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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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Tracy Nolen

E-Mail: tnolen@rti.org

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

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Revision includes addition of sections 7.1 - 7.5

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

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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 this comorbidity, along with mild to moderate TBI, is 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

Our primary objectives for the second year were:

- Complete activities in support of RFA#4.
- Launch new pre-clinical study funded through RFA#4.
- Launch and complete work on two planning grants, Yammine and Petrakis/Krystal.
- To launch the Lofexidine study protocol.
- Prepare and plan RFA#5.

3.0 PASA Core

The PASA Core research program continued in year 2 with the Requests for Research Applications (RFA) and oversight of the PASA Consortium.

3.0.a Primary objectives and milestones for the second year were:
At the start of year 2, we completed the review process for PASA RFA #4b. RTI received and conducted peer review on 10 applications that were reviewed by the Panel. The Panel agreed with RTI's recommendation and one pre-clinical was awarded funding.

A consortium team objective is to efficiently manage and monitor studies that lead to accurate, quality data for publication and dissemination. This is achieved though core management responsibilities such as regularly scheduled check-ins, follow-ups, data accountability, statistical analysis, quality control and assurance, and other various oversite 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.

One objective that formed over the course of the year, was to track COVID-19 barriers and delays. 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 #4.
- Dr. Verrico' s Lofexidine study was approved for funding under PASA 2; Dr. Roberto's pre-clinical study was approved under PASA 2.
- Begin activities in support of RFA #5.
- Developed manuscripts for 2 pre-clinical studies, one by Drs. Bardo and Hammerslag, and another by Dr. Haile.
- Hosted a virtual PASA Consortium wide Investigator Meeting.
- Maintain consortium and study progress despite the outbreak of the COVID-19 pandemic (i.e. transitioned to remote investigator meeting, stayed in close communications with and tracked sites' abilities to continue with study activities, revised protocols and submitted to regulatory entities to allow for telemedicine endeavors, etc.).
- Updated and maintained PASA website.

3.0.c Training and professional development provided:
The RTI PASA Core team met in January 2020 to discuss progress and improvements. This development session was attended by all key RTI PASA personnel.

The PASA Consortium also hosted a virtual Investigator Meeting via Adobe Connect in May 2020. This platform allowed for researchers to present current study accomplishments as well as provide an open platform for discussion of barriers and associated solutions for conducing ASUD research as well as potential future research concepts.

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 Investigator Meeting is another way pertinent informaiton was disseminated to communities of interest. This meetings allowed for PASA collaborators to discuss their own work as well as that of other researchers. Though the meeting was virtual due to COVID-19, the participants responded positively, stating it was beneficial and productive.

The PASA consortium was accepted to present a panel workshop at ASCP in May 2020. The workshop concept was to introduce more clinical investigators to the PASA funding mechanism and DoD funding in general. The conference was cancelled due to COVID-19.

The PASA consortium supported submission and acceptance of Dr. Haile's abstract for the Military Health Systems Research Symposium (MHSRS). Though this conference was cancelled due to COVID-19, the abstract is posted to the symposium's website.

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:
The core plans to continue providing support for our funded studies. We will finalize and release RFA#5 (an expansion RFA) specially geared towards our current investigators. The core plans to collaborate on manuscripts, encourage more conference participation, and host consortium-wide meetings as needed.

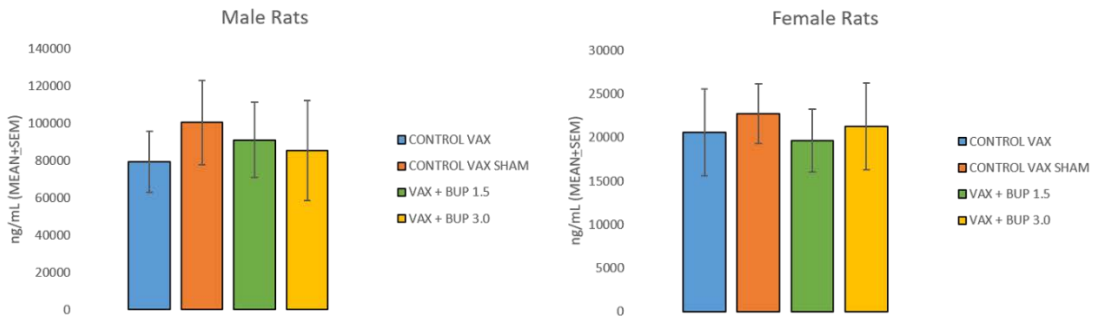
3.1 AS170014-A1 Novel Strategies for the Treatment of Opioid Use Disorder and Post-Traumatic Stress Disorder: Anti-Fentanyl Vaccine and Buprenorphine Combination Therapy
The objective of this project is to support the development of an anti-fentanyl vaccine targeting fentanyl assessed in combination 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 second year were:
One of the major goals we accomplished was to optimize the new formulation of our fentanyl vaccine. We also optimized the synthesis protocol for the fentanyl vaccine. Over the course of the year we completed vaccination in male (N=120) and female (N=120) rats at 0, 3, and 6 weeks. The study team was also able to surgically implant buprenorphine osmotic pumps and assess the effects of buprenorphine on predator odor-induced place aversion (animal model of PTSD). We completed our goal of pulling blood samples at 6, 8 and 10 weeks assess anti-fentanyl antibody levels (male rat samples). Also, we conducted fentanyl (two doses)-induced analgesic tests (tail flick, hot plate) and assessed brain and serum fentanyl levels following fentanyl challenge in all groups (male rat samples).

3.1.b Accomplishments under the goals include:

The study team optimized conjugate synthesized (FEN-CRM197) to be used for experiments in AIM1 and AIM2 purified for vaccination. AIMs 1&2: Two hundred forty (120 male, 120 female) rats received an initial vaccination (11/7/2019, 11/8/2019, 11/11/2019, 12/20/2019), 3 week boost (11/28/2019, 11/29/2019, 12/2/2019, 1/6/2020) and 6 week boost (12/19/2019, 12/20/2019, 12/23/2019, 2/3/2020) and blood samples were taken at 6, 8 and 10 weeks. Once blood samples were obtained from the vaccinated rats, anti-fentanyl antibodies were assessed.

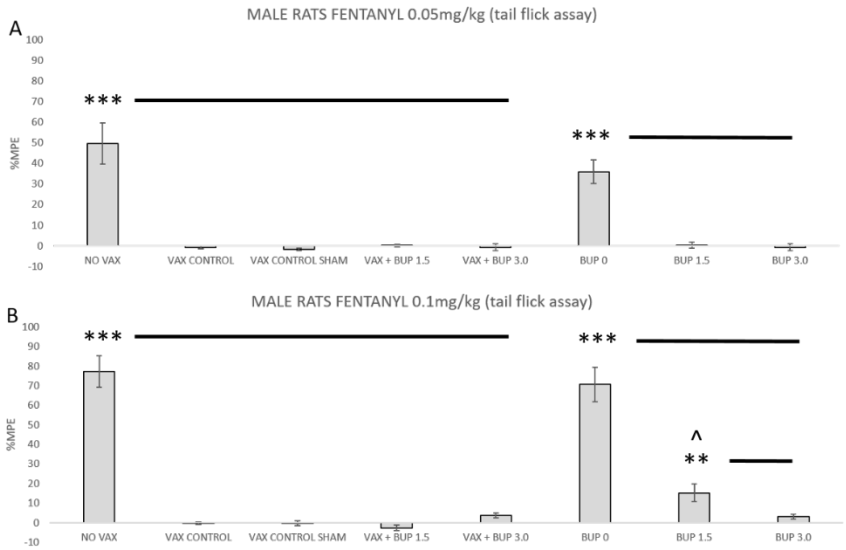
Figure 1. AIMS 1&2 Effects of optimized CRM-FEN (5ug)+dmLT (1ug) vaccine on anti-fentanyl antibody levels by group in Male and Female rats.



Rats were vaccinated at 0 and 3 and 6 weeks with the optimized CRM-FEN formulation (N=60 males, N=60 females) obtained at 8 weeks. Samples were then assessed for anti-fentanyl antibody levels using ELISA assay (see above). Other groups also were surgically implanted with mini-pumps that delivered buprenorphine (0, 1.5 or 3.0 mg/kg/day for 14 days).

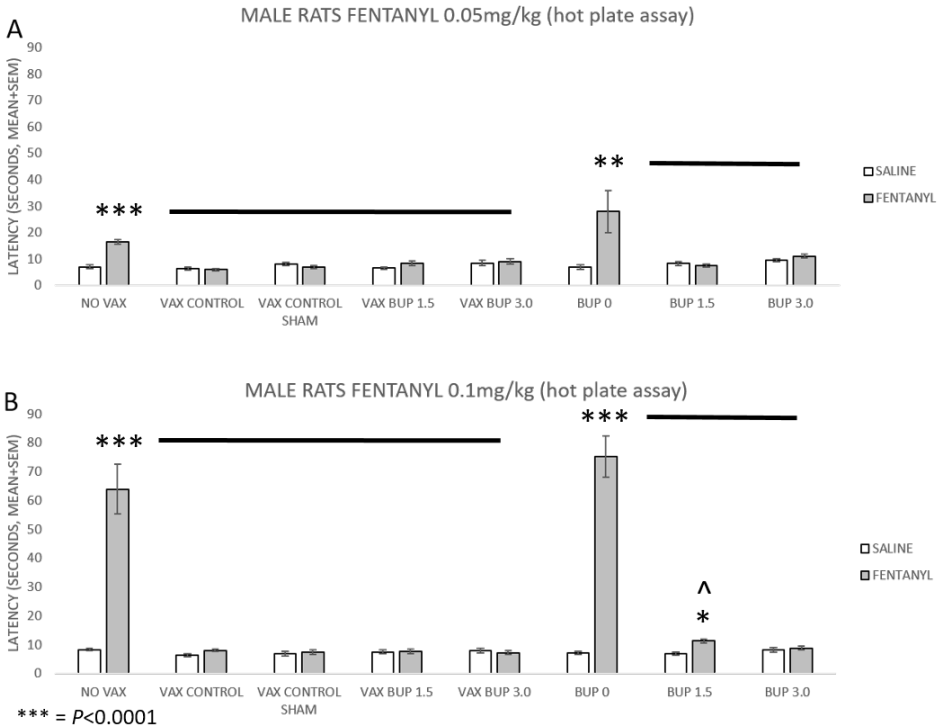
At approximately 10-12 weeks post initial vaccination the analgesic effects of fentanyl (0, 0.05 and 0.1mg/kg, SC) were assessed using the tail flick and hot plate assays.

Figure 2. The analgesic effects of fentanyl are completely blocked in vaccinated male rats compared to unvaccinated rats as determined with the tail flick assay.



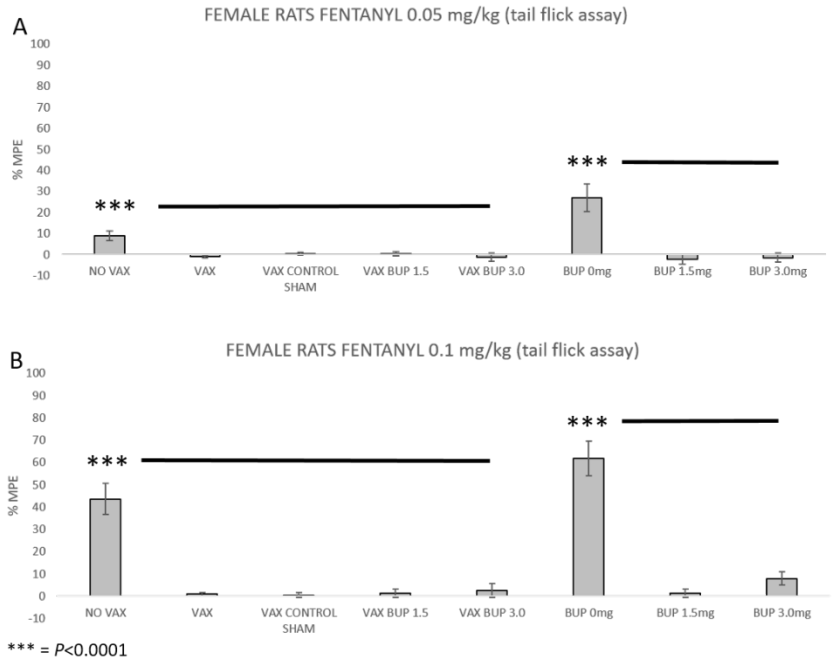
*** = *P*<0.0001
** =*P*<0.01
^ =P<0.01 vs VAX+BUP 1.5

Figure 3. The analgesic effects of fentanyl are completely blocked in vaccinated male rats compared to unvaccinated rats as determined with the hot plate assay.



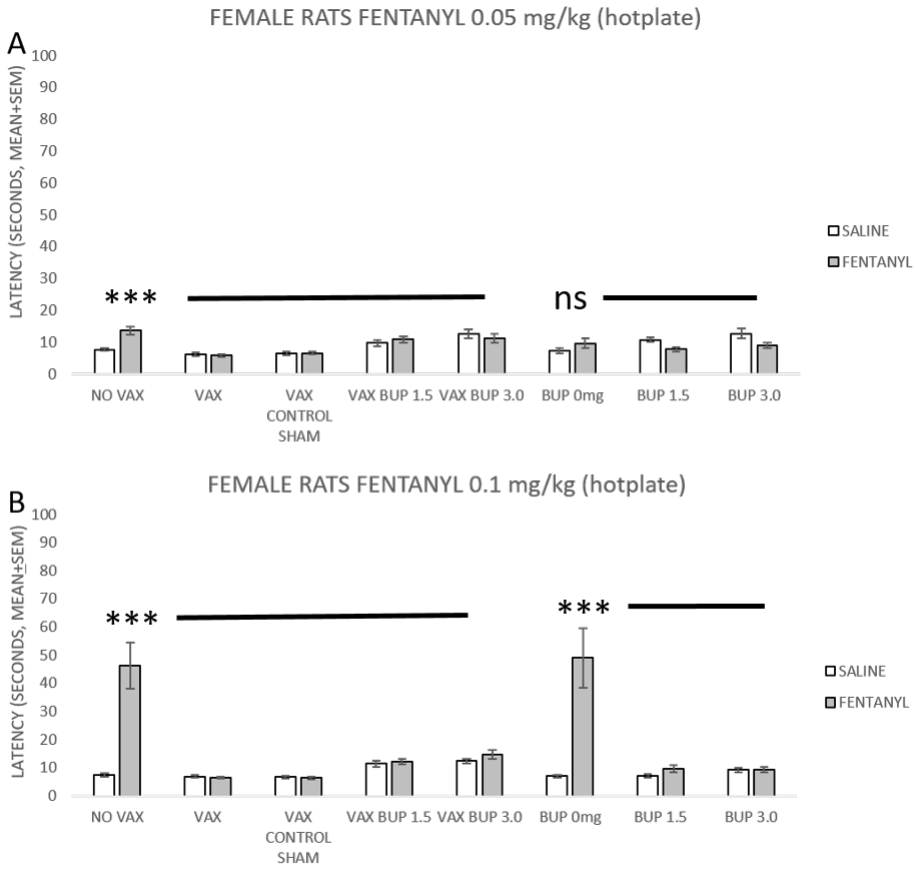
*** = *P*<0.0001
* =*P*<0.05
^ =P<0.01 vs VAX+BUP 1.5

Figure 4. The analgesic effects of fentanyl are completely blocked in vaccinated female rats compared to unvaccinated rats as determined with the tail flick assay.



*** = *P*<0.0001

Figure 5. The analgesic effects of fentanyl are completely blocked in vaccinated female rats compared to unvaccinated rats as determined with the hot plate assay.

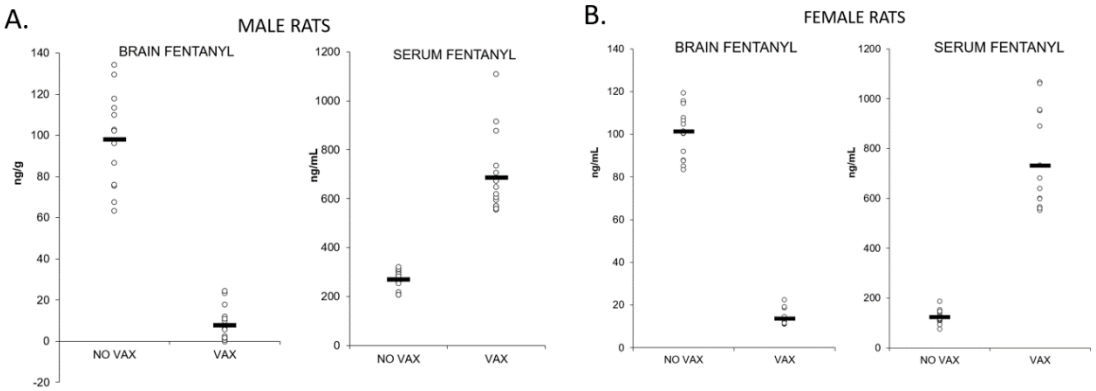


*** = $P<0.0001$

Fentanyl Brain and Serum Levels

Brain and serum samples were collected as we have done previously. For the final blood and tissue collection, rats were administered 0.1mg/kg. Thirty minutes later, rats were deeply anesthetized with isoflurane anesthesia. A bilateral thoracotomy was performed and whole blood removed via left ventricle puncture with a 22 gauge needle. The right ventricle was punctured then cold PBS (1X phosphate buffered saline, 5 ml) injected into the left ventricle over 2 minutes. Following perfusion, the brain was removed and washed in 1XPBS then immediately placed on dry ice. Samples were stored at -80°C until FEN levels were determined with ELISA (Immunalysis, Pamona, CA). Blood was allowed to coagulate for 24 hours at 4C then centrifuged at 3000 rpm for 15 minutes, supernatant removed and stored at 4C until assayed.

Figure 6. Brain and serum levels following administration of 0.1mg/kg (SC) FEN in male (A.) and female (B.) rats (N=13-15/group). FEN brain penetration was significantly attenuated in rats vaccinated with CRM-FEN+dmLT whereas high levels were seen in unvaccinated rats. High levels of FEN were sequestered in the periphery in vaccinated rats and were low in rats that did not receive the vaccine.



3.1.c Training and professional development provided:
Adopting new techniques to purify and concentrate vaccine preparations and utilize competitive ELISA assays offered an opportunity to learn new skills.

Attended PASA investigator meeting and presented study overview.

3.1.d Dissemination to communities of interest:
Through email and bi-weekly conference calls and online presentations.

3.1.e Plans for next reporting period to accomplish goals and objectives:
We plan to move forward with conducting the experiments proposed in the application. We plan to complete all AIMS in the coming months barring any further significant obstacles.

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 footshock) or pharmacologically (yohimbine) and reinstatement of fentanyl seeking was measured. Results showed efficacy for PT150. In Aim 2 (ongoing), we are determining if PT150 reduces fentanyl self-administration in individuals with co-morbid PTSD. Rats are being exposed to two different models of stress: (1) chronic social isolation and (2) acute stress induced by restraint/swim, which have been used to model PTSD. This aim will determine if oral PT150 reduces the effects of chronic social isolation and acute stress, either alone or in combination, on fentanyl self-administration.

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

Goal 1: Determine if PT150 reduces stress-induced reinstatement of fentanyl seeking. Male and female rats were trained to self-inject escalating doses of the potent opioid fentanyl using a standard 2-lever operant conditioning procedure. Following this, rats were treated daily with either PT150 or placebo while undergoing response extinction (abstinence). Stress was then being applied either environmentally (mild footshock) or pharmacologically (yohimbine) and reinstatement of fentanyl seeking was measured. The main objective of this experiment was to test the hypothesis that PT150 will reduce stress-induced reinstatement of fentanyl seeking.

Goal 2: Determine if PT150 reduces fentanyl self-administration in individuals with comorbid PTSD. In this ongoing experiment, rats are being 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 is being measured immediately before and after the acute stress. On the day after the acute stress treatment, rats are being 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 1: Determine if PT-150 reduces stress-induced reinstatement of fentanyl seeking. 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. Manuscript written, submitted and currently under review (abstract and graphs below):

Abstract

Rationale: Opioid use disorder (OUD) is highly comorbid with stress-related disorders, and stress can serve as a trigger for reinstatement of drug seeking. Glucocorticoid receptor (GR) antagonists such as mifepristone (RU-486) may be effective against stress-induced drug seeking. In the current study, PT150 (formerly ORG-34517), a more selective GR antagonist, was tested using two models of stress-induced drug seeking, namely footshock and yohimbine.

Methods: Adult male and female Sprague-Dawley rats were trained to self-administer fentanyl (2.5 µg/kg/infusion, i.v.) in a model of escalation. Rats then received 7 days of abstinence, during this time daily treatment with PT150 (0, 50 or 100 mg/kg; p.o.) was initiated. Following 14 days of extinction training, rats were tested for reinstatement following either footshock or yohimbine (0, 1 or 2 mg/kg; i.p.); daily PT150 treatment occurred prior to each extinction and reinstatement session.

Results: Prior to initiation of PT150 treatment, females acquired greater levels of fentanyl self-administration during 1-hr sessions compared to males; however, during 6-hr sessions, males and females escalated to the same level of self-administration. PT150 had no effect on extinction of self-administration. While both footshock and yohimbine reinstated fentanyl seeking, only footshock-induced reinstatement was decreased by PT150 (50 and 100 mg/kg). The effect of PT150 on footshock-induced reinstatement was driven by males in the sample.

Conclusion: The glucocorticoid antagonist PT150 reduces shock-induced fentanyl seeking,

suggesting it may be effective against stress-induced relapse, although the sex difference in response may need further exploration

Figures below

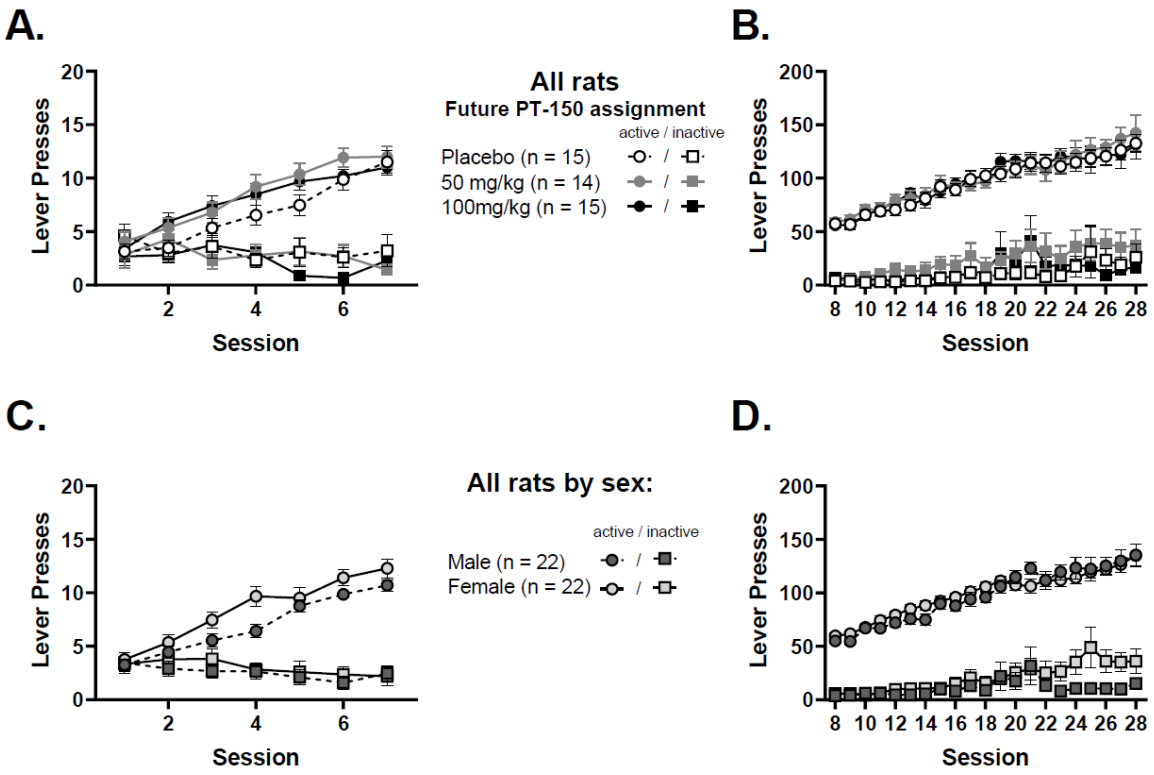


Figure 1: Number of lever presses (mean±SEM) across initial acquisition (1-hr) sessions (Panels A and C) and escalation (6-hr) sessions (Panels B and D) plotted as a function of treatment group (top panels) or sex (bottom panels); PT150 was not administered during acquisition or escalation. Collapsed across sex, active lever pressing increased significantly across 1-hr sessions (Panel A) and 6-hr sessions (Panel B). Broken down by sex, active lever pressing significantly increased more rapidly in females than males across 1-hr sessions (Panel C), but no sex differences were obtained across 6-hr sessions (Panel D). Females also emitted significantly more inactive lever presses than males across the 6-hr sessions.

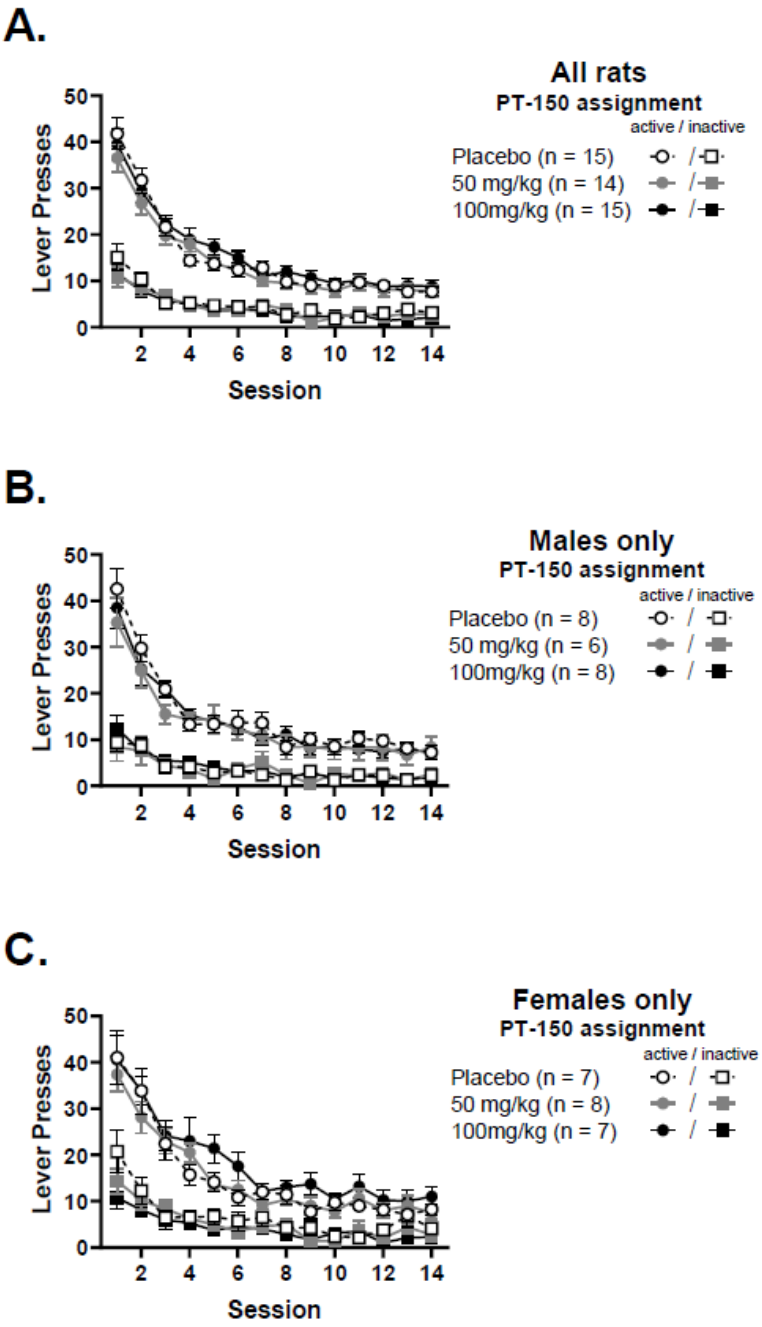


Figure 2: Responses on the active lever (mean±SEM) during extinction, plotted as a function of treatment group collapsed across sex (Panel A) or by treatment within males (Panel B) or females (Panel C). Responding decreased significantly across extinction sessions (Panel A), but there was no effect of PT150 treatment. Behavior was similar in males (Panel B) and females Panel C), although females pressed the inactive lever significantly more than males overall.

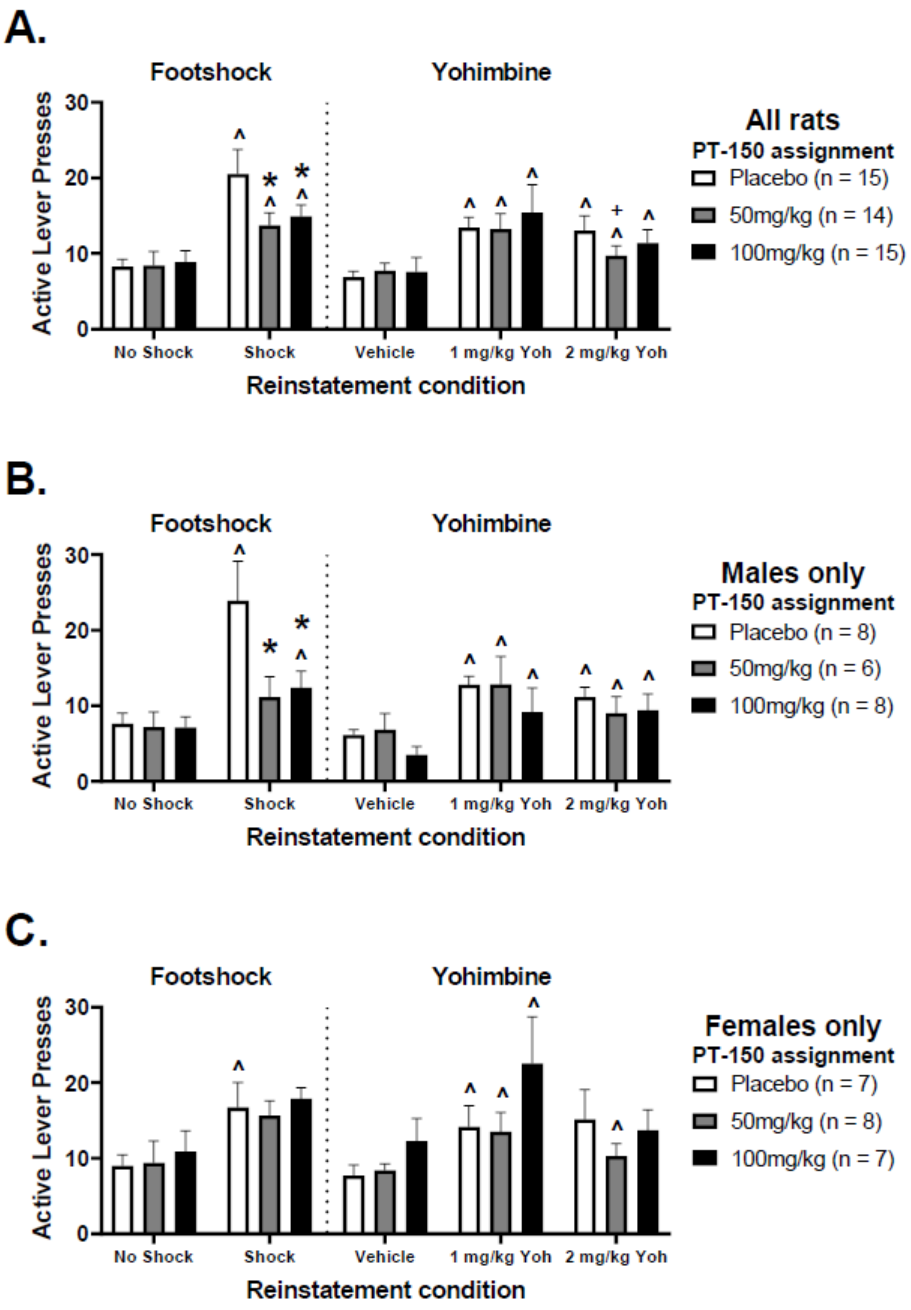
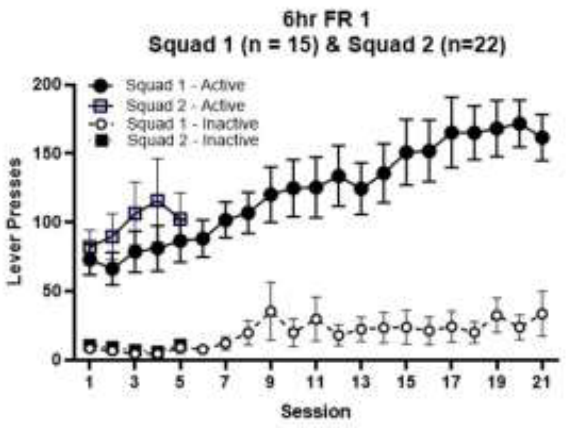


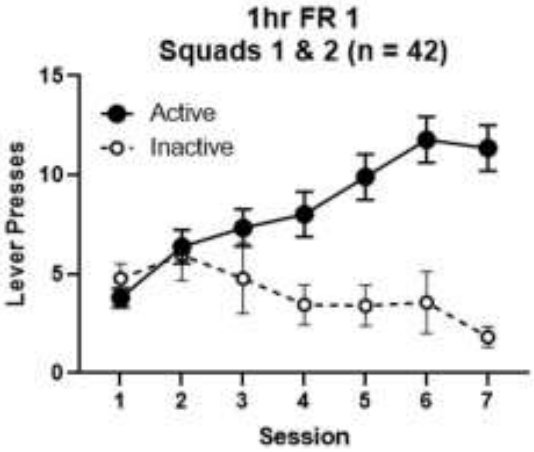
Figure 3: Reinstatement of active lever pressing (mean±SEM), plotted as a function of treatment group collapsed across sex (Panel A) or by treatment within males (Panel B) or females (Panel C). Footshock (Panel A, left) caused stress-induced reinstatement of active lever pressing that was significantly reduced by treatment with either dose of PT150. In contrast, yohimbine-induced reinstatement (Panel A, right) was not significantly altered by PT150. Sex differences in responding occurred across all test conditions, with males (Panel B) responding significantly less than females (Panel C). Exploratory examination of the effects of PT150 separately within males and females revealed that both doses of PT150 reduced footshockinduced reinstatement in males (Panel B), but not in females (Panel C). *FDR-adjusted planned comparisons:* ^$p < 0.05$ vs control condition (no shock or vehicle); *$p < 0.05$ vs placebo treatment; +$p < 0.05$ vs 1 mg/kg yohimbine.

Goal 2: Determine if PT150 reduces fentanyl self-administration in individuals with co-morbid PTSD.

The major activities accomplished under this goal are as follows:

a. Experimental design implemented
b. Data collection from Squad 1 completed.
c. Data collection from Squad 2 initiated.
d. Ongoing results complied below (not broken down by treatment group).





3.2.c Training and professional development provided:

Dr. Lindsey Hammerslag on the University of Kentucky team was on 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. Dr. Cassie Chandler on the University of Kentucky team has been on the bi-weekly teleconferences with the staff of RTI since June 2020 and will be replacing Dr Hammerslag. She has been 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.

Attended PASA investigator meeting and presented study overview.

3.2.d Dissemination to communities of interest:

Palisades therapeutics has reviewed the manuscript containing the results of Aim 1. The manuscript was submitted for publication and an editorial decision is pending.

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

For Aim 1, we will address any concerns from reviewers of manuscript and re-submit to the same journal or to a different journal. For Aim 2, we will complete data collection, analyze/graph all results, and prepare another manuscript for publication.

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 second year were:
One of the primary objectives was to develop a proof of concept trial study as well as a budget and Clinical Development Plan (CDP) for this study. We also had, and continue to work on, the goal of obtaining an IND through the FDA to use Dexmedetomidine HCI (BXCL501). The goal of obtaining Programmatic Panel approval is also still relevant as we hope to obtain approval within the next year.

3.3.b Accomplishments under the goals include:
During the past reporting year, the study team completed finalized the study protocol and assessment review. The group also drafted a preliminary budget for the potential study. Forms for submission to the FDA for an IND were finalized and sent in for review. The FDA required additional clarification, so the protocol was amended and resubmitted. After the resubmission the FDA, again, requested protocol edits. The study was presented to The Programmatic Panel; however, due to the clinical hold, the study is being deferred for Panel vote during the next fiscal year. Currently, the study team is working on addressing FDA IND questions and has plans to submit early October.

3.3.c Training and professional development provided:
Attended PASA investigator meeting and presented study overview.

3.3.d Dissemination to communities of interest:
Currently the study is under development but may produce a medication to potential treat comorbid PTSD and AUD

3.3.e Plans for next reporting period to accomplish goals and objectives:
Address the FDA IND requests. Present updated study and budget to the Programmatic Panel.

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 study protocol and IND application were submitted to the FDA. In May, the PI received notice of clinical hold from the FDA. The hold was related to SAEs (elevation in liver enzymes) reported in another (NIAAA) study utilizing ANS-6637. Despite the hold, the study team worked on achieving other milestones and tasks of this planning grant, i.e. preparing/revising the budget, drafting CDP, revising the protocol to address the FDA's safety concerns, and preparing complete response letter to the FDA. Thus, once the clinical hold is resolved, we will be able to promptly address any pending issues.

3.4.c Training and professional development provided:
Attended PASA investigator meeting and presented study overview.

3.4.d Dissemination to communities of interest:
N/A

3.4.e Plans for next reporting period to accomplish goals and objectives:
At this time, our pharmaceutical collaborator, ANS, is working with the FDA on resolving the hold. This is accomplished via close evaluation of the reported SAEs and additional in-vitro work, as requested by the FDA. Once this work is complete, ANS will request a Type A meeting with the FDA. This will allow to seek the FDA's input regarding any additional revisions to the protocol to address the FDA's concerns. The FDA's recommendations will guide any additional protocol revisions and IND resubmission during the next reporting period.

3.5 AS170014-A5 Preclinical testing of FKBP5 Inhibitors for alcohol use disorder-PTSD comorbidity
This study will examine a highly specific FKBP5 inhibitor (SAFit2) and a more broad-acting, but FDA-approved FKBP5 inhibitor (benztropine)that may potentially enhance the therapeutic effect for that PTSD/AUD comorbid-like phenotypes. This research will work to determine whether acute administration of FKBP5 inhibitors can restore normal behavior in rats exhibiting a PTSD/AUD-like Phenotype and whether Chronic administration of benztropine inhibitors can restore normal behavior in rats exhibiting a PTSD/AUD-like phenotype.

3.5.a Primary objectives and milestones for the second year were:
The major goals of this project are to investigate the effect of FKBP5 inhibitors on rats that displayed elevated PTSD/AUD-like comorbid behaviors. Our overarching hypothesis is 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 whether acute or 2) chronic administration of FKBP5 inhibitors (i.e., SAFit2-highly specific, or benztropine-broad acting) can restore normal behavior in rats exhibiting a

PTSD/AUD-like phenotype. Specific goals for this year included were development of protocol, obtaining IACUC and ACURO approval, development of Quality Assurance Plans and study launch.

3.5.b Accomplishments under the goals include:

The overall goal of this project is to examine whether FKBP5 inhibitors reduce AUD/PTSD comorbid-like behavioral phenotypes. During the current funding period, we submitted and obtained necessary regulatory approvals from The Scripps Research Institute Institutional Animal Care and Use Committee (IACUC) and the Animal Care & Use Review Office (ACURO), launched the study, accomplished key studies that address alcohol drinking, sleep disturbances, and hyperarousal. We completed a large aspect of Aim 1 related to experiment 1. 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, we exposed female and male rats (*n* = 24/sex) 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) in a between-subjects design 2 hr prior to 2BC testing. One week-later, rats were treated with the same dose of vehicle or benztropine (5 or 10mg/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 single dose of vehicle or benztropine (5 or 10mg/kg) and tested for acoustic startle responses.

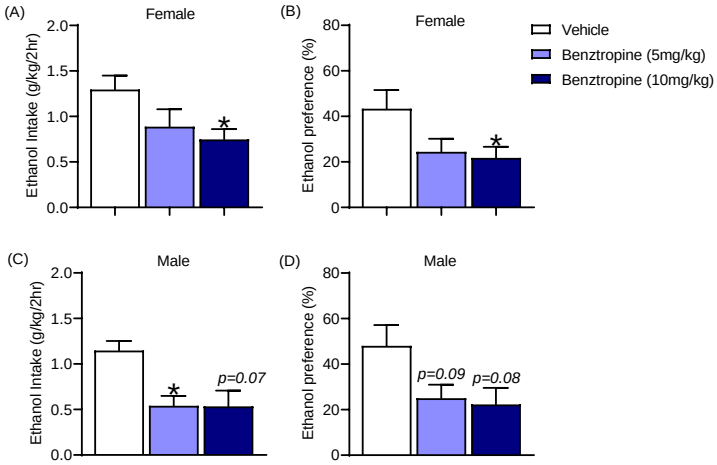


Fig. 1. Inhibition of FKBP5 via benztropine reduces ethanol drinking. Female (**A & B**) and male (**C & D**) were examined for ethanol intake and preference. (*) indicates significant difference from

As hypothesized, we have found that acute systemic benztropine administration reduced ethanol intake in PTSD-like rats (**Figure 1**). Specifically, female PTSD-like rats showed a significant 42% reduction in voluntary 2-hr ethanol intake (**Fig. 1-A**; *p<0.05), accompanied by a significant reduction in ethanol preference (**Fig. 1-B**; *p<0.05) following injection of the 10mg/kg dose of benztropine. In male PTSD-like rats, ethanol intake was significantly reduced by 52% at the 5mg/kg dose of benztropine (**Fig. 1-C**; *p<0.05).

Since core symptoms of PTSD involve sleep disturbances and hyperarousal (Cucciare et al., 2011), we measured sleep bout maintenance (**Fig. 2**) and acoustic startle responses (**Fig. 3**). Data collected so far do not indicate a significant mean increase in the duration of the longest sleep bout following an injection of acute benztropine at the 5 or 10mg/kg dose when compared to vehicle-treated controls in female (**Fig 2. A**) or male (**Fig. 2 B**) PTSD/AUD-like rats. However, Levene's test of inequality of variance demonstrated significantly unequal variability between the groups, reflecting larger variability in benztropine-treated female rats. Further analysis revealed that a subset of benztropine-treated females (*n*=2) showed a maximum sleep bout duration more than 3 standard deviations longer than that of vehicle-treated females. Thus, benztropine might increase sleep maintenance in a subset of females.

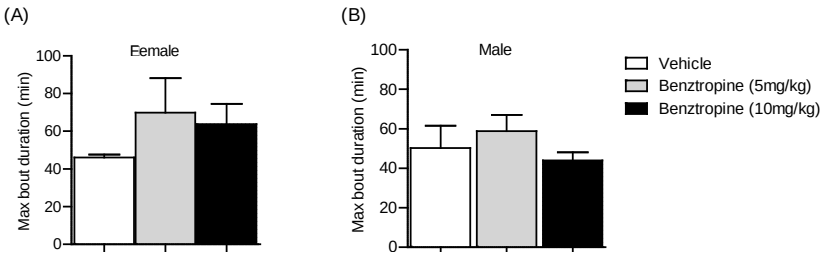


Fig. 2. Inhibition of FKBP5 via benztropine does not restore sleeping pattern. Female (**A**) and male (**B**) were examined for sleep cycle analysis following ethanol drinking.

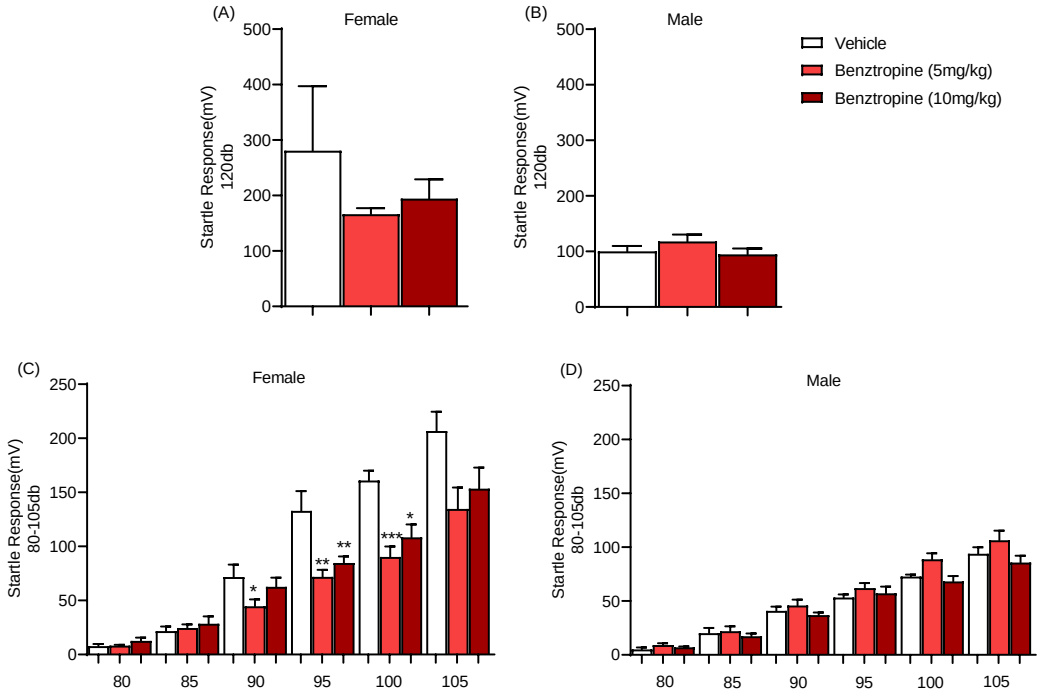


Fig. 3. Inhibition of FKBP5 via benztropine restores startle response in a sex-dependent manner. Female (**A & C**) and male (**B & D**) were examined for sleep cycle analysis following ethanol drinking. (*) indicates significant difference from vehicle-treated rats.

Interestingly, data so far indicate that benztropine altered mean acoustic startle responses in a sex-dependent manner in PTSD/AUD-like rats (**Fig. 3**). Specifically, we found that female PTSD/AUD-like rats treated with benztropine displayed reduced average acoustic startle responses across several acoustic intensities (**Fig.3-C;** *$p<0.05$). Benztropine did not similarly

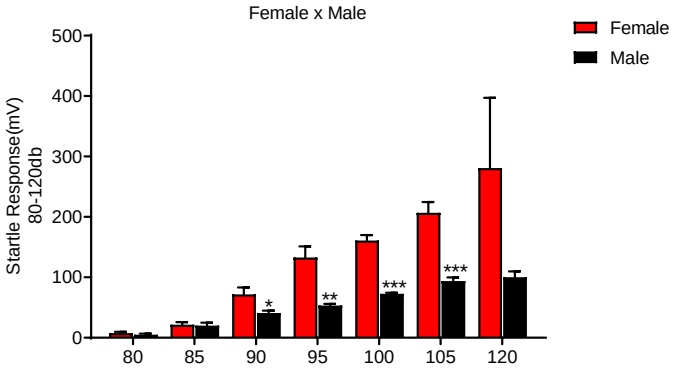


Fig. 4. PTSD/AUD produces higher startle responses in female versus male rats. Female and male were compared in acoustic startle test (*) indicates significant difference from female rats.

reduce startle in male PTSD/AUD-like rats (**Fig. 3-D**). A separate analysis comparing sex differences between PTSD/AUD-like rats showed that female rats exhibit higher startle responses across several intensities than males (**Fig. 4**). The results suggest that benztropine reduces acoustic startle in a sexually dimorphic manner (**Fig 3. B & D; Fig 4.**), perhaps via its inhibition of FKBP5.

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. A prior postbaccalaureate member of the research team, Shannon D'Ambrosio, also received training during the funding period and recently began her Ph.D. studies in Biomedical Sciences at the University of California-San Diego. The PI and co-PI are also well-positioned in mentoring and will continue to oversee these features develop.

Attended PASA investigator meeting and presented study overview.

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.

Protocols for the study were presented at the annual PASA Investigators Meeting by Drs. Zorrilla and Roberto.

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

Our 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. Our goals and objectives for the next reporting period are to complete the pharmacological studies that comprise **Aim 1**. First, this includes fear overgeneralization and amended irritability behavioral testing after acute benztropine administration. We also will complete additional subjects that may clarify whether or not benztropine increases sleep maintenance in some females. Second, we will test whether acute administration of the more specific FKBP5 inhibitor, SAFit2, can similarly restore normal behaviors in rats that exhibit a PTSD/AUD comorbid-like phenotype. Third, we will determine whether doses of the inhibitors that were effective in PTSD/AUD-like rats also alter drinking, hyperarousal and overgeneralization behavior in control rats. Finally, we plan to test whether chronic

administration of benztropine can robustly restore normal behaviors in rats that demonstrate PTSD/AUD comorbid-like phenotypes.

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). We plan 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.

3.6.a Primary objectives and milestones for the second 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 1.6mg/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 BCM IRB, R&D committee, and HRPO approval. The execution of a CRADA between Baylor College of Medicine and RTI was a major goal during this review period. Other goals for the year included: the finalization of the EDC system and study CRFs; the execution of a Certificate of Confidentiality; and ClinicalTrails.gov posting.

3.6.b Accomplishments under the goals include:
In response to the COVID-19 Pandemic, Baylor College of Medicine instituted college wide measures to help limit the spread of the virus and perform responsible conduct of research. Starting March 23, 2020 limited access to research facilities was implemented and all novel research protocols were suspended indefinitely. The study team plans to resume research activities once research restrictions have been lifted across the institution.

BCM IRB approval was initially granted on 12/17/2020, and again on 3/22/2020 following the submission of multiple amendments to the protocol. R&D committee approval has been granted as well. Additional amendments have been added to the protocol at the request of the Human Research Protection Office (HRPO) Office of Research Protections (ORP) United States Army Medical Research and Development Command (USAMRDC). The request of additional protocol modifications has delayed HRPO approval. The ClinicalTrials.gov posting was completed and EDC system/forms approval were completed as well. A Certificate of Confidentiality has been fully executed as well as a CRADA.

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.

Attended PASA investigator meeting and presented study overview.

3.6.d Dissemination to communities of interest:
Not applicable

3.6.e Plans for next reporting period to accomplish goals and objectives:
In order to expedite initiation of the protocol, the study team has modified the master protocol and resubmitted a revised telemedicine adapted version of the protocol to the local IRB. We anticipate commencement of the study by December 2020. The study team has generated a cumulative list of patients currently prescribed Buprenorphine with a co-occurring PTSD diagnosis to recruit from. Once open, those subjects will be contacted and screened for enrollment.

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 we continue to finalize and publish additional manuscripts, we strengthen our 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. We have refined our 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. We continue to build our template library as well as our website to allow for efficiency and consistency across studies. We have

also established excellent working relationships with several VAMCs across the USA for conducting our PASA clinical studies. We have used knowledge across studies conducted within the PASA consortium, as well as knowledge of clinical trials conducted outside of the PASA consortium by our established collaborators, to help inform initial and continued funding decisions for compounds being studied within PASA.

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

As noted above our anti-fentanyl vaccine has proven exceptionally effective with 100% blockade of the analgesic effects of fentanyl and significant decrease of brain levels of fentanyl following administration. This is a major accomplishment and our vaccine formulation innovative to the extent a provisional patent has been submitted with full support by the University of Houston. If efficacy of our vaccine is demonstrated in humans, it will have a profound impact on the ongoing opioid overdose epidemic in this country and abroad.

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 PT-150 ameliorates stress-induced escalation of fentanyl self-administration and stress-induced reinstatement, thus providing the impetus for a potential new avenue for treating co-morbid OUD and PTSD. Based on these results, we are working on a plan to apply for funding to conduct a study in humans in partnership with co-investigator Dr. Craig Rush at the University of Kentucky and the business entity holding rights to the drug (Palisades Therapeutics, a division of Pop Test Oncology LCC, Cliffside Park, NJ). Commercialization of this therapeutic would have widespread impact on treating veterans and other vulnerable populations who suffer from co-morbid OUD and PTSD.

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

Project is still in the development phase.

4.4 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

Project is still in the development phase.

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

We successfully used for the first time a new PTSD/AUD comorbidity model recently developed and characterized in our lab and accepted for publication (Steinman et al., 2020, in press). 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, we were able to identify effects of benztropine, an FDA-approved drug, to significantly reduce voluntary ethanol intake and preference and, in females, acoustic startle responses, a putative indicator of hyperarousal.

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

Project is still in the start-up phase.

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 studies did not experience drastic problems; however, there were some study delays and modifications due to the pandemic. To mitigate study problems as much as possible, the PASA core tracked each site's status and impacted abilities. Though there were some delays, sites are now re-launched and have adapted to the constraints inflicted by COVID.

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

We have overcome major obstacles: 1) COVID-19 University shutdown, 2) Limited to no staff, 3) limited accessibility to supplies needed to conduct the experiments.

Changes made to address obstacles:

1) COVID-19 University shutdown
   The PI arranged through University Administration to designate lab and lab personnel as "essential". The laboratory was never completely shut down which enable us to continue to conduct time-sensitive experiments and harvest tissues needed.
2) Limited to no staff
   The PI has continued to work at the University throughout the pandemic in order to conduct biochemistry experiments and process samples with the help of one part-time technician. An additional technician has been hired and student volunteers have started working on this project in a limited capacity.
3) Limited accessibility to supplies needed to conduct the experiments.
   Due to COVID-19 concerns the last six to eight months we have had difficult procuring surgical supplies, test drugs (e.g. fentanyl), ELISA assay reagents. We still cannot have supplies mailed directly to the laboratory but to a "central hub" where an appointment has to be made to acquire packages. Fentanyl has been back ordered till December however we have found an alternate source. Alternate suppliers have been found for other necessary supplies have also been identified.

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 in response to the COVID-19 pandemic, rats in Squad 1 of aim 2 had to be halted prior to completion of data collection. Due to this loss, we submitted and were approved for an IACUC amendment at the University of Kentucky to add additional animals for this project. We have also submitted and been approved for additional animals with ACURO.

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

The FDA issued two separate clinical holds that have delayed approval. The research team will continue to work to address these concerns and obtain necessary approvals. A revised protocol will be submitted during the next quarter.

5.4 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

As specified above, the clinical hold resulted in the delay of the IND receipt. This delay did not affect the expenditures of this planning grant. We anticipate that following the Type A meeting with the FDA, we should be able to promptly revise the protocol and resubmit the IND.

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

Our currently approved animal protocol stated that we 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. We 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, we have never previously tested these 3 tests in counterbalanced order. For that reason, after discussion with our PASA collaborators, we decided to test them in the order from least stressful (CLAMS-sleep) to most stressful (fear overgeneralization). Doing so helped us 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 we always have done previously for these 3 behavioral tests.

Also, in our past and recent work (Steinman et al., in press in Molecular Psychiatry), we have identified a 4th behavioral test (bottle brush irritability test) relevant to PTSD that also reliably distinguishes subjects in our model (aggressive and defensive-like behavior in the bottle-brush test of irritability). We therefore 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 at our home institution, new animal orders were not allowed until late June. As a result, the first cohort of animals for Aim 1 of these studies began in the middle phase of this report period. After ordering was allowed, 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. Nonetheless, we made significant progress via internal schedule planning and coordination with Animal Resource and IACUC colleagues. We will follow this approach to meet pandemic challenges in the coming year.

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.

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

Subjects will be recruited for the Michael E. Debakey Opioid Treatment Program (OTP) roster. Patients on the roster frequently attend Mental Health clinic visits at the MEDVAMC. By scheduling research

visits with co-occurring clinic visits, we drastically reduce the number of in-person visits to the MEDVAMC.

The COVID-19 pandemic and resulting closures of institutions/organizations has delayed opening the study up for recruitment. We have revised the protocol to include COVID-19 precautions for the subjects and study staff. Some of these revisions include limiting the number of in-person visits, removing the Fear Potentiated Startle test, and transitioning most visits to tele-medicine videoconferencing.

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

Haile, Colin, et al.; Effects of FEN-*CRM*^{197} conjugate vaccine + dmLT adjuvant with and without buprenorphine on fentanyl nociception in male and female Sprague Dawley rats, Military Health Systems Research Symposium. 2020 August.

Publications

Publications are in progress 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

The PI presented at the PASA Consortium online meeting 5/29/2020 “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 in coordination with the University of Houston to secure intellectual property rights for the anti-fentanyl vaccine.

“Adjuvanted Conjugate Opioid Vaccine” Colin N. Haile, Gregory D. Cuny, Elizabeth B. Norton, Therese A. Kosten, (5/27/2020).

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

1. Virtual PASA Consortium Investigator Meeting (May 29, 2020): Results from Aim 1 were presented by Drs. Bardo and Hammerslag.

2. Manuscript submitted to Psychopharmacology entitled “Effects of the glucocorticoid receptor antagonist PT150 on stress-induced fentanyl seeking in male and female rats”.

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

Project is still under the development phase.

6.4 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

N/A

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

Drs. Zorrilla and Roberto presented at the annual PASA Investigators Meeting.

Roberto, M.R. and Zorrilla, E.P., Preclinical testing of FKBP5 Inhibitors for alcohol use disorder-PTSD comorbidity. Pharmacotherapies for Alcohol and Substance Abuse Consortium (PASA) Investigator Meeting, May 29, 2020.

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

7. Participants and Other Collaborating Organizations

RTI International - Management Core

Nolen, Tracy	Principal Investigator	21%
Williams, Rick	Co-Principal Investigator	11%
Bradley, Lauren	Research Coordinator	19%
Baldi, Marjorie	Financial/Subcontracts Mgr	13%
Carper, Ben	Statistician	8%
Crawford, Meg	Research Coordinator	14%
Fain, Katie	Research Coordinator	27%
Hirsch, Shawn	Statistician	2%
Kendrick, Amy	Research Coordinator	26%
LeGrow, Keith	Programmer/Analyst	6%
Nowak, Kayla	Statistician	8%
Riggs, Callie	Financial/Subcontracts Mgr	5%
Roberts, Cheryl	Clinical Data Manager	12%
Tang, Yan	Programmer/Analyst	11%
Turner, Gene	Clinical Data Manager	18%
Vandergrift, Nathan	Statistician	6%
Whitworth, Ryan	Statistician	23%

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%
Quardri, Saif	Research Assistant	32%
Sanchez, Sergio	Research Assistant	80%
Baker, Miah	Research Assistant	80%
Duddupudi, Anantha	Post Doc	32%
Rodgers, Hailey	Graduate Student	16%

University of Kentucky

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

Bardo, Michael	Primary Investigator	5%
Prendergast, Mark	Co-Investigator	2%
Rush, Craig	Co-Investigator	2%
Dwoskin, Linda	Co-Investigator	2%

Hammerslag, Lindsey	Post doc	20%
Denehy, Emily	Facilities Manager	50%
Hamid, Usman	Laboratory Assistant	20%
Chandler, Cassie	Post doc	8%
Punzal, Emily	Laboratory Assistant	8%

University of Texas Health Science Center at Houston

An Aldehyde Dehydrogenase 2 Inhibitor for PTSD and AUD

Yammine, Luba	Principal Investigator	40%
Verrico, Christopher	Co-Principal Investigator	24% (no cost)
Kosten, Thomas	Co-Investigator	24% (no cost)

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	Post doc	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	25%
Kosten, Thomas	Co-Principal Investigator	25%
Vaughan, Adetola	Study Coordinator	45%

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	30%
Krystal, John	Co-Principal Investigator	1%
Levy, Lucienne	Research Assistant	20%
Newcomb, Jenelle	Coordinator/RA	10%

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:

Miah Baker (10 months) Research Assistant. Contributed by helping to conduct vaccination experiments, collect bloods, analgesic tests and process brain and blood samples, protein estimation and ELISAs.

Hailey Rodgers (2 months) Graduate student. Contributed by helping to conduct experiments described in AIM 2.

Sergio Sanchez (10 months) Research Assistant. Contributed by helping to conduct vaccination experiments, collect bloods, analgesic tests.

Anantha Duddupudi (4 months) Post-Doctoral Fellow. Synthesis optimization of the anti-fentanyl conjugate vaccine.
Saif Quadri (4 months) Research Assistant. Contributed by helping process brain and blood samples, protein estimation and ELISAs.

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

Tulane University School of Medicine: characterization of the vaccine formulation.

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): 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): No change, but effort ended in September.

Ms. Emily Denehy (facilities manager): No change.

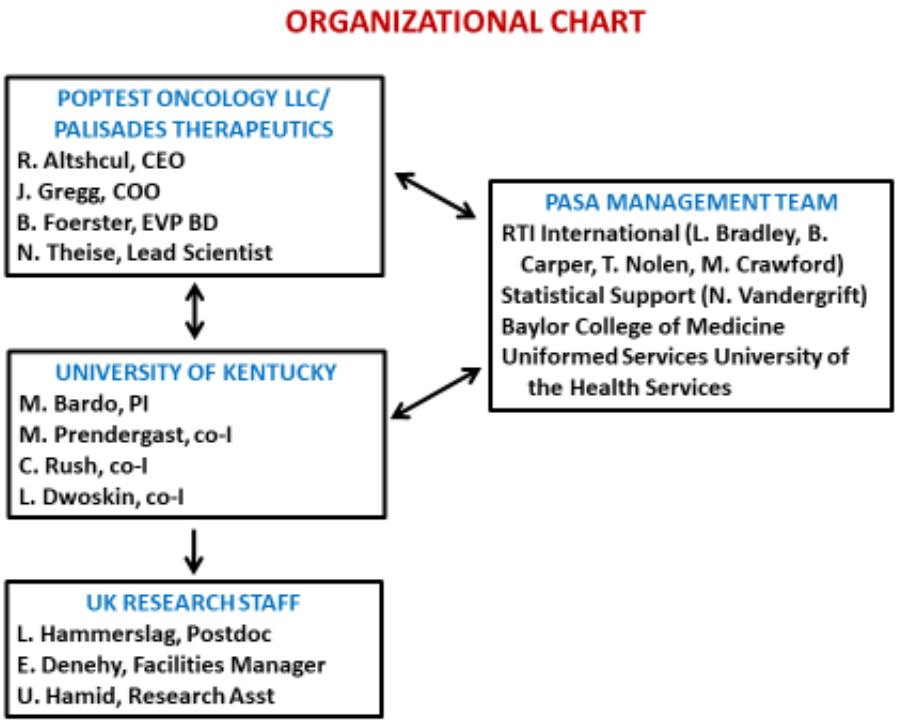
Mr. Usman Hamid (laboratory assistant): No change, but effort ended in March.

Dr. Cassie Chandler (postdoc): 1 mo; beginning July, served to oversee 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): 1 mo; beginning July, served as part-time hourly employee whose primary responsibility was to run the operant self-administration session on the weekends.

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

See organizational chart below:



7.3. AS170014-A3 Developing a proof of concept clinical trial to evaluate the use of a safe and highly selective α2a Adrenergic Receptor Agonist, BXCL 501, for the treatment of ASUD comorbid with PTSD and/or TBI – Planning Grant

7.3.a. Other organizations that have been involved as partners:

BioXcel Therapuetics, Inc.

7.4. AS170014-A4 An Aldehyde Dehydrogenase 2 Inhibitor for PTSD and AUD

7.4.a. Other organizations that have been involved as partners:

ANS Inc. is our pharmaceutical collaborator. ANS has demonstrated its full support for the subsequent clinical trial by (1) participating in biweekly meetings with PASA DCC (at RTI); (2) providing scientific input to the study team during protocol preparation; (3) granting access to reference the IND that ANS maintains for the study of ANS-6637 and (4) providing regular updates to the study team and PASA DCC (at RTI) regarding clinical hold and steps taken by ANS to resolve the hold. Once the study commences, ANS will participate in the study oversight in an advisory capacity and will provide ANS-6637 and matching placebo for the project.

7.5. AS170014-A5

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

Recent personnel added to this project:

Dr. Bryan Cruz, PhD, is a postdoctoral researcher in the Roberto Lab. He is also co-mentor with the co-PI Dr. Eric Zorrilla. He has prior experience with behavioral models involving stress and addiction disorders. Dr. Cruz earned his PhD in behavioral neuroscience from The University of Texas at El Paso under the guidance of the Dr. Laura E. O'Dell who studies the neurobiology of nicotine addiction.

Dr. Valentina Vozella, PhD, is a postdoctoral researcher in the Roberto Lab. She has previous experience with PTSD models and anxiety behavioral tests as well as expertise in small molecule drug discovery and development. Dr. Vozella earned her PhD in drug discovery from the University of Genova, Italy, under the mentorship of Dr. Daniele Piomelli. During her postdoc at the University of California Irvine she used animal models to bring small molecules from preclinical to clinical stages focusing on the use of FAAH inhibitors as potential treatments for PTSD.

DoD Alcohol and Substance Abuse Consortium Award

Pharmacotherapies for Alcohol and Substance Abuse (PASA) Consortium

PI: Tracy Nolen, DrPh, Rick Williams, PhD & Thomas Kosten, MD **Org:** RTI International

Study Research Planning Program

- Reviewed 10 applications and awarded one full study implementation awards for conduct of proof-of-principle basic research to determine which compounds are most appropriate for human research trials (RFA4b)
- Prepared RFA5 and received Programmatic Panel approval for new expansion award. This award will support the continued research of highly impactful studies that were previously funded by PASA. RFA will be released in study year 3.

Held first PASA Investigator meeting in May 2020. Researchers presented study accomplishments and an open forum was used to discuss potential future research concepts.

Timeline and Cost

Activities	Q1	Q2	Q3	Q4
Implementation of 3 new awards from RFA4				
Continued oversight for two preclinical studies (AS170014-A1 and AS170014-A2)				
Protocol development and study launch for one new preclinical study (AS170014-A5)				
Support of two planning grants (AS170014-A3 and A4)				
Protocol and systems development for one new clinical trial (AS170014-A6)				
PASA Investigator Meeting				
Costs per month	314k	258k	266k	439k
Total Costs: $1,277k to date; $9,761,851 remaining				

Year 2 Completed Objectives

- Launched one pre-clinical study.
- Launched and completed work on two planning grants.
- Ongoing preparation for 1 new clinical trial.
- Held PASA Investigator Meeting

Year 2 Objectives in Progress

- Ongoing study procedures/experimentation being carried out for 3 animal studies
- Fund and setup new expansion award(s) from RFA#5
- Launch new clinical trial by December 2020.