2. Keywords

- alcohol and substance use disorders
- post-traumatic stress disorder
- traumatic brain injury
- request for applications
- pharmacotherapy
- research consortium

3. Accomplishments

In addition to monitoring and supporting ongoing studies, our primary objectives for the third year were:

- To issue another request for applications for expansion studies (RFA#5) and award expansion study(s)
- Complete the Krystal and Petrakis BXCL501 study planning grant and gain approval for full study from the Programmatic Panel.
- Develop and launch the Petrakis and Krystal BXCL501 study.
- Complete preparations to implement the Lofexidine study protocol.
- Develop in-silico proposal for Programmatic Panel consideration

3.0 PASA Core

The PASA Core research program continued in year 3 with the Requests for Research Applications (RFA) and oversight of the PASA Consortium.

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

At the start of year 3, the PASA Core completed the study planning grant for the Krystal and Petrakis planning grant and successfully received Programmatic Panel approval to move towards a full study. An expansion request for application was also developed to award funds to support the continued research of highly impactful studies previously funded by PASA. Three applications were received and underwent independent review. In line with PASA Leadership recommendations, the Programmatic Panel awarded one pre-clinical study.

A PASA Core objective is to efficiently manage and monitor studies that lead to accurate, quality data for publication and dissemination. This is achieved through Core management responsibilities such as regularly scheduled check-ins, follow-ups, data accountability, statistical analysis, quality control and assurance, and other various oversight activities. Another objective of the Core is to ensure the PASA website remains a living entity with constant updates in order to ensure sites and the Consortium meet and maintain efficient feasible deadlines and milestones, as well as provide up to date, useful resources, and tools.

Consistent with the 3 Aims of this program as detailed in the Introduction (section I), the overall focus of the Core project is in (i) providing assistance 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. The PASA Core ensured close communication with all research sites and tracked status through shared internal documentation.

3.0.b Accomplishments under the goals include:

- Completed activities in support of RFA #5.
- Dr. Verrico's Lofexidine study enrolled its first participants.
- Dr. Petrakis and Krystal's BXCL501 study was approved for funding under PASA 2.
- Received IRB approvals for BXCL501 study.
- Developed systems for BXCL501 study.
- Developed 2 manuscripts for pre-clinical studies (Dr. Bardo).
- Subsequently, published 1 scientific article on the effects of the glucocorticoid receptor antagonist PT150 on stress-induced fentanyl seeking in male and female rats in the *Psychopharmacology* journal.
- Updated and maintained PASA website.

3.0.c Training and professional development provided:

The RTI data coordinating center staff performing study related activities on the PASA Consortium are responsible for complying with training requirements set forth by RTI and federally mandated regulations. All RTI staff performing study related activities on the PASA Consortium train on the PASA and BiostatEpi Division standard operating procedures (SOPs). Exceptions to this requirement are for staff who solely manage the PASA website and manage

the financial/subcontracting processes. Individual staff are responsible for providing clearly labeled documentation of relevant training files for PASA. The PASA Core calls are also a space dedicated to checking-in on study progress and development for RTI staff.

For study site staff, the PASA Consortium ensures personnel are adequately trained on all pertinent study documents including but not limited to the study protocol, manual of procedures (MOP), electronic data capture system (EDC), and all other applicable study materials. This is completed through meeting or communicating with the site study staff and having them acknowledge their participation in review of key study resources.

Due to the restrictions attributed to COVID-19 all training and professional development activities as described above were completed virtually.

3.0.d Dissemination to communities of interest:
The PASA consortium currently hosts a public and private website. The private side of the website is password protected and can only be accessed by specified researchers. Study specific templates, tools, dashboards, and trackers are disseminated via the private side of the portal. The public side also allows dissemination of various public recourses and provides updates and opportunities related to PASA to general society.

The PASA Consortium has also helped in dissemination of study data through collaboration on study specific manuscripts. Consortium personnel provide support in the development and/or finalization of all manuscripts.

3.1.e Plans for next reporting period to accomplish goals and objectives:
Over the next reporting period, one focus will be providing excellent support for our funded studies. The Core plans to collaborate on manuscripts and encourage the overall identification of eligible participants for study inclusion across all Consortium 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
The objective of this project is to support the development of an anti-fentanyl vaccine targeting fentanyl assessed in combination with buprenorphine, a medication indicated to treat opioid use disorder (OUD). The conjugated antigen is constructed using CRM197 carrier protein and a hapten with fentanyl-like domains, and will be combined with dmLT, an adjuvant tested in humans with demonstrated safety and efficacy. The anti-fentanyl vaccine will be tested in rats alone, and in combination with buprenorphine to determine its antigenicity and ability to block the analgesic effects of fentanyl in rodent models. A successful adjuvant/vaccine formulation will be slated for cGMP manufacturing, toxicology, stability testing, IND-filing, and a Phase 1 clinical trial. Other experiments associated with this project involves testing buprenorphine in our animal model of Post-Traumatic-Stress Disorder (PTSD).

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

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

Aim 2. *Determine whether the functional effects of fentanyl are blocked in rats vaccinated with CRM197-FEN alone and in combination with BUP.*

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

3.1.b Accomplishments under the goals include:

During the period of performance, approximately 1600 samples were assayed via ELISA to quantify anti-fentanyl antibodies, brain and serum fentanyl and buprenorphine levels. Additionally, a provisional patent application was submitted in coordination with the University of Houston to secure intellectual property rights for the anti-fentanyl vaccine. The University of Houston intellectual property committee met on December 14, 2020 and voted 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 (5/27/2020)].

Brain and Serum Fentanyl and buprenorphine levels were assessed in various groups of rats with ELISA. **See Table 1: Experimental Groups below.**

Table 1. Experimental groups.

No Vaccine Control	Vaccine Control	BUP	Combination
	CRM-FEN +dmLT	BUP(Saline, 0mg/kg/day)	CRM-FEN +dmLT + BUP (0mg/kg/day)
		BUP (1.5mg/kg/day)	CRM-FEN +dmLT + BUP (1.5mg/kg/day)
		BUP (3.0mg/kg/day)	CRM-FEN +dmLT + BUP (3.0mg/kg/day)
No Osmotic Pump	No Osmotic Pump	Osmotic Pump Implanted	Osmotic Pump Implanted
CRM=CRM197, FEN=fentanyl, BUP=buprenorphine			

Results

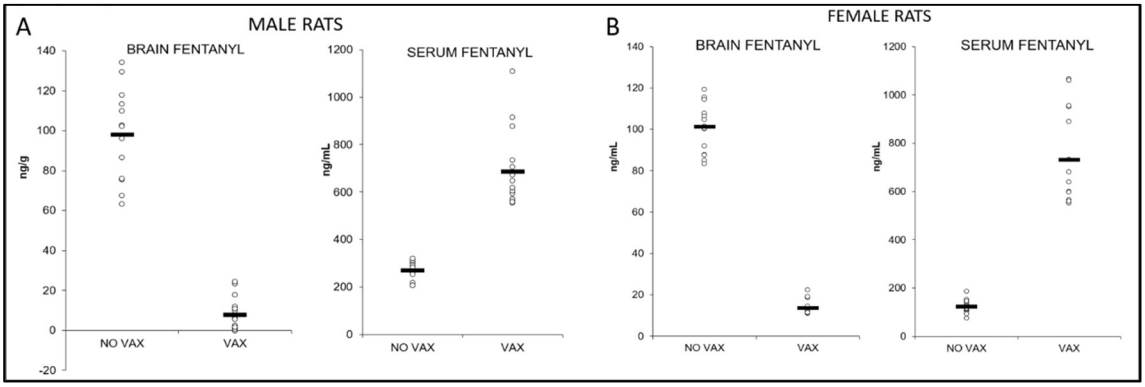


Figure 1: Vaccination with CRM-FED+dmLT prevents fentanyl from entering the brain and sequesters it in the periphery.

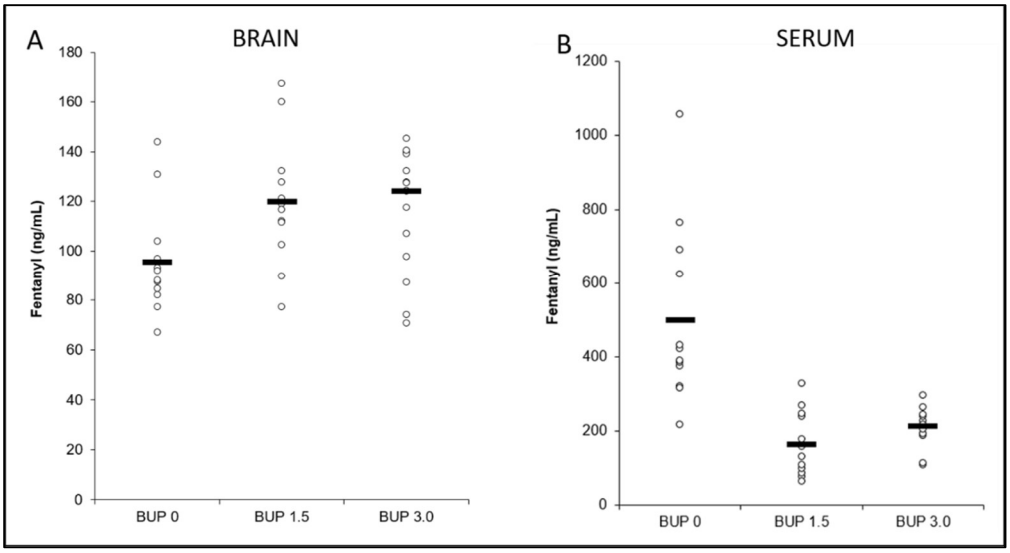


Figure 2: Buprenorphine may affect the elimination of fentanyl in male rats.

Conclusions

The vaccine produced anti-FEN antibodies that sequestered FEN in the periphery preventing the drug from penetrating the CNS. Buprenorphine alone blocked the analgesic effects of FEN; however, it may have also affected the elimination of the drug. Buprenorphine in combination with the anti-FEN vaccine produced robust sequestration of FEN in the periphery that resulted in very low brain levels. The study team has completed the aims of this award (**Figure 4**) and look to further characterize the anti-Fentanyl vaccine with additional funding.

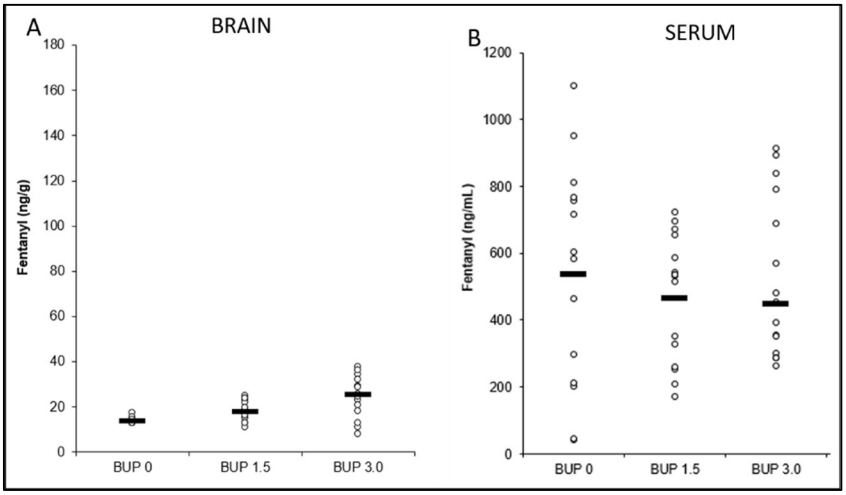


Figure 3: Fentanyl is sequestered in the periphery and prevented from entering the brain in vaccinated rats that also received buprenorphine (male rats).

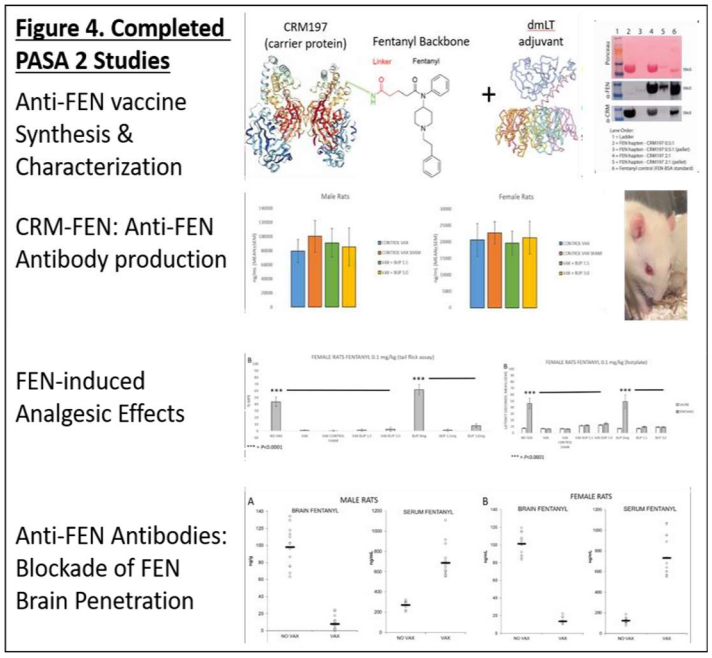


Figure 4: Completed PASA 2 Studies: Anti-FEN Vaccine Synthesis and Characterization, CRM-FEN Antibody Production, FEN-induced Analgesic Effects, and Anti-FEN Antibodies.

Additional accomplishments made during this period of the study:

- Determined the contribution of antibody isotypes in the efficacy of the vaccine between January and February 2021.
- Submitted application for expansion studies (RFA#5) that will assess the anti-FEN vaccine on FEN-induced reinstatement of drug seeking behavior in rats self-administering the drug IV. A second set of experiments will assess the effects of FEN-induced respiratory depression and decreases in other physiological measures in vaccinated and unvaccinated rats.
- The study team successfully worked with the University of Houston Office of Technology Transfer and Innovation, Commercialization Strategist and on March 5, 2021, signed a Non-Disclosure Agreement (NDA) to discuss interest in our vaccine with Orange Grove Bio, who runs a preclinical investment and development platform.
- During the period of performance, the study team re-assayed and confirmed anti-FEN antibody levels in various groups of rats during March through June 2021.
- Determined the contribution of antibody isotypes in the efficacy of the vaccine during the same period mentioned above.
- The study team received notification of funding for expansion studies that will assess the anti-FEN vaccine on FEN-induced reinstatement of drug seeking behavior in rats self-administering the drug IV. As well as funding for the second set of experiments mentioned in an earlier point.
- Submitted an amendment to the animal protocol to the university's IACUC and received approval on May 20, 2021.

- Submitted an amendment to the animal protocol to ACURO and received approval on June 10, 2021.
- Conducted ELISA assays showing specificity of the anti-fentanyl vaccine to produce antibodies that do not cross-react to a significant degree with morphine, methadone, and buprenorphine. The PASA Core is working with the study team to continue conducting ongoing data quality review and start initiating study analyses.

Methods and Results of isotypes

The immunoglobulin isotype IgG, IgG1, IgG2a and IgA were assayed in male and female Sprague Dawley rats vaccinated with our CRM-FEN (5ug) + dmLT (1ug) on 0, 3 and 6 weeks. As a final experiment, rats were administered high dose FEN (0.1, mg/kg, SC) and brains and bloods obtained to determine anti-FEN antibody concentrations and FEN brain and blood levels.

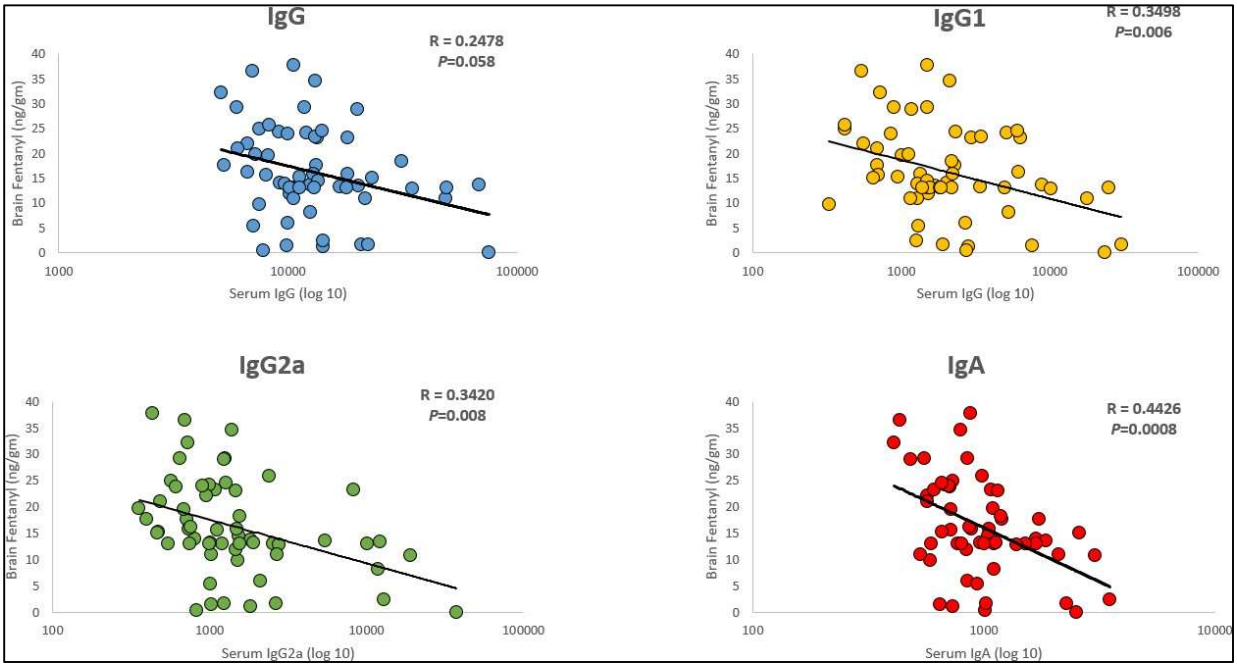


Figure 5: Antibody isotopes. Data show that all antibody isotypes correlated with brain fentanyl in male rats with the most robust correlation seen with IgA. These results replicate a series of studies the study team conducted in mice (Stone et al. 2021).

Method and Results of ELISA testing

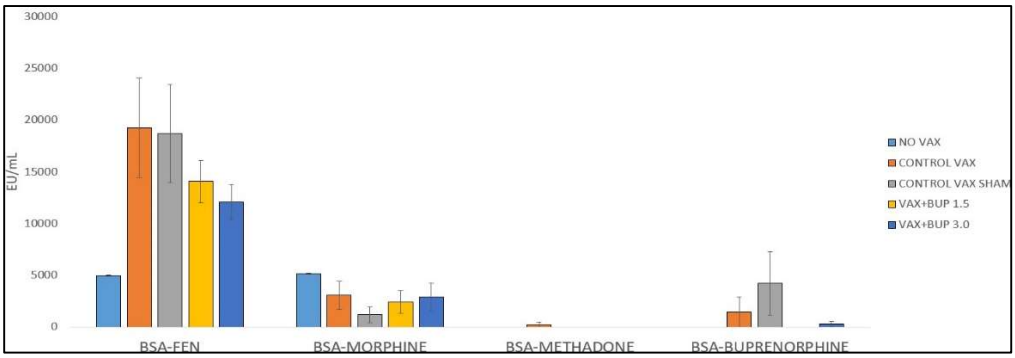


Figure 6: Anti-fentanyl Antibody Specificity. Individual ELISA plates were plated with BSA-FEN, BSA- Morphine, BSA-Methadone and BSA-Buprenorphine and serum samples from male (shown) and female (not shown) rats assayed for IgG antibodies according to our standardized protocol. Results show antibodies from vaccinated groups significantly bound to BSA-FEN and not to the other compounds above the group that did not receive the vaccine.

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

3.1.d Dissemination to communities of interest:
Information was delivered via presentations described below:

Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-fentanyl Vaccine and Buprenorphine Combination Therapy
Colin N. Haile, Alcohol and Substance Use IPR 9/23/2021

3.1.e Plans for next reporting period to accomplish goals and objectives:
This award will end November 30, 2021. The study team are in the process of pulling data together to generate a manuscript for publication.

3.2 AS170014-A2 Preclinical assessment of PT150 for opioid use disorder and PTSD
Stressful events can serve as a potent trigger for relapse among individuals who are being treated for opioid use disorder (OUD), as well as serving as basis for inducing an anxiety disorder (PTSD) that can predispose an individual to OUD. The overall working hypothesis of this preclinical study is that selective blockade of glucocorticoid receptors (GRs) in the brain with PT150 will serve as an effective pharmacotherapy for OUD and co-morbid PTSD. In Aim 1, we sought to determine if PT150 (0, 50 or 100 mg/kg, p.o.) reduces stress-induced reinstatement of fentanyl seeking using a reinstatement model of relapse in rats. Stress was applied either environmentally (mild foot shock) or pharmacologically (yohimbine) and reinstatement of fentanyl seeking was measured. As presented in our last annual report, results from that experiment (Aim 1) showed efficacy for PT150 in reducing foot shock-induced fentanyl seeking, an effect that was primarily driven by males in the sample. In the current annual report, the team presents results from an experiment (Aim 2) which sought to determine if PT150 reduces fentanyl self-administration in individuals with co-morbid PTSD. Rats were exposed to two different models of stress: (1) chronic social isolation and (2) single prolonged stress (SPS) induced by restraint/swim, which have been used to model PTSD. When subsequently tested for fentanyl self-administration, isolated rats showed more intake of fentanyl than group housed rats and males self-administered more than females overall. PT150 negated the sex difference by decreasing intake in males, while increasing it in females.

3.2.a Primary objectives and milestones for the second year were:
Goal 2: Determine if PT150 reduces fentanyl self-administration in individuals with comorbid PTSD. In this experiment, rats were raised in either social isolation or in group housing and then are receiving acute restraint/swim stress or control treatment. Previous work has shown that these stressful manipulations increase drug self-administration behavior. Plasma corticosterone was measured immediately before, after and 1 hour after the acute stress. On the day after the acute stress treatment, rats were treated daily with either PT150 or placebo and then are being trained to voluntarily self-administer fentanyl using a standard 2-lever operant conditioning

procedure. The main objective of this experiment is to test the hypothesis that PT150 will reduce the stress-induced increase in fentanyl self-administration.

3.2.b Accomplishments under the goals include:

Goal 2: Determine if PT150 reduced fentanyl self-administration in individuals with comorbid PTSD. The major activities accomplished under this goal are as follows:

a) All data collected and coded
b) All data analyzed statistically
c) Graphical representation of results completed
d) First draft of manuscript written and under review by co-authors

Figures from manuscript are below:

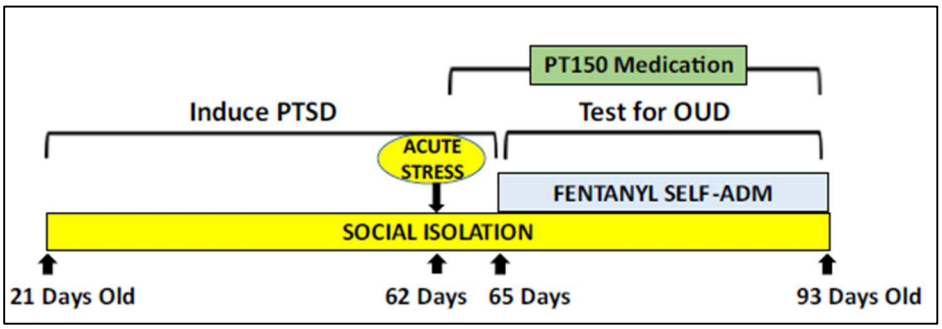


Figure 1: Schematic of Timeline. Timeline depicting sequence of procedures used to induce PTSD with social isolation and acute stress, followed by PT150 treatment and assessment of fentanyl self-administration.

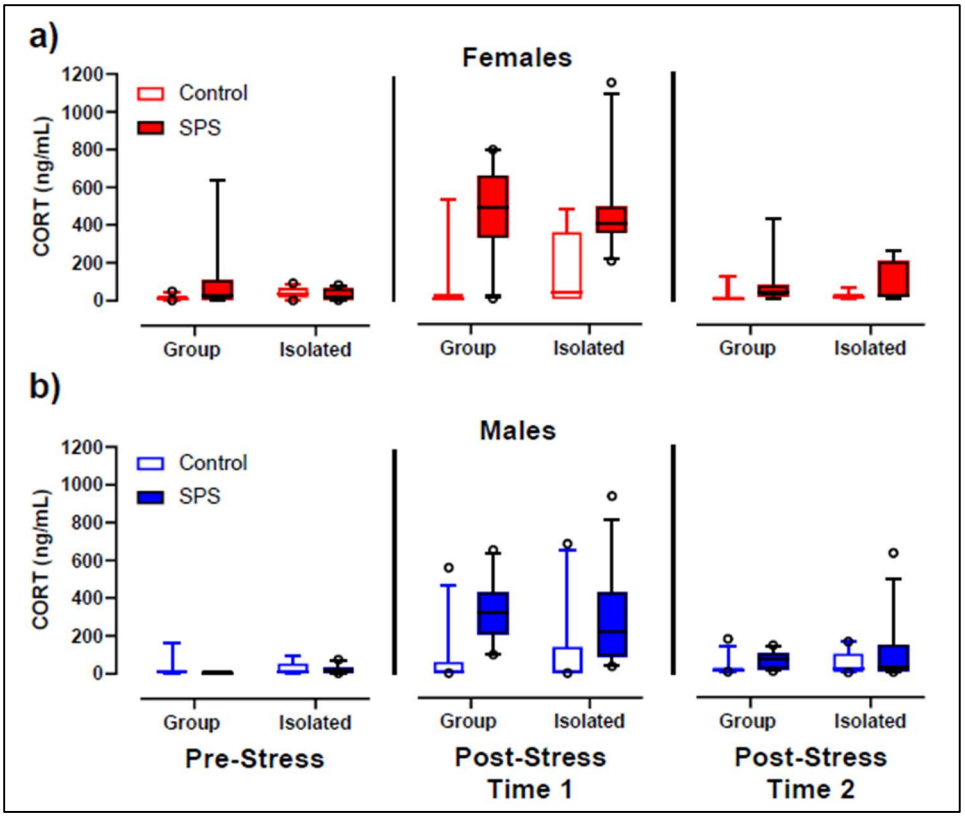


Figure 2: Corticosterone Levels. Boxplots for levels of plasma corticosterone measured in group- and isolate-housed males and females prior to (left panels), immediately after (middle panels), or 60 minutes after (right panels) SPS (filled boxes) or control (empty boxes) treatment. Boxplots depict the 10th, 25th, 50th (median), 75th, and 90th percentiles of the data with values below the 10th percentile and greater than the 90th percentile shown as empty circles.

Statistical Analysis: **Figure 2** uses a box plot to illustrate the effect of the single prolonged stress on plasma corticosterone levels in group- and isolate-housed males and females prior to any PT150 treatment. The repeated measure mixed model collapsed across PT150 treatment revealed an SPS x time interaction [$F(2,131) = 46.29$; $p < 0.0001$]; there was no main effect or interaction involving the housing condition. Subsequent least squares mean comparisons revealed that corticosterone levels immediately following SPS were elevated relative to the pre-SPS and 1-hour post-SPS timepoints. Further comparison between the pre-SPS and 1-hour post-SPS timepoint also revealed a significant difference ($p < 0.05$), indicating that corticosterone levels, while elevated most dramatically immediately after SPS application, continued to stay modestly elevated 1 hour after SPS termination. Finally, while females tended to show greater levels of corticosterone relative to males, this difference was not statistically significant.

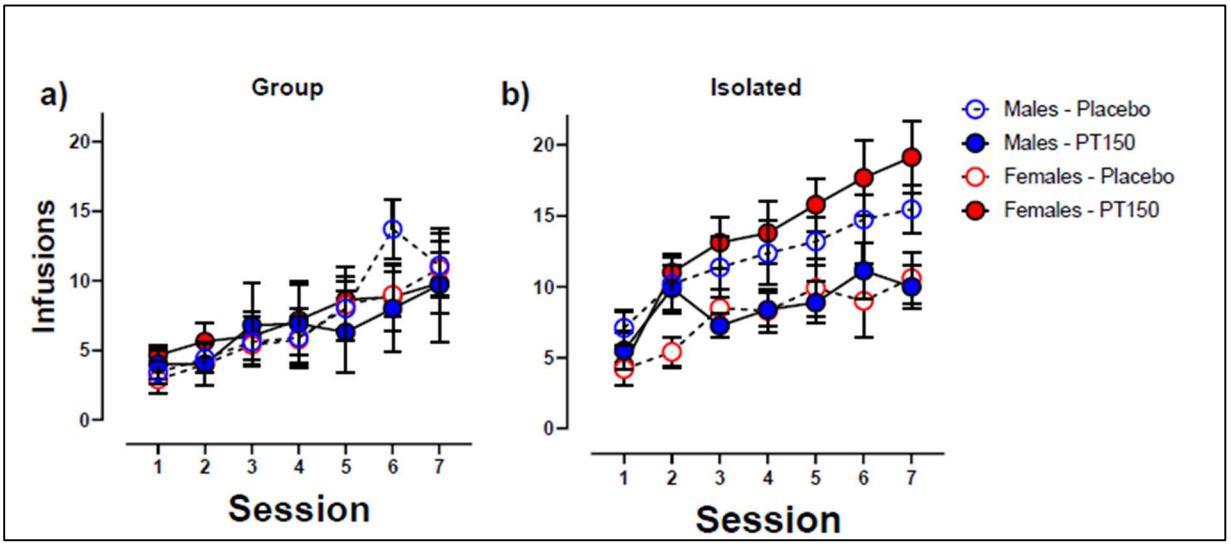


Figure 3: Acquisition of Fentanyl Self-Administration. Mean (±SEM) number of infusions earned in 1-hr self-administration sessions for group-housed males and females given either PT150 or placebo (panel a) and isolate-housed males and females given either PT150 or placebo (panel b). Results are collapsed across SPS treatment, as it was not statistically significant.

Figure 3 displays acquisition of fentanyl self-administration in group- and isolate-housed males and females treated with either PT150 or placebo across the 7 1-hr sessions that included autoshaped training. The Poisson regression model revealed significant overall linear and quadratic effects across sessions ($\chi2 = 22.06$, $p < 0.0001$ and $\chi2 = 11.59$, $p < 0.001$, respectively), indicative of acquisition of lever pressing. There was also a significant main effect of housing ($\chi2 = 8.36$, $p < 0.01$), with isolated rats self-administering more than group-housed rats. There was no significant effect of PT150 treatment or sex, although the sex x PT150 treatment interaction approached significance ($p = 0.069$).

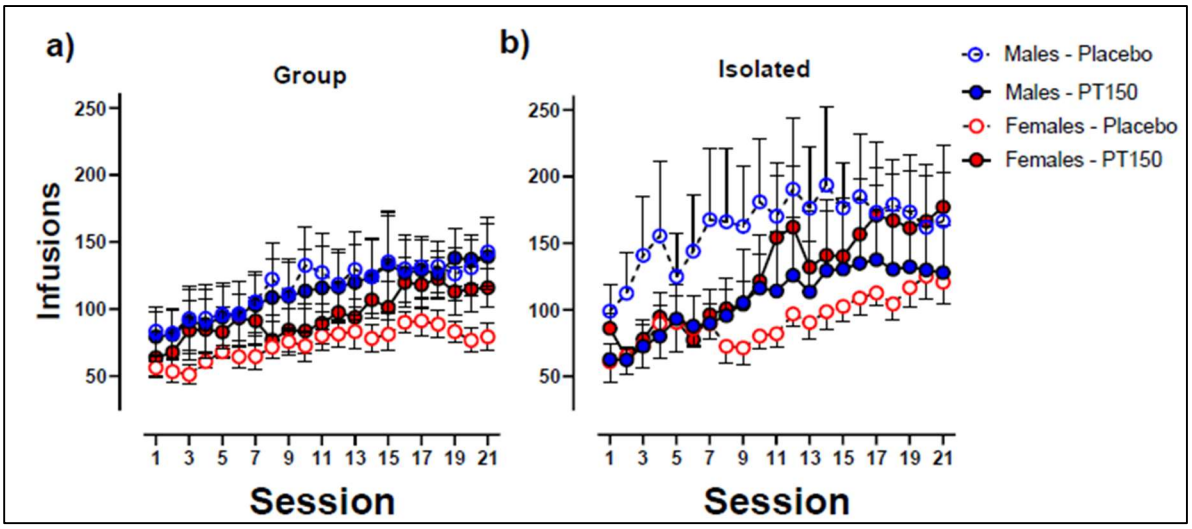


Figure 4: Escalation of Fentanyl Self-Administration. Mean (±SEM) number of infusions earned in 6-hr self-administration sessions for group-housed males and females given either PT150 or placebo (panel a) and isolate-housed males and females given either PT150 or placebo (panel b). Results are collapsed across SPS treatment, as it was not statistically significant.

Figure 4 displays escalation of fentanyl self-administration in group- and isolate-housed males and females treated with either PT150 or placebo across the 21 6-hr sessions. The Poisson regression model revealed significant overall linear and quadratic effects across sessions ($\chi2 = 18.37$, $p < 0.0001$ and $\chi2 = 9.10$, $p < 0.01$, respectively), indicative of escalation of intake. Similar to initial acquisition, the isolated rats self-administered more than group housed rats, although the effect in this phase only approached significance ($p = 0.069$). More important, the Poisson regression model revealed a significant sex x PT150 treatment interaction ($\chi2 = 4.97$, $p < 0.05$). Subsequent least squares mean comparisons revealed that males self-administered more than females within the placebo condition ($z = -3.14$, $p < 0.01$), while there was no significant sex difference within the PT150 condition. Further comparisons indicated that PT150 significantly increased fentanyl self-administration in females ($z = 2.08$, $p < 0.05$), but not in males. Thus, collapsed across housing and SPS conditions, males self-administered more fentanyl than females during the escalation phase, and this sex difference was negated by PT150.

3.2.c Training and professional development provided:

Dr. Cassie Chandler on the University of Kentucky team participated in the bi-weekly teleconferences with the staff of RTI. She was afforded the opportunity to gain insight into the workings of an independent nonprofit institute that provides research, development, and technical services to academic, government and commercial entities worldwide.

3.2.d Dissemination to communities of interest:

The study team published in the *Psychopharmacology* Journal.

Hammerslag, L. R., Denehy, E. D., Carper, B., Nolen, T. L., Prendergast, M. A. and Bardo, M. T. (2021). Effects of the glucocorticoid receptor antagonist PT150 on stress-induced

fentanyl seeking in male and female rats. *Psychopharmacology* (Berl). 2021;238(9):2439-2447. doi:10.1007/s00213-021-05865-0

Dr. Bardo presented the study at the MOMRP meeting:

Preclinical assessment of PT150 for opioid use disorder and PTSD
Michael Bardo, Alcohol and Substance Use IPR 9/23/2021

3.2.e Plans for next reporting period to accomplish goals and objectives:
The team plans to submit a second manuscript to *Psychopharmacology* journal.

3.3 AS170014-A3 Effect of Sublingual formulation of Dexmedetomidine HCl (BXCL501) on Ethanol in Heavy Drinkers with PTSD

The objective for this planning grant was to accomplish milestones necessary to obtain approval and launch a new 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.

3.3.a Primary objectives and milestones for the third year were:
The primary objectives were to develop a proof-of-concept trial study as well as a budget and Clinical Development Plan (CDP) for this study. These were presented to the Programmatic Panel.

3.3.b Accomplishments under the goals include:
During the past reporting year, the Programmatic Panel approved this study to move forward. See section 3.7 for additional details.

3.3.c Training and professional development provided:
None to report.

3.3.d Dissemination to communities of interest:
None to report.

3.3.e Plans for next reporting period to accomplish goals and objectives:
This planning grant is now closed. Study updates appear in section 3.7.

3.4 AS170014-A4 An Aldehyde Dehydrogenase 2 Inhibitor, ANS-6637, for Reducing Symptoms of Post-Traumatic Stress Disorder (PTSD) and Alcohol Use Disorder (AUD) in Veterans

The objective for this planning grant was to accomplish milestones necessary for the completion of a proof-of-concept study that will examine the safety and potential efficacy of an aldehyde dehydrogenase 2 inhibitor, ANS-6637, as a treatment for comorbid posttraumatic stress disorder and alcohol use disorder.

3.4.a Primary objectives and milestones for the second year were:

The primary milestones for this planning grant included the development of the study protocol and submission of IND application to FDA. Additional tasks included development of Clinical Development Plan and preparation of the study budget.

3.4.b Accomplishments under the goals include:

The protocol was placed on clinical hold by the FDA and the planning grant has been closed out and no new study will be planned.

3.4.c Training and professional development provided:

None.

3.4.d Dissemination to communities of interest:

None.

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

None.

3.5 AS170014-A5 Preclinical testing of FKBP5 Inhibitors for alcohol use disorder-PTSD comorbidity

Military personnel show high susceptibility to alcohol use disorder (AUD) and post-traumatic stress disorder (PTSD). AUD is found among those with a military history more frequently than the general population (Hoerster, et al., 2012). AUD and PTSD display high comorbidity, as most veterans diagnosed with AUD have PTSD (Seal et al., 2010). Unfortunately, there is a lack of FDA approved pharmacotherapies for the treatment of PTSD/AUD comorbidity (Ralevski et al., 2014). Recently, the stress-related marker, FK506-binding protein 51 (FKBP5) might serve as a promising target in alleviating stress-related disorders (Pohlmann et al., 2017; Wang et al., 2018) and may assist in reducing AUD among patients with PTSD. This proposal examined the effects of FKBP5 inhibitors by using a highly specific FKBP5 inhibitor (SAFit2) or a more broad-acting, but FDA-approved FKBP5 inhibitor (benztropine) in a rodent model of PTSD/AUD comorbidity. The team hypothesized that FKBP5 inhibitors would reverse PTSD/AUD-like behaviors in our recently developed rat model of PTSD/AUD comorbidity (Steinman et al., 2020, in Molecular Psychiatry). This model increases voluntary alcohol intake and generates PTSD-like behavior responses in males and females in a manner relevant to the sexual dimorphism seen in human PTSD (Brown et al., 1995; Hourani et al., 2015), AUD (Zilberman et al., 2003), and comorbid PTSD/AUD (Lehavot et al., 2014; Sonne et al., 2003).

3.5.a Primary objectives and milestones for the second year were:

The major goals of this project were to investigate the effects of FKBP5 inhibitors on rats that displayed elevated PTSD/AUD-like comorbid behaviors. The study team hypothesized that pharmacological inhibition of FKBP5 can ameliorate PTSD/AUD-like comorbid behaviors such as alcohol drinking, hyperarousal, and fear overgeneralization. The objectives of this study consist

of 1) investigating acute or 2) chronic administration of FKBP5 inhibitors (i.e., SAFit2-highly specific, or benztropine-broad acting) in restoring normal behavior in rats exhibiting a PTSD/AUD- like phenotype.

3.5.b Accomplishments under the goals include:

The overall goal of this project was to examine whether FKBP5 inhibitors reduce AUD/PTSD comorbid-like behavioral phenotypes. During the current funding period (September 15, 2020-September 14, 2021), the team accomplished key studies that address alcohol drinking, sleep disturbances, and hyperarousal. The team completed a large aspect of Aim 1 related to experiment 1a & 1b. Briefly, rats were first exposed to "2-hit" stress in which a foot shock was elicited upon crossing a dark compartment on two occurrences of a chamber of a similar context. Next, the team exposed male and female rats to 2-bottle choice (2BC), limited access, intermittent ethanol drinking (20%) for 4 weeks. On the last day of drinking, the rats received acute intraperitoneal injections of vehicle or benztropine mesylate (hereafter benztropine: 5 or 10mg/kg) or SAFit2 (10 or 20mg/kg) in a between-subjects design prior to 2BC testing. One week-later, rats were treated with the same dose of vehicle or benztropine (5 or 10mg/kg) or SAFit2 (10 or 20 mg/kg) and underwent a sleep cycle analysis via bout analysis of automated photobeam interruptions. Finally, one week after the sleep analysis, rats were treated again with the same dose of vehicle or benztropine (5 or 10mg/kg) or SAFit2 (10 or 20mg/kg) and tested for acoustic startle responses. *The work to date demonstrated that acute administration of the FKBP5 inhibitor benztropine to rats in our PTSD/AUD-like model significantly reduced ethanol intake and preference and, in females, also reduced acoustic startle responses. The work for this reporting period reflects data from additional assessments of FKBP5 influence in unstressed ethanol drinking animals.*

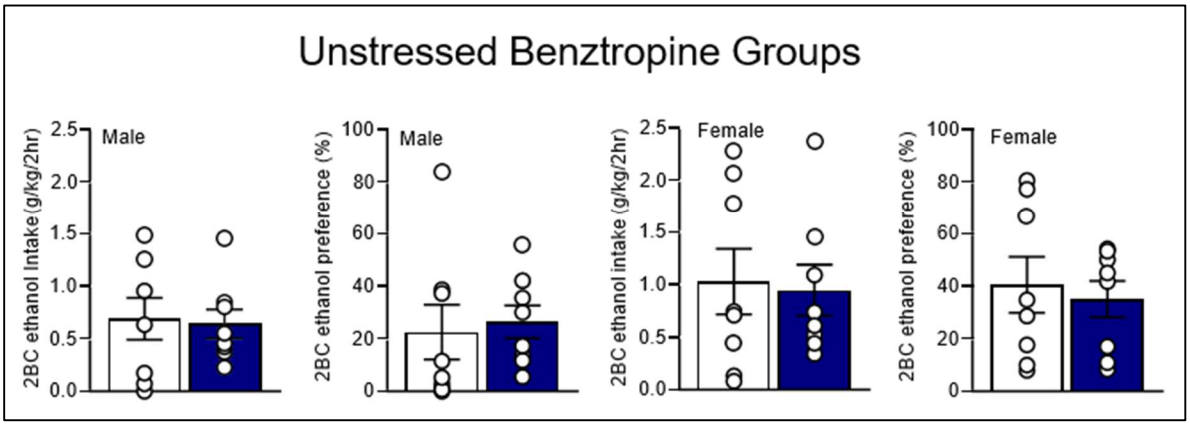


Figure 1. 2BC ethanol drinking in unstressed rats that received an acute administration of benztropine. The groups of rats consisted of unstressed males and females that received vehicle (white bars; n=8) or 10mg/kg of Benztropine (Blue bars; n=8). In general, acute benztropine administration did not alter 2BC ethanol drinking in male and female rats. Data were analyzed using analysis of variance techniques with drug treatment as between- subjects factor.

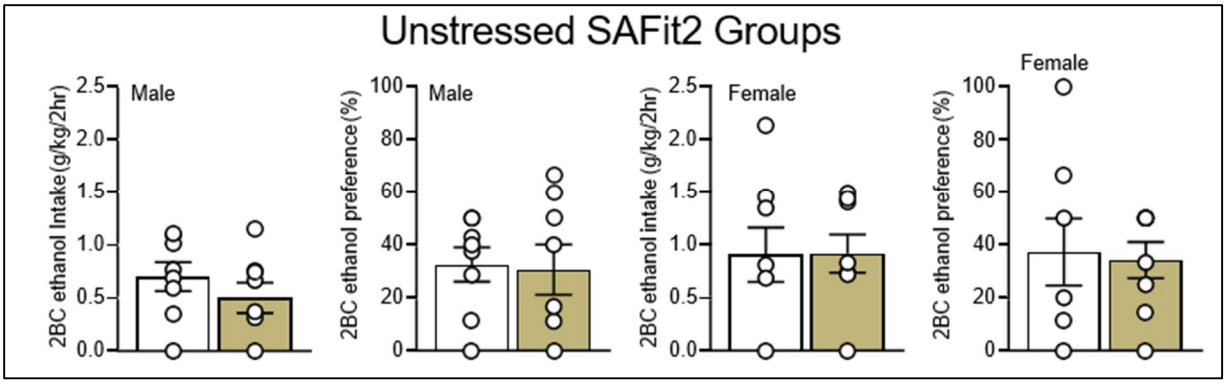


Figure 2. 2BC ethanol drinking in unstressed rats that received an acute administration of SAFit2. The groups of rats consisted of unstressed males and females that received vehicle (white bars; n=8) and/or 20mg/kg of SAFit2 administration (brown bars; n=8). In general, acute SAFit2 administration did not alter 2BC ethanol drinking in male and female rats. Data were analyzed using analysis of variance techniques with drug treatment as between- subjects factor.

3.5.c Training and professional development provided:

This project has provided extensive training and professional development opportunities during this funding period. For the post-graduate fellows, Drs. Cruz and Vozella, this included learning novel comorbid PTSD/AUD models, experimental design, studying clinically relevant compounds that inhibit FKBP5, and preparing and communicating the group's results for PASA meetings. The PI and co-PI are also well- positioned in mentoring and will continue to oversee these features develop.

3.5.d Dissemination to communities of interest:

Study data were uploaded to PASA web portal and the PASA Management Core via a secure website. These data transfers are required to allow for study status reporting and data quality and consistency checks to be performed by the Management Core.

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

The team's goals and objectives for the next reporting period are to complete the pharmacological studies that comprise the unstressed SAFit2 behavioral studies. This includes CLAMS, acoustic startle, fear overgeneralization and amended irritability behavioral testing after acute SAfit2 administration. The study team plan to analyze all the data and compile them in a manuscript for peer review.

The studies will continue to provide training opportunities for post-doctoral fellows and student interns (that will be appropriately added to the IACUC animal protocol and, if involved with the actual PASA, experiment amended to the ACURO prior to work). The team plans to disseminate results through conference presentations (approved by PASA collaborators) and draft a manuscript with our PASA collaborators for publication of results during the coming funding period.

3.6 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.

3.6.a Primary objectives and milestones for the third 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. Our 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 submission of additional IRB amendments to lessen the exclusionary criteria and extend study related visit windows. The research group is working to reach the anticipated monthly recruitment goal of enrolling 5 PTSD and OUD veterans monthly.

3.6.b Accomplishments under the goals include:
Administrative goals accomplished during the past year include HRPO approval granted on October 10, 2020, CRF and MOP finalization completed on March 09, 2021, EDC launch completed on March 09, 2021, and an BCM IRB amendment/modification approval was granted on August 20, 2021, following the submission of multiple amendments to the protocol (initial BCM IRB approval was granted in January 2020). In May 2021, the research team initiated bi-weekly meeting sessions between MEDVAMC suboxone providers and research collaborators to synergize recruitment efforts.

Recruitment goals accomplished during the past year include protocol initiation in March 2021. The first study subject was enrolled in April 2021. 11 subjects have completed the preliminary screening visit. Of the five study subjects consented and enrolled, three have been randomized to the study conditions. Two subjects have completed all study related visits.

3.6.c Training and professional development provided:
Baylor College of Medicine and the Michael E. DeBakey VA Medical Center regularly provides training courses for research personnel. Trainings seminars at Baylor College of Medicine are conducted by the Office of Research and Sponsored Programs Office and are SoCRA approved training programs.

3.6.d Dissemination to communities of interest:
The study is in early data collection with no plans to disseminate information until more data are available.

3.6.e Plans for next reporting period to accomplish goals and objectives:
In order to increase recruitment, the study team modified the master protocol and submitted and received approval from the local IRB. The approved modifications focused on reworking some of the inclusion/exclusion criteria to allow for previously prohibited medications (e.g. common diabetes medications, etc.) that resulted in candidates being excluded from eligibility requirements. The research team continues to collaborate with MEDVAMC suboxone prescribers in efforts to increase recruitment rates. The research team plans to expand the number of suboxone providers currently listed as co-investigators.

3.7 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.

3.7.a Primary objectives and milestones for the third year were:

The overall objectives for the third year were to obtain the IND through the FDA to use Dexmedetomidine HCl (BXCL501), finalize the study's budget, develop plan for recruiting subjects, and prepare for the study to run at VA Connecticut.

3.7.b Accomplishments under the goals include:

In September 2020, the FDA requested more information and changes for the IND. By October 2020, the study team sent responses to the FDA IND Clinical Hold and completed the budget for the study. The FDA Clinical Hold removal notification was received a month later. In December 2020, the study team began processing the West Haven VA IRB submission package and finalized documents by January 2021 and submitted in February 2021. The VA IRB reviewed the protocol and associated materials in March 2021 and requested minor changes but gave overall VA IRB approval. The study team began working with RTI on reviewing study assessments, programming alcohol infusions and creation of study data collection forms. In April 2021, licenses were purchased for study assessments. Yale IRB approval was obtained in May 2021 which required an amendment to the budget to include transportation for participants. The study team was able to purchase the alcohol used during infusion test days in June 2021 and remaining study supplies were purchased by July 2021. Also, in June 2021, the initial HRPO submission was sent in for review. Since then the site has provided all requested updates and additional materials. Preparations for study start up at the VA began in August 2021 when the team was able to hire /onboard a research coordinator, set up the VA pharmacy and finalize study data collection forms. Currently, the site is awaiting HRPO approval to launch the study.

3.7.c Training and professional development provided:

Emily Pisani, the new coordinator for the study, has started training on the study protocol, associated study assessments, and EDCs. Ms. Pisani will continue to train until ready to coordinate the study.

3.7.d Dissemination to communities of interest:

Currently the study is under development but may produce a medication to potential treat comorbid PTSD and AUD.

3.7.e Plans for next reporting period to accomplish goals and objectives:

The study team is addressing the HRPO requests but have regulatory approval at VA Connecticut. Waiting on Yale University IRB to approve our amendment and COVID Safety Guidelines. The team plans to start recruitment once the study team has approval from Yale University IRB and HRPO start the study by the fall.

4. Impact

4.0 PASA Core

The work, findings, and specific products of the projects sponsored through PASA are still in progress, but collaboration on manuscripts and publications has provided quality data to push innovations forward. As the PASA management and study leaders continue to finalize and publish additional

manuscripts, this strengthens the Consortium's impact. Another important impact during this reporting period has been with our pharmaceutical company partners. These partners have favorably noted our major accomplishments, innovations, and successes for identifying promising new medications for substance use disorders. The PASA Core 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. The Core continues to build our template library as well as the PASA website to allow for efficiency and consistency across studies. The Core has also established excellent working relationships with several VAMCs across the USA for conducting our PASA clinical studies. The PASA Core has used knowledge across studies conducted within the PASA Consortium, as well as knowledge of clinical trials conducted outside of the PASA Consortium with the PASA established collaborators, to help inform initial and continued funding decisions for compounds being studied within PASA. To further expand on our ability to select novel compounds efficiently and effectively, the PASA Consortium has recently pursued funding of an In-silico project. This work will help to generate a formalized catalog of promising compounds that can then be incorporated into clinical or preclinical 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 team has made significant progress in the development of the anti-fentanyl vaccine during the reporting period to the extent in which the plans include to manufacture of clinical grade vaccine for toxicology testing and a Phase 1 clinical trial. With this progress and as mentioned above, the University of Houston intellectual property committee has voted 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 (5/27/2020)].

4.2 AS170014-A2 Preclinical assessment of PT150 for opioid use disorder and PTSD

The results of this preclinical project provide the initial proof-of-principle evidence that PT150 has differential effects in males and females in ameliorating stress-induced escalation of fentanyl self-administration, thus providing the impetus for a potential new avenue for treating co-morbid OUD and PTSD, at least in males. Further work is needed to determine the mechanism(s) underlying the differential effect of PT150 in males and females. This will be critical for potential translation to humans.

4.3 AS170014-A5 Preclinical testing of FKBP5 Inhibitors for alcohol use disorder-PTSD comorbidity

The study team successfully used for the first time a novel PTSD/AUD comorbidity model recently developed and characterized in our laboratory and accepted for publication (*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 for the current study as the team continues to work towards publication and future studies to potentially pursue.

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

This project is in early stages of recruitment; 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.

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

Project is nearing the end of the development phase and planned to transition into implementation pending final regulatory approval. Once launched, the study team anticipated this trial will be impactful at laying the safety and efficacy groundwork for larger studies to come.

5. Changes/Problems

5.0 PASA Core

The main challenge in the past year has been impact of the COVID-19 pandemic on research. Overall, most of the pre-clinical studies did not experience drastic setbacks; however, there were some study delays and modifications to some pre-clinical study and clinical trial protocols/procedures due to the pandemic. To mitigate study barriers as much as possible, the PASA Core tracked each site's status and routinely assessed for impacted abilities at the site level. Though there were some delays, sites are now fully reopened and operational and have adapted to the constraints inflicted by COVID. Of important note is that regulatory approvals from FDA and DoD advisory boards and local IRB and VA R&D committees remain on track for successful resolution toward clinical projects since COVID-19 restrictions were lifted.

A change to note would be the addition of Dr. Nathan Vandergrift as the PASA Consortium co-PI as replacement for previous Co-PI, Dr. Rick Williams. Dr. Vandergrift is a statistician with more than 15 years of experience in collaborative research in diverse areas such as public health–related infectious disease research, translational medicine from bench science, vaccine development, infectious disease treatment, community-level intervention for substance use disorder, pharmaceutical trials for substance use disorder, and large-scale educational and child development. His areas of statistical expertise are structural equation modeling, nonlinear and linear mixed-effects modeling, generalized linear models, nonparametric statistics, missing data, and statistical matching. Dr. Vandergrift has a depth of experience applying for, receiving, and executing large multisite UM1 and P01 grants and contracts and R01-level grants. He has led statistical teams and been a part of large collaborative research groups spanning many sites, countries, and continents. These collaborations have resulted in publications in highly regarded peer-reviewed journals and make him well suited for the Co-PI position.

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 team experienced significant delays in procuring reagents and general supplies. The team remedied this to an extent by ordering from vendors that other researchers have obtained supplies from.

5.2 AS170014-A2 Preclinical assessment of PT150 for opioid use disorder and PTSD
Due to the shutdown of the University of Kentucky in March 2020 in response to the COVID-19 pandemic, project completion is delayed but an extension resolved that delay. COVID-19 delays have also increased project expense; however, supplemental funding resolved the issue.

5.3 AS170014-A5 Preclinical testing of FKBP5 Inhibitors for alcohol use disorder-PTSD comorbidity
The team's currently approved animal protocol stated that they would perform behavioral tests in counterbalanced order. This would have been undesirable, however, because the 3 tests used are of qualitatively different stressfulness from one another and some involve conditioned re-experiencing of the past trauma context, which could influence later behaviors. The team have always followed a set order of testing in our behavioral models beginning from least stressful (e.g., observing their natural sleep patterns) to most stressful (acoustic startle testing and re-exposing them to contexts somewhat reminiscent of their initial traumatic context) and, by design, the team have never previously tested these 3 tests in counterbalanced order. For that reason, after discussion with our PASA collaborators, the study team decided to test them in the order from least stressful (CLAMS- sleep) to most stressful (fear overgeneralization). Doing so helped the team to: 1) avoid contaminating stress or conditioning effects of test procedures across endpoints, which may confound our experimental stress history, and 2) avoid unexpected effects of changing the model from what the team has always done previously for these 3 behavioral tests.

Also, in the study teams past and recent work (*Steinman et al., 2020 in Molecular Psychiatry*), they have identified a 4th behavioral test (bottle brush irritability test) relevant to PTSD that also reliably distinguishes subjects in their model (aggressive and defensive-like behavior in the bottle-brush test of irritability). Therefore, the team added this 4th test one week after completing the previously approved PASA protocol. This additional test does not impact the already completed experiments that were previously approved by the ACURO in any way. The 4th procedure is being performed under a non-DoD-funding source with all appropriate IACUC regulatory approvals.

Due to pandemic (COVID-19) restrictions, personnel conducting the proposed studies were working at a limited capacity due to institutional constraints on personnel density as well as vivarium housing density constraints. This protracted the duration of the project, therefore, extending personnel cost that was originally budgeted for a shorter time. After discussions with the PASA Consortium, focus was geared towards having proper controls for studies involving Aim 1. As a result of the cost of running additional controls for the SAFit2 studies and the pandemic circumstances of reduced animal and personnel density extending project duration, funds were exhausted to perform Aim 2 regarding the chronic studies.

There have been no changes that have impacted on expenditures of this project. There have been no significant changes that have impacted the use of animals involving this project.

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

The COVID-19 pandemic and resulting organizational changes have had a drastic impact of recruitment efforts. Most substance abuse clinic appointments have transitioned to telehealth visits, completely removing the opportunity for the research staff to recruit patients from in-person substance abuse appointments. The research team and suboxone prescribers have transitioned to providing the study flyer virtually to potential subjects. The study team has increased the number of study advertisements that are posted/visible in locations frequented by VA patrons.

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

Once all Yale and HRPO approvals are obtained, the study team plans to begin recruitment.

6. Products

6.0 PASA Core

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

Presentations

Presentations are as noted above.

Publications

Publications are as noted above

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

A provisional patent application was submitted and approved on September 23, 2021, in coordination with the University of Houston to secure intellectual property rights for the anti-fentanyl vaccine.

6.2 AS170014-A2 Preclinical assessment of PT150 for opioid use disorder and PTSD

One presentation of results from Aim 1 to Military Operational Medicine Research Program (MOMRP) annual meeting was given called “Effects of the glucocorticoid receptor antagonist PT150 on stress-induced fentanyl seeking in male and female rats.” Additionally, one scientific article was published, and one manuscript was prepared:

a. Hammerslag, L. R., Denehy, E. D., Carper, B., Nolan, T. L., Prendergast, M. A. and 9 Bardo, M. T. (in press). Effects of the glucocorticoid receptor antagonist PT150 on stress-induced fentanyl seeking in male and female rats. *Psychopharmacology*. [PMCID in progress].
b. Bardo, M. T., Chandler, C. Denehy, E. D., Carper, B., Prendergast, M. A. and Nolen, T. L. Effect of the glucocorticoid receptor antagonist PT150 on fentanyl self-administration in rats following early life stress. Manuscript in preparation.

6.3 AS170014-A5 Preclinical testing of FKBP5 Inhibitors for alcohol use disorder-PTSD comorbidity

Dr. Bryan Cruz presented at the Research Society on Alcoholism annual meeting (June 2021). See citation below.

Cruz B, Vozella V, Kirson D, Bradley L, Fain K, Crawford M, Carper B, Nolen T, Kosten T, Zorrilla EP, & Roberto M. Benztropine, an FKBP5 inhibitor, reduces ethanol drinking and stress-associated phenotypes in rat model of comorbid post-traumatic stress and alcohol use disorder (June 2021). *Research Society on Alcoholism, Virtual Meeting.*

Dr. Bryan Cruz presented at the MOMRP Alcohol & Substance Use IPR. See citation below:

Preclinical Testing of FKBP5 Inhibitors for Alcohol Use Disorder-PTSD Comorbidity (September 2021) *MOMRP Alcohol & Substance Use IPR virtual meeting.*

Dr. Bryan Cruz also presented virtually at the Research Society on Alcoholism meeting in June. See citation below:

Cruz, Bryan, et al.; Benztropine, an FKBP5 Inhibitor, Reduces Ethanol Drinking and Stress-Associated Phenotypes in a Rat Model of Comorbid Post-Traumatic Stress and Alcohol Use Disorder, *Research Society on Alcoholism*. 2021 June.

There were no publications, papers, inventions, patents, technologies, techniques, or products developed under this study during the September 15, 2020 – September 14, 2021, funding period.

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

The project is newly started, and no products have been reported at this time.

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

Project is still under the development phase and looking to launch soon.

7. Participants and Other Collaborating Organizations

RTI International - Management Core

Nolen, Tracy	Principal Investigator	15%
Williams, Rick	Co-Principal Investigator	4%
Bradley, Lauren	Research Coordinator	13%
Baldi, Marjorie	Financial/Subcontracts Mgr	13%
Arafat, Dana	Financial/Subcontracts Mgr	5%
Carper, Ben	Statistician	7%
Crawford, Meg	Research Coordinator	21%
Fain, Katie	Research Coordinator	20%
Hirsch, Shawn	Statistician	15%
Jones, Alexis	Research Coordinator	6%
LeGrow, Keith	Programmer/Analyst	8%
Roberts, Cheryl	Clinical Data Manager	10%
Smith, Emily	System Analyst	7%
Tang, Yan	Programmer/Analyst	15%
Turner, Gene	Clinical Data Manager	8%
Vandergrift, Nathan	Statistician	8%
Whitworth, Ryan	Statistician	17%
Glass, Kendra	Clinical Data Manager	14%

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%
Quardri, Saif	Research Technician	75%
Baker, Miah	Research Technician	75%
Duddupudi, Anantha	Post Doc	16%

University of Kentucky

Preclinical assessment of PT-150 for opioid use disorder and PTSD

Bardo, Michael	Primary Investigator	5%
Prendergast, Mark	Co-Investigator	0%
Rush, Craig	Co-Investigator	0%
Dwoskin, Linda	Co-Investigator	0%
Hammerslag, Lindsey	Post doc	0%
Denehy, Emily	Facilities Manager	50%
Hamid, Usman	Laboratory Assistant	0%
Chandler, Cassie	Post doc	100% (no cost)
Punzal, Emily	Laboratory Assistant	75%

The Scripps Research Institute

Preclinical 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%

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	50%
Kosten, Thomas	Co-Principal Investigator	25%
Fermo, John	Co-Investigator	58%
Yang, Fang	Co-Investigator	33%
Asif Khan, Mohammad	Co-Investigator	33%
Vaughan, Adetola	Study Coordinator	50%
Chii, Philip	Study Clinician	58%

Yale University

Developing a proof-of-concept clinical trial to evaluate the use of a safe and highly selective a2a Adrenergic Receptor Agonist, BXCL 501, for the treatment of ASUD comorbid with PTSD and/or TBI – Planning Grant

Petrakis, Ismene	Co-Principal Investigator	20%
Krystal, John	Co-Principal Investigator	1.5%
Levy, Lucienne	Research Assistant	10%

Emily Pisani	Coordinator/RA	100%

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. Colin Haile (PI): He directed all phases of the study including but limited to conducting experiments, generating, and interpreting data, animal protocol approval, providing updates and disseminating results. Dr Kosten has provided input into the design of the studies. Dr Cuny with his post-doc Dr Duddupudi synthesized the conjugate vaccine. Mr. Quadri and Ms. Baker conducted experiments and utilized numerous types of ELISA assays for fentanyl and anti-fentanyl antibody quantification.
Miah Baker (RA): Contributed by helping to conduct vaccination experiments, collect bloods, analgesic tests and process brain and blood samples, protein estimation and ELISAs.
Hailey Rodgers (Student): Contributed by helping to conduct experiments described in AIM 2.
Sergio Sanchez (RA): Contributed by helping to conduct vaccination experiments, collect bloods, analgesic tests.
Anantha Duddupudi (Post-Doctoral Fellow): Synthesis optimization of the anti-fentanyl conjugate vaccine.
Saif Quadri (RA): Contributed by helping process brain and blood samples, protein estimation and ELISAs.

7.1.b. Change in other active support for active support of PIs

The PI obtained additional funding (expansion) to conduct additional experiments and produce clinical grade vaccine for a potential Phase 1 Clinical Trial.

7.1.c. 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-A2 Preclinical assessment of PT-150 for opioid use disorder and PTSD

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

Dr. Michael Bardo (PI): Academic salary charge; no change
Dr. Mark Prendergast (co-I): No change.
Dr. Craig Rush (co-I): No change.
Dr. Linda Dwoskin (co-I): No change.
Dr. Lindsey Hammerslag (postdoc): Effort ended in September 2020.
Ms. Emily Denehy (facilities manager): Annual salary charge. Conducted surgeries, daily animal runs and participated in the bi-weekly teleconferences with RTI.
Dr. Cassie Chandler (postdoc): 12 month overseeing data collection,

data transfer and graphical presentation. She also assisted in surgeries, daily animal runs and participated in the bi-weekly teleconferences with RTI.
Ms. Emily Punzal (laboratory assistant): Hourly charge over 12 month; served as part-time hourly employee whose primary responsibility was to run the operant self-administration session on the weekends, effort ended in May 2021.

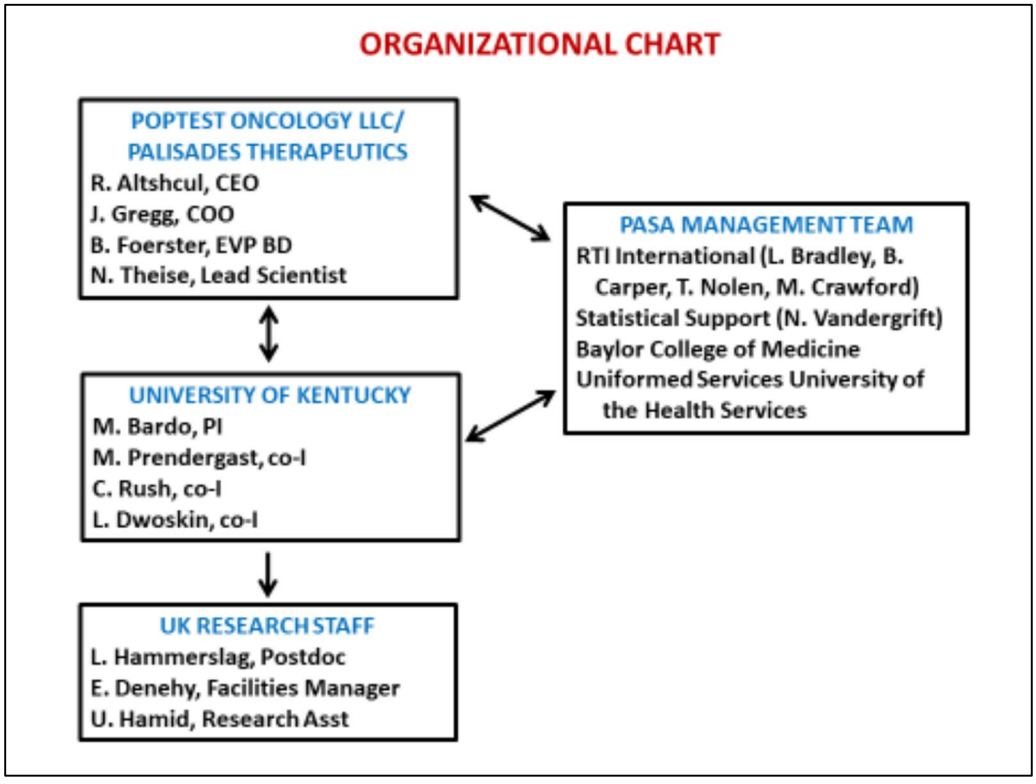
7.2.b. Change in other active support for active support of PIs

Newly funded grants:
NIH R01 DA053070
Bardo (mPI with Turner and Ortinski)
03/15/21-02/31/24
Functional and genomic signatures of escalated fentanyl use
Role: mPI

NIH U01 DA051377
Prisinzano (PI)
09/01/21-07/31/24
Development of agents for synthetic opioid overdose
Role: co-I

7.2.c. Other organizations that have been involved as partners:

See organizational chart below:



7.3 AS170014-A5 Preclinical testing of FKBP5 Inhibitors for alcohol use disorder-PTSD comorbidity

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

Dr. Marissa Roberto (PI): no change
Dr. Eric Zorrilla (Co-PI): no change
Dr. Michal Bajo (Co-PI): no change
Dr. Bryan Cruz (Study Coordinator): no change
Dr. Valentina Vozella (Study Coordinator): no change
Dr. Kerry Ressler (Consultant): no change

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

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

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

7.5.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 (RA): no change
Ms. Emily Pisani (Coordinator/RA): added to the study; replaced Jenelle Newcomb.

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

BioXcel Therapeutics, Inc remains partners in this study.