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Protocol Number: NCIG-007R

Randomized, Double Blind, Placebo-Controlled Trial of the Efficacy and Safety of Intranasal Oxytocin for the Treatment of Alcohol Use Disorder

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STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CRF Part 46, 21 CFR Part 50, 21 CFR Part 56 and 21 CFR Part 312)
- International Conference on Harmonisation (ICH) E6

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

1. Protocol Synopsis

Name of Sponsor/Company: National Institute on Alcohol Abuse and Alcoholism (NIAAA)	
Name of Investigational Product: Oxytocin Nasal Spray	
Name of Active Ingredient: Oxytocin	
Protocol Number: NCIG-007R	
Study Title: Randomized, Double Blind, Placebo-Controlled Trial of the Efficacy and Safety of Intranasal Oxytocin for the Treatment of Alcohol Use Disorder	
NIAAA Principal Investigator: Raye Litten, Ph.D.	
Study Centers: Boston University Medical Center, Johns Hopkins University School of Medicine, University of California at Los Angeles, and University of Virginia	
Study Period: ~ 12 months	**Phase of Development:** 2
Objectives: **Primary:** The primary objective of the study is to compare the efficacy of intranasal oxytocin in reducing the weekly percentage of heavy drinking days over the 10 weeks of maintenance treatment among subjects with moderate to severe Alcohol Use Disorder (AUD). A "heavy drinking day" is 4 or more drinks per drinking day for women and 5 or more drinks per drinking day for men. **Secondary:** Secondary objectives include assessment of other measures of the effects of oxytocin compared with placebo on reduction of alcohol use as well as effects on psychological assessments, alcohol craving, alcohol-related consequences, cigarette smoking and other nicotine use, retention in the study, safety, and application site (nares) tolerability throughout the study.	
Methodology: This study is a double-blind, randomized, placebo-controlled, parallel group, multi-site clinical trial designed to assess the efficacy of oxytocin compared with placebo to reduce drinking in 100 subjects (50 in each group) who report 4 or more Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5™) symptoms of AUD. This study will be conducted at 4 clinical sites. If eligible for the study, subjects will be randomized using a stratified permuted block randomization procedure with "clinical site" as a stratification variable in an approximate 1:1 ratio (targeting 50 subjects per group) to receive either intranasal oxytocin or placebo for 12 weeks. If eligible for the study, subjects will receive blinded intranasal oxytocin at 35 IU once per day or intranasal placebo (same volume once per day) for 2 weeks (initial tolerability assessment period), then for the next 10 weeks (maintenance period) subjects will receive oxytocin at 35 IU/ twice per day (BID, totally daily dose 70 IU) or intranasal placebo (same volume twice per day). Subjects will be seen in the clinic at screening visit(s), at randomization and 7 other times during the study (Weeks 2, 3, 4, 6, 8, 10, and 13). During the Weeks 5, 7, 9, 11, and 12 subjects will be contacted once during the week by telephone at non-clinic visit weeks to encourage study drug compliance and to assess withdrawal, adverse events (AEs), and concomitant medication use. A final follow-up telephone interview will occur during Weeks 14/15 (1-to-2 weeks after the end of dosing). Enrollment will be halted if more than 2 treatment-related SAEs occur. If this occurs, a DSMB will be convened and a decision in conjunction with the study Sponsor will be made regarding stopping the study.	
Number of Subjects (Planned): Estimated 250 to obtain 100 eligible and randomized.	
Main Inclusion/Exclusion Criteria: Subjects will be male and female at least 21 years of age with 4 or more DSM-5™ symptoms of AUD. They must also be seeking treatment for alcohol problems and if male, report drinking an average of 28 drinks per week or if female report drinking an average of 21 drinks per week and at least one heavy drinking day per week in the 28-day period prior to consent and at least one in the 7-day period prior to randomization.	

Investigational Product, Dosage and Mode of Administration: Oxytocin, 35 IU per dose, intranasally once-daily for 2 weeks then twice-daily (BID) (morning and evening) for 10 weeks. Oxytocin is formulated to contain 70 IU/mL. One dose is defined as intranasal spray of 100 μL per each nostril x 5 sprays in alternating nostrils with a 30 second wait between sprays for a total dose volume of 500 μL.

Reference Therapy, Dosage and Mode of Administration: Identically matched placebo administered intranasally once-daily for 2 weeks then BID (morning and evening) for 10 weeks. The placebo spray is formulated identically to oxytocin without the addition of oxytocin. One dose is defined as intranasal spray of 0.1 mL per each nostril x 5 sprays in alternating nostrils with a 30 second wait between sprays for a total dose of 0.5 mL.

Duration of Study: Each subject will participate in the study for up to approximately 17 weeks, including up to 2 weeks of screening, 12 weeks of treatment (2 weeks for initial tolerability and 10 weeks of maintenance dosing for safety and efficacy), one follow-up visit after completing treatment, and a final telephone contact 1-to- 2 weeks after completing treatment.

Criteria for Evaluation:

Primary: The primary efficacy endpoint is the weekly percentage of heavy drinking days during the 10-week maintenance treatment period. A "heavy drinking day" is 4 or more drinks per drinking day for women and 5 or more drinks per drinking day for men. Drinking data will be collected by the Timeline Followback (TLFB) method.

Secondary: Secondary efficacy endpoints will be analyzed over the entire 10-week treatment period including:

1. Percentage of subjects with no heavy drinking days during treatment
2. Percentage of subjects abstinent from alcohol during treatment
3. Percentage of subjects with a World Health Organization (WHO) drinking risk category decrease during treatment of:
   a) at least 1-level
   b) at least 2-levels
4. Endpoints analyzed monthly and with 1- and 2-month grace periods:
   a) Percentage of subjects with no heavy drinking days during the period
   b) Percentage of subjects abstinent from alcohol during the period
   c) Percentage of subjects with WHO drinking risk category decrease during the period of:
      i. at least 1 level
      ii. at least 2 levels
5. Percentage of days abstinent from alcohol per week
6. Weekly mean number of drinks per week
7. Weekly mean drinks per drinking day
8. MINI AUD Score at end of study
9. Cigarettes smoked per week among smokers
10. Abstinence from cigarette smoking among smokers
11. Other nicotine product use per week among other nicotine product users
12. Experiences in Close Relationships—Relationship Structures Questionnaire (ECR-RS) scores (attachment-related anxiety)
13. PROMIS sleep disturbances scores
14. PROMIS alcohol-related negative consequences scores
15. PROMIS pain interference scores
16. Profile of Moods States (POMS) scores (including subscale scores)
17. Urge to Drink Scores

Exploratory Endpoint:

1. Hyperkatifeia Scale

Safety Endpoints Safety endpoints will be evaluated over the entire treatment and follow-up periods including:

1. Vital signs
2. Physical exam of the nasal mucosa

3. Smell testing with the University of Pennsylvania Smell Identification Test (UPSIT)
4. Blood chemistries
5. Urine drug screen results
6. Blood alcohol concentration (BAC) by breathalyzer
7. AEs/SAEs
8. Electrocardiogram (ECG)
9. Clinical Institute of Withdrawal – Alcohol Revised (CIWA-AR) scores
10. Frequency of subjects with suicidal ideation at any time during the treatment period –Columbia-Suicide Severity Rating Scale (C-SSRS)
11. Change in appetite – Simplified Nutritional Appetite Questionnaire (SNAQ) scores and percentage of subjects with scores ≤14.
12. Buss-Perry Aggression score

Compliance: Self report of compliance with study drug self-administration.

Statistical Methods (Data Analysis):

Analysis Populations:

Full Analysis Set: The full analysis set is defined as subjects randomized to participate in the study who took at least one dose of investigational product. The full analysis set will be used to evaluate all efficacy and safety endpoints.

Sample Size: With an intake sample of 100 subjects (50 subjects per arm) and 12% attrition, it is projected that the sample size for primary endpoint analyses will be 88 (44 subjects per arm). The alpha level for the primary analyses will be 0.05, two-tailed. An estimate of the effect size (Cohen's d) for the investigational products is 0.60. Equal variances in both groups are assumed. These assumptions lead to a projected power of 0.80.

Analysis of the Primary Efficacy Endpoint: The analysis of the primary endpoint, percentage of heavy drinking days, will be a mixed-effect model for repeated measures during the last 10 weeks of the treatment period. Treatment group, study week, treatment group by study week interaction, clinical site and baseline percentage of heavy drinking days will be covariates in the mixed effects model. Additional covariates may be selected as specified in the SAP. The primary endpoint will be performed on the Full Analysis Set. A sensitivity analysis will be performed using multiple imputation for missing drinking data. Specification of the multiple imputation models will be provided in the statistical analysis plan (SAP). Descriptive statistics will be used to present these data during the initial 2-week period as well.

Analysis of the Secondary Efficacy Endpoints: Analysis of the continuous secondary endpoints (weekly mean drinks per week, weekly mean drinks per drinking day, percentage of days abstinent, number of cigarettes smoked per week, number of days of other nicotine product use per week, POMS scores, ED-RS scores, PROMIS scores, Urge to Drink Scores will be analyzed in a similar fashion to that of the primary outcome (except substituting baseline equivalent of the outcome as a covariate). Analysis of the dichotomous secondary outcomes (percentage of subjects with no heavy drinking days, percentage subjects abstinent from alcohol, percentage of subjects with 1- and 2-level decreases in WHO drinking categories, and percentage of smokers abstinent from smoking) will be conducted via logistic regression of the endpoint measured across the entire treatment period (monthly and with grace periods, as specified). Secondary analyses to satisfy statistical hypotheses will be performed on data collected during the 10-week maintenance period; however, as appropriate, descriptive statistics will be used to present these data during the initial 2-week period as well. Covariate selection beyond treatment group, clinical site, and baseline equivalent of the endpoint will be specified in the SAP. No imputation will be used for the analysis of secondary endpoints.

Exploratory Analysis: A number of variables will be tested as potential moderators of the medication treatment effect on the percentage of heavy drinking days primary endpoint. Weeks 3-12 will be the period of interest. Continuous moderator variables will be dichotomized. The potential moderator variables that will be examined include measures of anxiety and depression (ECR-RS subscales of attachment and anxiety, POMS subscales of depression and anxiety, Spielberger Trait Anxiety Inventory, and Barratt Impulsiveness Scale), and those suggestive of alcohol withdrawal (i.e., withdrawal question on the MINI for alcohol use disorder), and alcohol-related treatment goal.

There is interest in exploring the use of a new Hyperkatifeia Scale as a predictor and moderator of drug response Exploratory analyses utilizing Hyperkatifeia Scale scores will include: 1) conducting moderator analyses using total

score; 2) comparison of change from baseline between treatment groups for total score and subscales.

***Ad Hoc* Analyses:** *Ad hoc* analyses may be performed on the Hyperkatifeia Scale as a planned part of exploring the utility of this scale including total scores, subscales, and individual items. *Ad hoc* analyses utilizing Hyperkatifeia Scale scores may include: 1) identification of baseline subsets and relationship to drinking outcomes; 2) conducting mediator analyses; 3) factor analysis to better understand the underlying dimensions of hyperkatifeia; and, 4) validity of the Hyperkatifeia Scale by comparing to other measures (e.g., POMS and PROMIS scales or subscale scores). The data from this study could be used for meta-analyses with results of other studies to expand data availability for the proposed *ad hoc* analyses

Safety Analyses:

The severity, frequency, and relationship of AEs to investigational product will be presented by Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class (SOC) and Preferred Term (PT) grouping. AE summary data will be presented by dose (all weeks, first two weeks and last 10 weeks). Listings of each individual AE including start date, stop date, severity, relationship, outcome, and duration will be provided. Clinical chemistry, urine drug screen results, pregnancy test results, and BAC results, will be reported as summary statistics. Vital signs will be presented as summary statistics and change from baseline. The proportions of ECG results considered clinically significant will also be provided. In addition, change from baseline (shift tables) will also be presented for clinical chemistry data. The numbers and proportion of subjects who reported CIWA-AR scores ≥ 10 at any time after the start of dosing will be presented. Buss-Perry Aggression scores and SNAQ scores will be compared using repeated measures ANOVA. The numbers and proportions of subjects mild, moderate, severe microsomia or any smell loss (anosmia) compared with screening assessment using the UPSIT will also be presented.

Compliance and Retention Analyses:

Medication compliance, defined as the amount of investigational product taken as a proportion of the total amount prescribed per protocol, will be evaluated for the oxytocin and placebo groups based on subject's self-report. Average amounts of investigational product taken overall and weekly will be reported for the oxytocin and placebo groups. In addition to self-reports, the volume self-administered will be determined by weighing each of the bottles of investigational product dispensed to the subject before and after use and subtracting the difference. The research participation rate, defined as percentage of subjects with complete drinking data, will be compared between treatment groups. In addition, the percentage of subjects discontinuing medication or early withdrawal from the study and a listing of these reasons for discontinuation will be provided.

Baseline Descriptive Statistics:

Summaries of the characteristics of the subjects in each of the study groups at baseline will be prepared. Baseline characteristics will be compared between the oxytocin and placebo groups using appropriate statistical methods.

2. Table of Contents, List of Tables, and List of Figures

Table of Contents

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3. List of Abbreviations and Definition of Terms

Abbreviation	Definition
5-HT	Serotonin
AA	Alcoholics anonymous
AE	Adverse event
AEDs	Antiepileptic drugs
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
AUD	Alcohol Use Disorder
AWSC	Alcohol Withdrawal Symptom Checklist
BAC	Blood alcohol concentration
BID	Twice daily
BIS	Barratt Impulsiveness Scale
BPAQ-SF	Buss Perry Aggression Questionnaire – Short Form
CAP	College of American Pathologists
CeA	Central nucleus of the amygdala
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Act
CIWA-AR	Clinical Institute Withdrawal Assessment for Alcohol-revised
CoC	Certificate of confidentiality
CORT	Corticosterone
CrCL	Creatinine clearance
C-SSRS	Columbia-Suicide Severity Rating Scale
eCRF	Electronic Case Report Form
CTCAE	Common terminology criteria for adverse events
CSF	Cerebral spinal fluid
dL	Deciliter
DNA	Deoxyribonucleic acid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
ECR-RS	Experiences in Close Relationships-Relationship Structures
EDMS	Electronic data management system
EDTA	Ethylenediaminetetraacetic acid
EMA	European Medicines Agency
EOS	End of study
EtOH-CPP	Ethanol-induced conditioned place preference
FDA	Food and Drug Administration
g	Gram

Abbreviation	Definition
GABA	Gamma aminobutyric acid
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability Accountability Act
hr	Hour
ICH	International Conference on Harmonization
IDS-30	Inventory of Drinking Situations – 30 item
IM	Intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
IWRS	Interactive web response system
kg	Kilogram
L	Liter
MAOI	Monoamine oxidase inhibitors
MDMA	3,4-methylenedioxy-methamphetamine
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter
µg	Microgram
µL	Microliter
min	Minutes
MINI	MINI Neuropsychiatric Interview
mL	Milliliter
mm	Millimeter
mmol	Millimole
mmos	Milliosmols
NIAAA	National Institutes on Alcohol Abuse and Alcoholism
NIH	National Institutes of Health
OTC	Over-the-counter
oz	Ounce
pg	Picograms
PI	Principal Investigator
PK	Pharmacokinetic
POMS	Profile of Mood State
PROMIS	Patient-Reported Outcomes Measurement Information System
PRN	when necessary
PSNHDD	Percentage of subjects with no heavy drinking days
PT	Preferred Term
PVN	Paraventricular nuclei
QID	Three-times-per-day
SAE	Serious adverse event

Abbreviation	Definition
SAP	Statistical analysis plan
SD	Standard deviation
SDU	Standard drinking unit
SOC	System Organ Class
SON	Supraoptic nuclei
SOS	Secular organizations for sobriety
SNAQ	Simplified Nutritional Appetite Questionnaire
SNRI	Serotonin-norepinephrine reuptake inhibitors
STAI	Spielberger Trait Anxiety Inventory
SSRI	Selective serotonin reuptake inhibitors
THC	Tetrahydrocannabinol
TLFB	Timeline followback
TSST	Trier Social Stress Test
ULN	Upper limit of normal
U	Units
UPSIT	University of Pennsylvania Smell Identification Test
US	United States
WHO	World Health Organization

4. Introduction

4.1. Alcohol Use Disorder

Alcohol use disorder (AUD) is one of the most prevalent substance use disorders (SUDs) in the world. An estimated 95,000 people die of alcohol-related causes in the United States each year (Esser-2020). AUD is responsible for a myriad of medical, psychological, social, economic and personal problems (Litten-2012). Tragically, more than 2.5 million individuals including 80,000 Americans die each year from alcohol-related events. The total economic cost to society is a staggering $229 billion each year in the United States (US) (NIAAA-2019). Mintz (2021) reported that overall 5.8% of persons with AUD received treatment in the past year with breakdown by severity of 2.7% with mild AUD, 6.0% with moderate AUD, and 20.7% with severe AUD.

Pharmacologic treatment of AUD has mostly focused on altering the reinforcing effects of alcohol use. Medication development has focused on several neurotransmitter systems that interact with the corticomesolimbic dopamine pathway, which can mediate reinforcement. Many available or promising compounds appear to act by modulating the function of opioids, glutamate (with or without gamma aminobutyric acid, GABA), and serotonin (5-HT) (Heilig and Egli-2006).

During the past decade, advances have been made in medications development for AUD. Currently, there are four medications approved by the US Food and Drug Administration (FDA) to treat alcohol dependence: disulfiram, oral naltrexone, long-acting injectable (depot) naltrexone, and acamprosate. As these medications do not work for everyone or in all situations, additional research is vital to develop more efficacious and safe medications to treat AUD.

4.1.1. Oxytocin

Oxytocin is a nine amino acid peptide synthesized in the magnocellular neurons of the paraventricular (PVN), supraoptic (SON), and accessory magnocellular nuclei of the hypothalamus and is released into the bloodstream from axon terminals of these neurons that are located in the posterior pituitary.

Oxytocin is also centrally released into the brain by various mechanisms. There is dendritic release from the PVN and SON in the hypothalamus, with subsequent passive diffusion to oxytocin receptors that are located throughout the brain. Parvocellular neurons of the PVN also produce oxytocin and these neurons project centrally to diverse regions including the olfactory bulb, tubercule, medial and central amygdala, lateral septum, hippocampus, brainstem, and spinal cord (Lee and Weerts-2016). Oxytocin is also widely distributed into peripheral tissues (Gimpl and Farenholz-2001).

4.1.2. Rationale for Studying Oxytocin

The neurochemical neurocircuits in drug rewards particularly those due to alcohol have been described (Koob-2013). The medial forebrain bundle represents ascending and descending projections between the ventral forebrain (nucleus accumbens, olfactory tubercle, and septal area) and ventral midbrain (ventral tegmental area). Alcohol activates GABA receptors or

enhances GABA release in the ventral tegmental area, nucleus accumbens, and amygdala. Alcohol is also hypothesized to facilitate the release of opioid peptides in the ventral tegmental area, nucleus accumbens, and central nucleus of the amygdala. Alcohol facilitates the release of dopamine in the nucleus accumbens via an action either in the ventral tegmental area or nucleus accumbens. The intersection of the central release of oxytocin with many of these structures gives anatomic support for the idea that oxytocin might influence the neurocircuits in drug rewards particularly those related to alcohol abuse (Lee and Weerts-2016).

There have been two main threads of investigation of oxytocin on neuropsychology. One follows the initial discovery that this peptide influences forms of neuroadaptation, including learning and memory and drug addiction. The other thread is linked to the effects of oxytocin on maternal, social and other forms of affiliative behavior. These two pathways of investigation are summarized by Sarnyai and Kovács (2014).

Oxytocin has gained significant interest for the treatment of AUD and other drug use disorders (reviewed recently by Lee and Weerts-2016, Lee-2016 and Pederson-2017). This is primarily related to research of oxytocin's effects in emotional regulation, pain, and stress, and its purported ability to modulate response to rewarding behaviors promoted by food, sex, and drugs (Meyer-Lindenberg-2011; Onaka-2012). Lee and Weerts-2016 speculate that, "The comodulation of both stress and motivational processes is believed to be because of the important role of oxytocin to shift salience to social, affiliative processes, both by increasing the salience itself of rewarding stimuli and/or by reducing stress, allowing for attention to social bonding (Baskerville and Douglas-2010). This is obviously relevant to addiction, where salience of drug stimuli overshadows motivation for social affiliation and where stress may trigger drug seeking and relapse (Sinha-2008)." Lee and Weerts-2016 speculation is based on their following observations: "Heavy alcohol drinking is associated with dysregulation of HPA-axis activity, as shown by episodes of hypercortisolism between drinking bouts and a blunted corticosterone (CORT) response to stress during early abstinence (Kemper-1990; Wand and Dobs-1991; Errico-1993; Vescovi-1997; Boschloo-2011). A blunted CORT response has been associated with increased anxiety and craving during acute abstinence and subsequent relapse to heavy drinking (Higley-2011; Sinha-2011; Walter- 2006)."

4.1.3. Pharmacokinetics in the Natural State (non-drug treated)

Most of the oxytocin in the body is stored in the posterior pituitary. In humans, the pituitary oxytocin content has been estimated at 14 U (28 µg). This gland lies outside the blood-brain barrier, so peptide released from this gland readily enters the blood. There is no barrier to the passage of peptides between the blood and interstitial fluid of the body, so the distribution volume for oxytocin is much larger than the plasma volume.

Circulating concentrations (except in pregnancy) are 1–10 pg/mL, and the pharmacokinetics (PK) after intravenous injection fit a two-compartment model, with a distribution volume of 33 L, a distribution half-life of 3.2 minutes, and an elimination half-life of 20 minutes. Oxytocin is stable in plasma (except in pregnancy, when oxytocinase is abundant) and is cleared from the blood via the kidneys and liver. Oxytocin is thought to be cleared from cerebral spinal fluid (CSF) by a combination of flow into the subarachnoid space and active transport into blood.

4.1.4. Pharmacokinetics after Intranasal Administration

4.1.4.1. Non-clinical

When 12 µg (mice) or 20 µg (rat) were administered to rodents, analysis of microdialysates showed that oxytocin levels in the amygdala and hippocampus increased by approximately 100%, demonstrating evidence that nasally applied oxytocin indeed reaches behaviorally relevant brain areas, and this uptake is paralleled by changes in plasma oxytocin (Neumann-2013). Studies performed in rhesus macaques have also demonstrated that intranasally administered oxytocin resulted in increased concentrations in both CSF and plasma as compared with saline (Dal Monte-2014). When nasal spray delivered oxytocin was compared with nebulizer delivery, similar elevations of CSF oxytocin concentration were observed; however, elevations of plasma oxytocin were greater with the nasal spray administration.

Lee (2018) showed that plasma and CSF concentrations were increased after intravenous and intranasal administration in Rhesus macaques and that this increase was due to the administered oxytocin and not to increases in endogenous levels. That oxytocin was detected in the CSF, supports that intranasal oxytocin penetrates through the blood-brain barrier.

4.1.4.2. Clinical

Measurement of oxytocin plasma levels following intranasal administration of 24 U of oxytocin showed elevations in both plasma and CSF oxytocin levels. Quantitative analysis demonstrated that plasma levels peaked 15 min post administration, whereas CSF levels did not peak until 75 min after administration (Striepens-2013).

In a recent review, Leng and Sabatier (2016) have suggested that two routes may exist for the passage of oxytocin from nose to brain. The first route is internalization of peptide into olfactory or trigeminal neurons, followed by axonal transport and exocytosis. The second is passage through intercellular clefts into the subarachnoid space. If vast amounts of peptide accumulate in the subarachnoid space, the concentration difference across the blood-brain barrier might support nonspecific passage. It is further suggested that peptides may cross the blood–brain barrier in small amounts either by extracellular active transport or by transcellular diffusion.

4.1.5. Nonclinical Efficacy

4.1.5.1. Mouse Models

King (2017) reported that oxytocin in a dose dependent manner reduces ethanol self-administration in C57BL/6 mouse model of binge-like drinking. In this report, male C57BL/6J mice were assessed for reduction in binge-like drinking after administration of oxytocin (0, 0.3, 1, 3, or 10 mg/kg) given access to ethanol (20% v/v) using a model of binge-like drinking ("drinking in the dark") that included the use of lickometer circuits to evaluate the temporal pattern of intake as well as 2-bottle choice drinking in the home cage. They concluded that the effects of oxytocin on reduction of ethanol drinking were not due to a sedative effect and that oxytocin receptors had a role in mediating the effects. In a subsequent C57BL/6 mouse study, King (2019) evaluated alcohol relapse-like behavior using lever responses in operant conditioning chambers for alcohol in daily self-administration sessions. Once lever responding and alcohol intake stabilized mice were tested under extinction conditions for 14 days before

reinstatement testing. Oxytocin at doses of 0.1, 0.5, 1 mg/kg, attenuated alcohol-seeking behavior in a dose-related manner in male and female mice in response to acute challenge with a predator odor. Additionally, oxytocin administration of 1 mg/kg produced a similar decrease in alcohol relapse-like behavior triggered by the pharmacological stressor yohimbine in both sexes.

Mice develop tolerance to the hypothermic effect of ethanol by the second day of alcohol administration and mice do not become as hypothermic as on Day 1. One injection of oxytocin (1 U per animal) prior to the first injection of ethanol did not change temperature sensitivity in response to alcohol, but daily treatment with oxytocin did block the development of tolerance (Szabo-1987). In addition, Szabo (1987) showed that picrotoxin-induced seizures in alcohol dependent mice were ameliorated by oxytocin. Mice were made physically dependent on ethanol, and the severity of withdrawal symptoms was assessed by picrotoxin administration. Oxytocin-treated mice displayed milder withdrawal symptoms in response to increasing doses (0.2-2.0 U). Earlier studies confirmed that oxytocin attenuated tolerance to ethanol-induced hypothermia, and also attenuated tolerance to ethanol-induced myorelaxation and akinesia (Jodogne-1991).

In another study (Bahi-2015), ethanol-induced conditioned place preference (EtOH-CPP) in mice was established by rewarding the choice of one chamber with an injection of alcohol. The effects of oxytocin receptor pharmacological modulation, using the oxytocin analog Carbetocin, and genetic overexpression in the nucleus accumbens, using lentiviral-mediated gene transfer technology, was investigated. In the first experiment, results showed that Carbetocin administration and nucleus accumbens oxytocin receptor-overexpression reduced EtOH-CPP establishment. In the second experiment, systemic Carbetocin treatment and oxytocin receptor overexpression resulted in decreased time spent in the ethanol paired compartment following completion of a 7-day extinction protocol. Finally, the third experiment showed that Carbetocin and oxytocin receptor-overexpression suppressed primed reinstatement of EtOH-CPP. The authors concluded that pharmacological and genetic modulation of the oxytocin receptor can modulate the acquisition, extinction, and reinstatement of conditioned reinforcing effects of ethanol (*Bahi-2015*).

4.1.5.2. Rat Models

Studies were conducted in animals to better understand how oxytocin affected consumption of a sweet alcohol-containing beverage that is popular with young Australian humans (Raspberry Vodka Cruiser, 5% ethanol v/v; *McGregor and Bowen-2012*). Alcohol-preferring rats were given a choice between this beverage and a non-alcoholic sweet solution (3% sucrose) in daily sessions in a “lickometer” apparatus. A single administration of oxytocin (1 mg/kg) resulted in a long lasting decline (at least 6 weeks) in the preference for the alcoholic beverage relative to sucrose. When rats were given a choice between 0.05% saccharine and 10% ethanol in 0.05% saccharine, there was a decrease in total ethanol intake of about 40% on days when oxytocin (0.05, 0.1, 0.3, and 0.5 mg/kg was administered intraperitoneally (*MacFadyen-2016*).

Tunstall (2017) used an established rat model of alcohol dependence to investigate oxytocin’s effects on dependence-induced alcohol drinking, enhanced motivation for alcohol, and altered GABAergic transmission in the central nucleus of the amygdala (CeA). In this model, when oxytocin was administered intraperitoneally, intranasally, or into the brain, it blocked enhanced alcohol drinking at doses that did not alter non–alcohol-related behaviors or alcohol drinking in

nondependent rats. These data suggest that the effect was specific to alcohol drinking in alcohol dependence. They also examined *ex vivo* electrophysiological recordings from CeA neurons that indicated that oxytocin decreases evoked GABA transmission in nondependent but not in dependent rats, but oxytocin decreased the amplitude of spontaneous GABAergic responses in both groups. They concluded that "Oxytocin blocked the facilitatory effects of acute alcohol on GABA release in the CeA of dependent but not nondependent rats. Together, these results provide converging evidence that oxytocin specifically and selectively blocks the enhanced motivation for alcohol drinking that develops in alcohol dependence likely via a central mechanism that may result from altered oxytocin effects on CeA GABA transmission in alcohol dependence."

4.1.5.3. Prairie Vole Models

Prairie voles are an interesting species to study the effects of medications to treat AUD, as this species both consumes high amounts of alcohol and forms oxytocin dependent social bonds in a manner similar to humans. Oxytocin treatment (1.0, 3.0, and 10.0 mg/kg, i.p.) reduced alcohol consumption in male and female prairie voles in animals that had access to 15% ethanol vs water every other day for 12 alcohol drinking sessions (Stevenson-2017). In an open field locomotor test, oxytocin (1.0, 3.0, and 10.0 mg/kg, i.p.) did not affect overall locomotor activity.

4.1.5.4. Conclusions from Animal Models

Overall, in animal models there is evidence that oxytocin, administered systemically or centrally, reduces self-administration of alcohol and reinstatement of responding induced by exposure to drug cues and stress. The mechanisms seem to involve the midbrain dopaminergic as well as medial prefrontal glutamatergic pathways (Lee and Weerts-2016, Pederson-2017, Tunstall-2017).

4.1.6. Clinical Efficacy in Alcohol Use Disorder

Ryabinin and Fulenwider (2021) have recently reviewed the extensive literature on rodent and human studies evaluating the effects of oxytocin on alcohol's effects and alcohol-related behaviors. They present studies reporting both positive and no effects. They concluded that most studies support that oxytocin reduces alcohol consumption in both males and females, but that more studies are needed to examine oxytocin's effects on alcohol-related tolerance, withdrawal, craving, anxiety and social affiliations in subjects of both sexes.

The following summarizes studies relevant to the design of this trial with respect to dose selection and regimen, safety and efficacy on intranasal oxytocin.

Pederson's group completed 3 small randomized-controlled trials of intranasal oxytocin in patients with AUD (Pederson-2013, Pederson-2017) suggesting that intranasal oxytocin may attenuate alcohol withdrawal symptoms, reduce very heavy drinking, reduce anxiety scores, and can be safety administered at doses of 80 IU/day for 12 weeks.

Pederson (2013) reported the results of a small pilot study of very heavy drinking subjects with alcohol dependence. This study was a randomized, placebo-controlled, double-blind pilot study of 3 days of BID intranasal oxytocin (24 IU /dose) versus placebo in 11 alcohol-dependent inpatient subjects undergoing inpatient detoxification with symptom-triggered lorazepam for withdrawal symptoms. Outcome measures included the amount of lorazepam received, Alcohol

Craving Visual Analog Scale, Alcohol Withdrawal Symptom Checklist (AWSC), Clinical Institute Withdrawal Assessment for Alcohol scale (CIWA), Penn Alcohol Craving Scale (PACS), and Profile of Mood States (POMS). For the 7 oxytocin treated patients versus the 4 placebo patients, lorazepam use was less (3.4 mg ± 4.7 mg (SD) vs 16.5mg ±4.4; P=0.0015), CIWA scores were less on Days 1 and 2, AWCS scores were less on Day 1, and POMS tension/anxiety subscale was significantly lower in the oxytocin group (P=0.007). The authors concluded that the results are the first evidence that oxytocin may block alcohol withdrawal symptoms in humans. In another study of the effects of intranasal oxytocin on the need for oxazepam for alcohol detoxification, a 3-day course of a lower dose 24 IU BID, did not show statistically significant differences in oxazepam use, although there was a trend for a lower amount (Melby-2019). There were no SAEs or oxytocin-related AEs in participants with alcohol dependence.

The Pederson (2013) study was replicated in a substantially different cohort of patients with low socioeconomic status heavy drinking (Pederson-2017). Intranasal test treatments (24 IU oxytocin or placebo three times daily – total daily dose 72 IU) were initiated within the first 36h of their inpatient stay in individuals who had not received more than a total of 6mg of lorazepam to control withdrawal symptoms since admission. CIWA ratings were obtained every 4h in patients admitted for alcohol detoxification. During the 48h after initiation of randomly assigned intranasal test treatments, mean CIWA scores declined in oxytocin recipients (N=4) but increased in placebo recipients (N=4) (P=0.025). The sum of lorazepam doses (each 2 mg) given for elevated CIWA scores during the 48-h period trended toward being lower in oxytocin recipients (P=0.076).

In a 12-week randomized-controlled trial comparing twice daily intranasal oxytocin to intranasal placebo (40 IU of oxytocin/dose or placebo three times daily for the first 2 days and then twice daily for the remainder of the 12-week treatment period), Pederson (2017) evaluated treatment effects in patients with AUD who were very heavy drinkers (≥35 standard drinks/week for men, ≥28 standard drinks/week for women). Twelve patients received intranasal oxytocin and 10 patients received placebo of which 13 were males and 9 were females. Statistical analyses revealed that the oxytocin compared to the placebo treatment group had significantly fewer heavy drinking days (P=0.047; about 15 fewer heavy drinking days during the 12-week trial, d=0.97) and drank very significantly fewer drinks/drinking day (P=0.0008; about 2.6 fewer drinks/drinking day during the trial, d=1.83). No significant oxytocin effect on abstinent days was noted. Spielberger state anxiety self-ratings trended weakly toward being lower in the oxytocin compared to the placebo group (P=0.098), corresponding to an on-average 3.5-point difference. There were no group differences in alcohol craving scores. No severe adverse events occurred. Also, vital signs remained stable and laboratory measures remained within normal limits in all subjects except for transient, asymptomatic, and mild hyponatremia in three oxytocin subjects.

In a human laboratory double-blind, crossover study of 32 non-treatment seeking alcohol abusing (defined as per Diagnostic and Statistical Manual of Mental Disorders, 4th Edition criteria), the effects of single dose intranasal oxytocin (40 IU) were evaluated on measures social perceptual ability, cue-induced craving, and approach bias for alcohol and appetitive imagery (Mitchell-2016). Oxytocin significantly improved recognition of easier items on a social perception task. Although there was no main effect of oxytocin on cue-induced craving for alcohol, a subsequent analysis revealed that oxytocin's effect on craving was moderated by

attachment anxiety, such that oxytocin reduced craving in more anxiously attached individuals and increased craving in less anxiously attached individuals. Subjects did not display an approach bias to alcohol images on the placebo day, preventing meaningful analysis of the effects of oxytocin on this measure. However, subjects did display an approach bias to appetitive images on the placebo day, which was significantly reduced by oxytocin administration. No adverse reactions were reported.

4.1.7. Clinical Efficacy and/or Safety of Intranasal Oxytocin in Other Substance Abuse and Mental Health Disorders

In a double-blind placebo-controlled study of 16 cannabis-dependent subjects, pretreatment with oxytocin (40 IU) reduced marijuana craving and stress scores after a human laboratory session but did not alter anxiety scores (McRae-Clark-2013).

In a double-blind, placebo-controlled study of 31-cocaine dependent subjects, Flanagan (2015) examined the effect of oxytocin on neuroendocrine reactivity to a laboratory social stress paradigm. Participants were randomized to receive a single dose of oxytocin (40 IU) or placebo before the Trier Social Stress Test (TSST). Administration of intranasal oxytocin induced a significant decrease in both cortisol and DHEA levels (Cortisol: Δ= -0.29±0.13, p=0.035 and DHEA: Δ=−0.40±0.13, p=0.004) prior to administration of the TSST. These changes were not significant for subjects who received placebo (Cortisol: Δ = -0.12±0.13, p=0.348 and DHEA: Δ= -0.11±0.12, p=0.368). There was no effect of the administration of intranasal oxytocin on cortisol or DHEA response levels immediately following the TSST (Cortisol: Δ=0.20±0.15, p=0.166 and DHEA: Δ=0.10±0.13, p=0.419).

Feifel (2010) conducted a randomized, double blind cross over study in 19 schizophrenia patients with residual symptoms despite being on a stable dose of at least one antipsychotic drug. Intranasal oxytocin was dosed at 20 IU (5 sprays) twice a day for the first week and 40 U (10 sprays) twice a day for the following 2 weeks. Analysis of the 15 subjects who completed all the study visits revealed that oxytocin significantly reduced scores on the Positive and Negative Symptom Scale and Clinical Global Impression-Improvement Scale ($p < .001$) compared with placebo at the 3-week end point. Oxytocin was well tolerated and produced no adverse effects based upon patient reports or laboratory analyses.

A double-blind, placebo-controlled study of 12 obsessive-compulsive patients examined the effect of 18 IU of oxytocin intranasally daily for 6 weeks on obsessive compulsive behaviors (Den Boer-1992). No reductions in the number of compulsive behaviors were observed in either treatment group. Two additional patients were treated with 54 IU of oxytocin intranasally of which only one patient had a slight reduction in obsessive-compulsive behavior. Side effects were mild and transient. In the placebo group, 1 patient complained of headache. In the oxytocin group, 4 patients had mild dizziness and 2 patients reported dry mouth and mild headache.

Epperson (1996) also conducted a placebo-controlled of intranasal oxytocin in 7 patients with obsessive compulsive disorder administered higher doses of oxytocin (320 IU oxytocin intranasally per day for 7 days). No cardiac or serum osmolarity AEs were reported; and as reported by Den Boer (1992), there was no clinical benefit.

In a double-blind, placebo-controlled multicenter study, 59 women with chronic constipation were administered placebo (30 subjects) or intranasal oxytocin (29 subjects) twice daily (five

nasal inhalations in each nostril) for a total daily dose of 80 IU per day for 13 weeks (Ohlsson-2005). There was no significant advantage on constipation. In addition, no effects on blood pressure or electrolyte concentrations were seen in either the treated or placebo group. Eleven subjects in the oxytocin group (48%) and 12 subjects in the control group (47%) reported side effects including headache (11), nausea (6), abdominal pain (6), weight gain (4) and local irritation in the nasal mucosa (8). The distribution of side effects was equal in the two groups.

4.1.8. Safety of Single Dose and Multiple Dose Intranasal Oxytocin

Macdonald (2011) published a systematic review of 38 randomized controlled trials conducted between 1990 and 2010 that investigated the central effects of intranasal oxytocin. In the 1529 subjects included in this review, 79% of subjects were male and 8% were participants with developmental or mental health difficulties. Single dosages ranged from 18 U to 40 U. Most were single doses except for the reports of Epperson-1996 (320 U oxytocin intranasally per day for 7 days) and Ohlsson-2005 (80 U per day in divided doses of 40 U twice daily for 13 weeks). The data from this review are summarized in Table 1. Side effects were not different between oxytocin and placebo. Participants were unable to accurately report on whether they had received oxytocin or placebo.

Table 1: Summary of Reported Adverse Events

Symptom	Total Number of Times Reported		
	Total	Placebo	Oxytocin
Light headedness/vertigo	21	10	11
Drowsiness/sleepy	38	22	16
Dry throat/mouth	12	6	6
Nasal irritation	14	8	6
Runny nose	13	6	7
Abdominal/stomach pain	8	3	5
Anxious/worried/uncomfortable	18	11	7
Euphoric/energized/uplifted	14	5	9
Calm/relaxed/comfortable	59	27	32
Headache	29	14	15

Three SAEs related to hyponatremia were cited among 3 patients receiving oxytocin. One was in an obsessive-compulsive patient who was treated with ~12 U oxytocin intranasally daily for 4 weeks and who had a marked decrease in plasma sodium (126 mmol/L) and osmolarity (252 mosm/kg which was down from 290 mosm/kg prior to therapy), in addition to an SAE involving psychotic symptoms (Ansseau-1987). Two reports involved long-term use of intranasal oxytocin plus excessive water intake to facilitate lactation (Ansseau-1987). Hyponatremia in response to oxytocin may be due to the chemical similarity between oxytocin and the other pituitary hormone, argentine vasopressin (anti-diuretic hormone).

4.1.9. Safety of Multiple-Dose Intranasal Oxytocin

Studies of 1-4 Weeks Duration: Four studies provided safety information for oxytocin twice daily (BID) dose regimens of 1-4 weeks duration, with dose regimens ranging from 20 IU (*Feifel-2010*) to 72 IU (*Finger-2015*). In the 3 studies which listed specific AEs (*Feifel-2010*, *Finger-2015*, *Parker-2017*), those AEs that were reported in more than 1 patient included: inappropriate sexual behavior/ hypersexuality; sleep impairment; headache; dyspepsia /nausea; dizziness/lightheadedness; nasal irritation; and nasal congestion. There appeared to be no dose relationship with respect to any of these AEs. Nasal AEs (nasal irritation or nasal congestion) were the only AEs that were reported in more than 1 study (*Feifel-2010*, *Parker-2017*), and nasal discomfort from the intranasal spray was also a reason for 1 subject withdrawal (*Feifel-2010*). In one study in patients with dementia (*Finger-2015*), inappropriate sexual behaviour/ hypersexuality was reported in 5 (31%) of the 15 patients receiving oxytocin, compared with 1 (14%) of the 7 placebo patients ($p = 0.4$). However, there was no clear dose-response relationship, as this behaviour was seen only at the 2 lower doses of oxytocin (24 or 48 IU BID) and not at the highest dose (72 IU BID). Information on vital signs effects was available from 3 studies, with 2 of the 3 studies showing no oxytocin effect on vital signs (*Finger-2015*, *Pedersen-2011*), and the third study (*Parker-2017*) suggesting that systolic standing blood pressure increased in subjects treated with oxytocin 24 IU BID for 4 weeks. Oxytocin 24 IU - 40 IU BID for 2 weeks was not associated with changes in blood chemistry or urine parameters in two studies (*Pedersen-2011*, *Feifel-2010*).

Studies of 5-8 Weeks Duration: Eight studies provided safety information for oxytocin BID or three-times-per-day (QID) dose regimens of 5-8 weeks duration. Dosing regimens varied widely in these studies, with 3 of the studies investigating an oxytocin regimen of 24 IU BID (*Anagnostou-2012, Watanabe-2015, Cacciotti-Saija-2014*) and 2 studies employing doses up to 40 IU BID (*Einfeld-2014, Modabbernia-2013*). The highest daily dose of oxytocin (24 IU QID) and the highest cumulative exposure (96 IU/day x 56 days for a total of 5376 IU) was an 8-week study of oxytocin use against binge eating disorder (*Agabio-2016*). Only one of the 8 studies reported SAEs associated with oxytocin treatment. That was the study by *Yatawara (2016)*, where 2 (13.3%) of 15 autistic patients treated with oxytocin 12 IU BID developed hyperactivity and aggression. Although many different types of AEs were reported in these studies, it was difficult to appreciate any major trends. AEs that occurred in more than 1 subject in more than 1 study included constipation, thirst, increased urination, nausea, palpitations, and dizziness. Among these, the most frequently reported was constipation, which occurred in more than 1 subject in 3 different studies *(Yatawara-2016, Cacciotti-Saija-2014, Modabbernia-2013)*.

Studies of 12-13 Weeks Duration: Three studies provided safety information for oxytocin BID dose regimens of 12-13 weeks duration. In 2 of the 3 studies, oxytocin was administered at a dose of 40 IU BID (*Pederson-2017, Ohlsson-2005*), while in the third study, oxytocin daily dosing was titrated to as high as 72 IU BID (*Feifel-2013*). No SAEs were reported in any of these studies. Furthermore, the study by *Pederson (2017)* reported no AEs of any type in the oxytocin treatment group. In the 2 studies that did report AEs (*Feifel-2013*, *Ohlsson-2005*), the 3 AEs that occurred in more than 1 subject in both studies were headache, nausea, and weight gain. Two studies (*Feifel-2013*, *Ohlsson-2005*) found that oxytocin had no adverse effects on blood chemistry/electrolytes, while a third study found that oxytocin did not affect urine (*Feifel-2013*). Only one study (*Ohlsson-2005*) addressed changes in vital signs, concluding that oxytocin did not affect blood pressure.

Most Frequently Reported AEs: For all of the multiple-dose studies described above, AEs that were reported in more than 1 subject in more than 1 study of 1-13 weeks duration included: nausea (5 studies), headache (5 studies), dizziness (3 studies), constipation (3 studies), nasal irritation (3 studies), thirst (2 studies), increased urination (2 studies), palpitations (2 studies), increased appetite (2 studies), weight gain (2 studies), dry mouth/throat (2 studies), and somnolence/drowsiness (2 studies). Table 2 presents these AEs grouped by increasing cumulative dose of oxytocin. In the majority of cases, there was no clear evidence of dose-dependent relationship. The one possible exception was the AE of "palpitations", which was reported in 11.1% of subjects at a cumulative oxytocin dose of 2016 IU in one study and in 25% of subjects at a higher 5376 IU cumulative dose in a second study. However, because data for this AE is available from only 2 studies, the ability to identify a true trend is very limited.

Table 2: Adverse Events Reported in More than One Subject in More than One Oxytocin Study of 1-13 Weeks Duration: Percentages of Adverse Events Reported at Selected Cumulative Doses of Intranasal Oxytocin

Adverse Events	No. AEs/ No. Subjects (Percentage) at Cumulative Dose of Oxytocin							
	739 IU	768 IU	1400 IU	2016 IU	4200 IU	5376 IU	7280 IU	Cumulative Dose Not Calculable
Nausea			4/15 (26.7%)	3/27 (11.1%)	4/20 (20%)		3/23 (13.0%)	2/13 (15.4%)
Headache			4/15 (26.7%)	7/27 (25.9%)	4/20 (20%)		6/23 (26.1%)	2/13 (15.4%)
Dizziness	4/6 (66.7%)		4/15 (26.7%)		3/20 (15%)			
Constipation		10/15 (66.7%)		2/27 (7.4%)	2/20 (10%)			
Nasal irritation			4/15 (26.7%)	2/10 (10%)			4/23 (17.4%)	
Thirst		10/15 (66.7%)		8/27 (29.6%)				
Increased urination		10/15 (66.7%)		5/27 (18.5%) daytime 4/27 (14.8%) night time				
Palpitations				3/27 (11.1%)		2/8 (25.0%)		
Increased appetite					5/25 (25.0%)			3/13 (23.1%)
Weight gain							2/23 (8.7%)	2/13 (15.4%)
Dry mouth/throat					2/20 (10.0%)			2/13 (15.4%)
Somnolence/ drowsiness					6/20 (30.0%)			2/13 (15.4%)

Given that the cumulative dose of oxytocin administered in the current protocol is 5320 IU over 12 weeks, AEs reported at cumulative doses of 4200 IU – 7280 IU in Table 2 are especially relevant. These include nausea, headache, dizziness, constipation, nasal irritation, palpitations, increased appetite, weight gain, dry mouth/throat, and somnolence/drowsiness. Based on the AEs percentages shown in Table 2 at increasing cumulative doses of oxytocin, there was no suggestion of a dose-response for any of these AEs. In addition, 4 of the AEs (drowsiness, dry throat/mouth, nasal irritation, and headache) were also seen in the systematic review of single-dose studies (Table 1) but occurred at comparable or lower frequencies than in Placebo subjects.

In conclusion, oxytocin appears safe at a variety of doses and in a range of clinical populations as reported in the literature. However, because oxytocin can modify heart rate and the anti-diuretic

effect might lead to seizures, *Macdonald (2011)* recommended screening potential recipients of intranasal oxytocin for allergies, significant cardiovascular disease, and neurological disorders.

4.1.10. Rationale for Selection of Doses for this Study

As presented above, intranasal oxytocin has been studied in small clinical trials of patients with mental health disorders at doses ranging from 18 IU to 320 IU given as single doses or in divided doses given BID or QID for as long as 13 weeks. The 3 small randomized controlled trials examining the effects of intranasal oxytocin in patients with AUD reported by Pederson (*Pederson-2013, Pederson-2017*) suggested that intranasal oxytocin may attenuate alcohol withdrawal symptoms, reduce very heavy drinking, reduce anxiety scores, and can be safely administered at doses of 80 IU/day for 12 weeks. This Phase 2 trial proposes to study a dose of 70 IU/day in a larger group of patients, with a run-in period of 2-weeks of once-daily intranasal oxytocin at 35 IU, followed by 10-weeks of twice daily oxytocin at 35 IU for a total daily dose of 70 IU that is similar to the dose shown to be safe and effective in reducing drinking, craving, anxiety, and withdrawal by *Pederson (2017)*.

4.2. Study Design

This study is a double-blind, randomized, placebo-controlled, parallel group, multi-site clinical trial designed to assess the efficacy of oxytocin compared with placebo to reduce drinking in 100 subjects (50 in each group) who report 4 or more Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5™) symptoms of AUD. This study will be conducted at 4 clinical sites. If eligible for the study, subjects will be randomized using a stratified permuted block randomization procedure with "clinical site" as a stratification variable in an approximate 1:1 ratio (targeting 50 subjects per group) to receive either intranasal oxytocin or placebo for 12 weeks.

If eligible for the study, subjects will receive blinded intranasal oxytocin at 35 IU once-per-day or intranasal placebo (same volume once-per-day) for 2 weeks (initial tolerability assessment period), then for the next 10 weeks (maintenance period) subjects will receive oxytocin at 35 IU/ BID, (total daily dose 70 IU) or intranasal placebo (same volume BID).

Subjects will be seen in the clinic at screening visit(s), at randomization (Week 1), and at Weeks 2, 3, 4, 6, 8, 10 and 13. During the alternate weeks when the subjects will not be coming into the clinic they will be contacted once during that week by telephone to encourage study drug compliance and to assess withdrawal, adverse events (AEs), and concomitant medications. A final follow-up telephone interview will occur during Week 14/15 (1-to-2 weeks after the end of dosing).

The overall study schema is provided in Figure 1. The study schedule of visits and assessments is shown in Table 3.

Enrollment will be halted if 2 or more at least possibly related SAEs as judged by the investigator or medical monitor occur. If this occurs, a DSMB will be convened and a decision in conjunction with the study Sponsor will be made regarding stopping the study.

Figure 1: Overview of Study Design

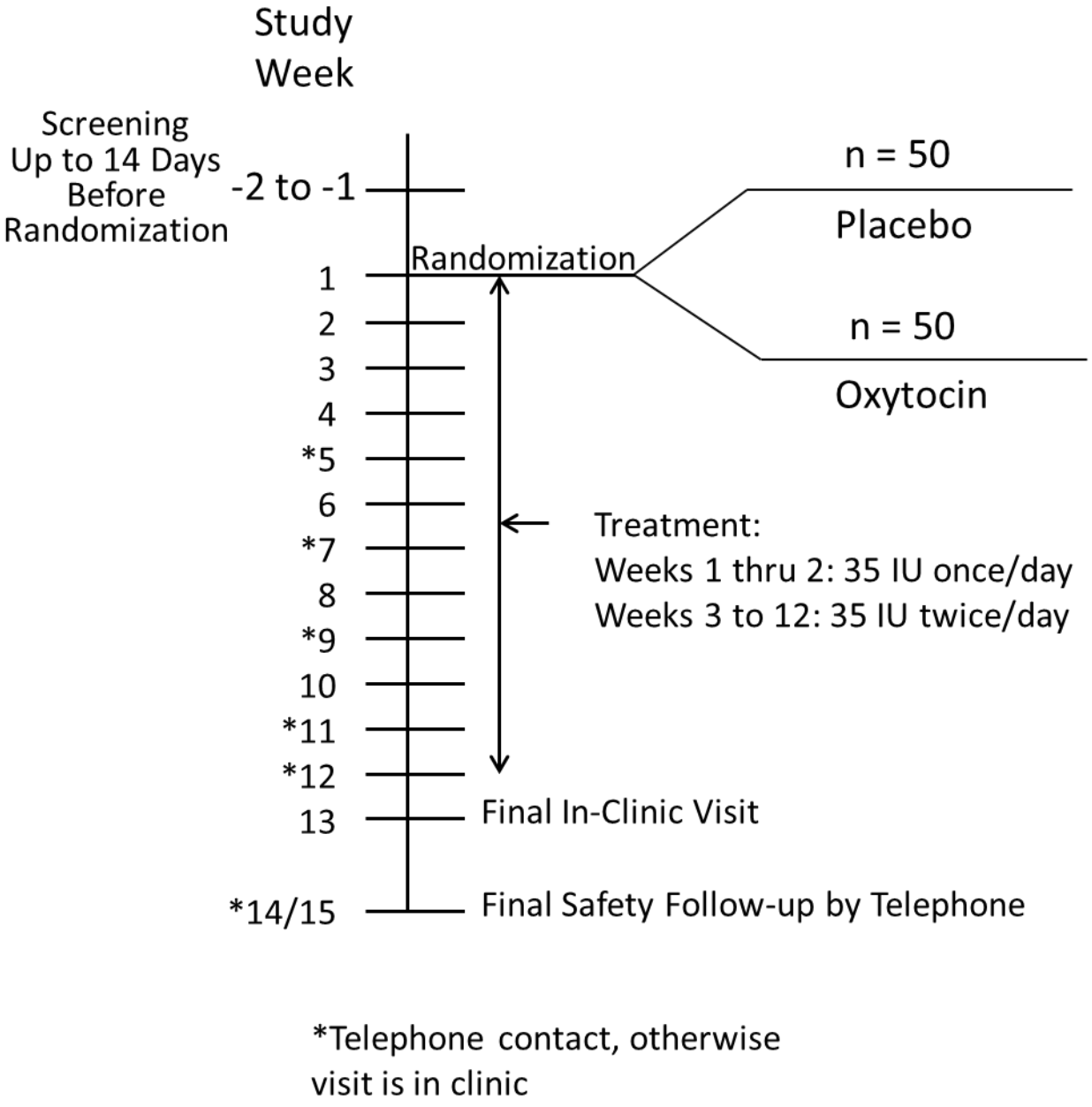

Table 3. Schedule of Assessments

	Screen			Maintenance										EOS[a]	Safety Follow-up
Study Week	**-2 to -1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14/15**
Clinic Visit #	**1**	**2**	**3**	**4**	**5**		**6**		**7**		**8**			**9**	
Informed Consent	X														
Alcohol Breathalyzer	X	X	X	X	X		X		X		X			X	
Urine Drug Screen[b]	X	X	X	X	X		X		X		X			X	
Locator Form	X														
Demographics	X														
Medical/Surgical History	X	X[c]													
Physical Exam	X[d]	X	X	X	X		X		X		X			X	
MINI V 7.0.2 (AUD module at EOS)	X													X	
C-SSRS		X	X				X		X		X			X	
Clinical Chemistry[e]	X						X		X		X			X	
Vital Signs	X	X	X	X	X		X		X		X			X	
ECG (12-lead)	X													X	
Prior and Concomitant Meds	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CIWA-AR	X	X	X	X	X		X		X		X			X	
Eligibility Checklist	X	X[c]													
Drug Compliance – Diary review		X	X	X	X		X		X		X			X	
Drug Accountability (vial weight)		X	X	X	X		X		X		X			X	
Pregnancy Test/Female Birth Control Methods	X	X	X[f]				X		X		X			X	
Weight	X						X		X		X			X	
Drinking Goal	X														
AEs/SAEs			X	X	X	X	X	X	X	X	X	X	X	X	X
Other Services Used for Alcohol Use Problems[g]		X												X	
RANDOMIZATION		X													
Brief Telephone Interview[h]						X		X		X		X	X		
Take Control		X	X	X	X		X		X		X				
Exit Interview														X	
Treatment Referral														X	
Follow-Up Telephone Interview															X
Final Subject Disposition															X

	Screen			Maintenance										EOS[a]	Safety Follow-up
Study Week	**-2 to -1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14/15**
Clinic Visit #	**1**	**2**	**3**	**4**	**5**		**6**		**7**		**8**			**9**	
Subject Reported Outcomes															
Hyperkatefia Scale		**X**												**X**	
Barrett Impulsivity Scale		**X**													
Spielberger Trait Anxiety Inventory		**X**													
BPAQ - SF[i]		**X**					**X**		**X**		**X**			**X**	
Simplified Nutritional Appetite Questionnaire		**X**					**X**		**X**		**X**			**X**	
Cigarette and other nicotine use		**X**					**X**		**X**		**X**			**X**	
ECR-RS[j]		**X**					**X**		**X**		**X**			**X**	
IDS-30[k]		**X**													
POMS		**X**					**X**		**X**		**X**			**X**	
PROMIS – alcohol negative consequences		**X**					**X**		**X**		**X**			**X**	
PROMIS – sleep disturbances		**X**					**X**		**X**		**X**			**X**	
PROMIS – pain interference		**X**					**X**		**X**		**X**			**X**	
UPSIT[l]	**X**			**X**			**X**		**X**		**X**			**X**	
Urge to drink questionnaire		**X**					**X**		**X**		**X**			**X**	
Timeline followback (TLFB)	**X**	**X**	**X**	**X**	**X**		**X**		**X**		**X**			**X**	
Brief Drinking Questionnaire[m]														**X**	

[a] EOS=end of study. These assessments are to be done at Week 13 or if the subject discontinues early and agrees to a final clinic visit.
[b] Test for opioids, cocaine, amphetamines, methamphetamine, THC, buprenorphine, methadone, benzodiazepines, oxycodone, 3,4-methylenedioxy-methamphetamine (MDMA), and barbiturates.
[c] Updated prior to randomization.
[d] Complete physical exam at screening including examination of the nares, plus examination of the nares at all other visits as indicated.
[e] AST, ALT, total bilirubin, creatinine, sodium, and potassium.
[f] Only birth control methods are collected at this visit.
[g] At baseline asks about lifetime treatment use and at EOS asks “since beginning this study
[h] AEs, concomitant medications, and drug compliance reminder.
[i] Buss Perry Aggression Questionnaire – Short Form
[j] Experiences in Close Relationships—Relationship Structures Questionnaire (ECR-RS) (attachment related anxiety)
[k] Inventory of Drinking Situations
[l] University of Pennsylvania Smell Identification Test
[m] Only asked to subjects who request to withdraw from the study and are not willing to provide TLFB drinking data. This questionnaire will be asked at whatever constitutes the EOS visit.

4.3. Benefit/Risk Assessment

Intranasal oxytocin is remarkably well tolerated and 80 IU per day has been administered for up to 13 weeks in two studies (*Ohlsson-2005, Pederson-2017*). Infrequent hyponatremia, probably secondary to an anti-diuretic effect, and infrequent nasal irritation have resulted in cessation of treatment. To minimize this risk, patients with significant gastrointestinal, cardiac, neurological, and renal disorders as well as pregnant or lactating women will be excluded. Sodium levels in addition to other liver enzyme and creatinine levels will be monitored over the course of treatment. A physical examination of the nares and throat will also be performed for signs of irritation; and sense of smell will be regularly evaluated. Dose reductions are permitted in the event of intolerance. The benefit of participating in this study is that all subjects will be asked to view the "Take Control" behavioral platform. The intervention is derived from a self-help approach developed by NIAAA that provides evidence-based, field tested information for individuals with alcohol problems, and suggestions for making changes in their drinking. In addition, it is possible that subject randomized to receive Oxytocin nasal spray may reduce their alcohol drinking which can have positive overall health benefits.

5. Study Objectives

5.1. Primary Objective

The primary objective of the study is to compare the efficacy of oxytocin in reducing the weekly percentage of heavy drinking days over 10 weeks of maintenance treatment among subjects with moderate to severe AUD. A "heavy drinking day" is 4 or more drinks per drinking day for women and 5 or more drinks per drinking day for men.

5.2. Secondary Objective

Secondary objectives include assessment of other measures of the effects of oxytocin compared with placebo on reduction of alcohol use as well as effects on alcohol craving, alcohol-related consequences, cigarette smoking and other nicotine use, psychological assessments, retention in the study, and safety and tolerability throughout the study.

6. Study Interventions

6.1. Investigational Products: Oxytocin and Placebo

Investigational products will be manufactured under contract with University of Iowa and will be packaged and distributed in drug kits by Catalent, Inc. Oxytocin and Placebo liquid formulations will be packaged in 20 mL vials with a fill volume of 18 mL with a Nemera P270 pump spray actuator device crimped to the vial. Oxytocin is supplied at a concentration of 70 IU/mL. The pump spray actuator delivers 100 µL in a single spray (equivalent to 7 IU of oxytocin).

Oxytocin and placebo will be administered once daily (morning) for 2 weeks (Weeks 1 and 2) and then twice daily (morning and evening) for 10 weeks (Weeks 3 through 12). One dose is defined as the intranasal administration of 100 µL per each nostril for a total of 5 insufflations alternating between nostrils with a 30 second wait between insufflations for a total dose of 500 µL equivalent to 35 IU. Thus, subjects will receive oxytocin 35 IU per day for 2 weeks, then 35 IU twice per day for 10 weeks for a total daily dose of oxytocin 70 IU.

6.2. Investigational Product Packaging, Labeling, and Distribution of Drug Kits

Investigational products will be provided in drug kits containing 6 bottles of either oxytocin or placebo in each kit with each bottle containing 18 mL of oxytocin or placebo. Kits will be shipped periodically to sites during the study depending on enrollment.

The study number and the words “oxytocin or placebo study” will be preprinted on each vial label. The label will also have a unique kit number, the volume contained in the bottle, storage conditions, and the words “Caution: New Drug – Limited by Federal (or United States) Law to Investigational Use” and “Keep Out of Reach of Children**”**. A separate label will be supplied to affix to the vial each time a bottle is dispensed. This label will contain the clinical site name, clinical site phone number and 24/7 emergency phone number, and places to record the subject number and the date dispensed.

6.3. Investigational Product Storage

Kits should be stored under refrigeration (within the range of 36°F to 46°F; 2°C to 8°C) in a secured area at the clinical site. After dispensing subjects will be instructed to store the vials under refrigerated conditions; however, stability at room temperature (68°F to 77°F; 20°C to 25°C) has been demonstrated for one month, so excursions to room temperature for 30 days or less should not impact the product.

6.4. Investigational Product Dispensing

Prior to dispensing the study drug vials, each vial will be weighed on an analytical balance that measures to the 0.01g or lower level and the weight will be recorded. The person dispensing the drug will prime the pump by depressing the actuator 5 times and will weigh the vial again. This will be done each time a new vial is dispensed. Repriming of the pump between uses is not necessary.

The subject will be instructed to return study vials at each clinic visit. Upon return of the used study drug vial, the vial will be weighed a second time to estimate the amount of drug insufflated

during the dosing period. Bottle weights will be collected at each visit when the subject returns the vial.

Dispensing will be in accordance with the plan in Table 4, unless the subject does not return within the scheduled windows around visits, then other arrangements can be made. Vials contain sufficient volume of investigational product in one vial for 16 to 17 days of dosing at 2 doses per day. As the first two weeks are one per day dosing, then vial #1 will have enough for the first two weeks, plus approximately another 7 days at twice daily dosing. Vial #1 can serve as a back-up if needed. When vials are dispensed, the subject will be provided with a card that shows which numbered vial to use over the date range covered by the amount in the vial.

Table 4: Investigational Product Dispensing Plan

Study Week[a]	Vial # Dispensed	Vial #s Returned
1	1	NA
2	1	1 (weighed and redispensed)
3	2	1 (weighed)
4	2 + 3	2 (weighed and redispensed)
6	4	2 + 3 (weighed)
8	5	4 (weighed)
10	6	5 (weighed)
13	NA	6 (weighed)

[a] Only weeks where clinic visits are scheduled are shown.

6.5. Investigational Product Accountability and Compliance

The site principal investigator (PI) or designated study personnel will maintain a log of the receipt of all investigational products and record of dispensing of all investigational products to the subject. Investigational product for each subject will be inventoried and accounted for throughout the trial. The site PI or his/her staff will count the drug kits/individual vials remaining at the end of the study and record the vial count on the appropriate drug accountability form. Subjects will be asked to return any used and/or unused vials of investigation product at each clinic visit for accountability. Subjects will be provided with a Diary Card (Appendix A) to complete each time they self-administer a dose and will be asked to bring the card back at each clinic visit for site staff to review and confirm dosing compliance.

6.6. Used/Unused Investigational Product Supplies

Unused investigational products will be retained at the clinical sites until the end of the study, at which time they will be shipped back to Catalent for destruction.

6.7. Investigational Product Administration

To control dosing and absorption of investigational product, subjects will be instructed to administer the nasal spray with their head in an upright position closing one nostril with one finger while administering the spray in the other nostril and sniffing during administration. At the first administration on Study Day 1, subjects will be provided with instruction on how to self-

administer their first dose, will be observed while administering the first dose, and will be given an instruction sheet to take home. At subsequent visits, subjects will be asked to return the nasal spray devices.

Missed Doses: Subjects will be instructed if the dose is not taken at the recommended time, to skip this dose, and the next dose should be taken at the time of next scheduled dose; doses should never be doubled up.

Dose Reductions and Discontinuation. If the subject is not tolerating the investigational product, the dose can be reduced by 14 IU or 200 μL increments per dose (2 less sprays) to determine if the subject can tolerate a lower dose. For example, one spray in each nostril can be omitted for each reduction. Then the subject should try to continue to give one dose in the morning and one dose in the evening if during the maintenance period. If the dose is reduced during the initial 2-week tolerability period, then subjects will not escalate to the maintenance dose and will stay at the tolerated dose. If the subject develops a medical condition requiring discontinuation of the investigational product, he/she can simply discontinue immediately. In the event that a female subject becomes pregnant, she will be instructed to discontinue taking the investigational product immediately.

6.8. Take Control Behavioral Platform

The behavioral platform "Take Control" consists of a series of 7 computerized modules. Subjects will view each module of "Take Control" at clinic visit starting at Week 1 after randomization. Thereafter, it will be as shown in Table 3. If a visit is missed, missed modules will be reviewed at the next visit. The paper versions of the modules are not to be given to the subject to take home and must remain at the clinical site. The intervention is derived from a self-help approach developed by NIAAA that provides evidence-based, field tested information for individuals with alcohol problems, and suggestions for making changes in their drinking. The NIAAA material is publicly available in a NIAAA booklet entitled "Rethinking Drinking" and on a NIAAA website http://rethinkingdrinking.niaaa.nih.gov. Delivering these materials in a computerized method in this trial has the advantage of standardizing the amount of educational material received by the subject.

6.9. Concomitant Medications

For study inclusion, subjects cannot be taking oxytocin for any reason and must agree to not take non-study oxytocin during the study. In addition, subjects cannot have taken any anti-convulsants, hypnotics, barbiturates, antipsychotics, psychomotor stimulants (such as methylphenidate), or benzodiazepines within 5-half-lives prior to the date of randomization. In addition, if a subject is taking a medication for depression or anxiety, he or she must have been taking a stable dose in the 2-months prior to randomization and plan to continue during the study. This includes drugs such as the following:

- selective serotonin reuptake inhibitors (SSRIs)
- dual uptake inhibitors
- serotonin-norepinephrine reuptake inhibitors (SNRIs)
- tricyclic antidepressants

- monoamine oxidase inhibitors (MAOIs)
- bupropion

Pharmaceutical treatments approved for treatment of alcoholism or treatments known to be used off-label or experimentally for treatment of alcoholism are prohibited during the study. The following drugs are thus prohibited:

- Oral naltrexone (Revia, Depade)
- Depot naltrexone (Vivitrol)
- Disulfiram (Antabuse)
- Acamprosate (Campral)
- Nalmefene (Selincro)
- Baclofen
- Varenicline

Also, if a subject reports using a drug or having been prescribed a drug to treat alcoholism during the trial, they will be asked to discontinue its use.

The use of nicotine nasal sprays will be prohibited during the study. The use of other intranasal vasoconstricting products is allowed; however, subjects will be instructed to not use these within 2 hours before or 1 hour after intranasal investigational products are self-administered. Examples are decongestant nasal sprays containing oxymetazoline, epinephrine or phenylephrine.

Subjects will be instructed to check with study staff before taking any new medications or stopping current medications. Subjects will be informed that starting any new medication without consulting study staff could pose health risks and/or result in their discontinuation for the study drug.

Management of investigational products and concomitant medications during the study is at the discretion of the PI or designated medical doctor. The PI or designee may consult with the medical monitor if there are questions.

When oxytocin has been given intravenously to induce labor, severe hypertension has been reported when oxytocin was given three to four hours following prophylactic administration of a vasoconstrictor in conjunction with caudal block anesthesia. Cyclopropane anesthesia may modify oxytocin's cardiovascular effects, so as to produce unexpected results such as hypotension. Maternal sinus bradycardia with abnormal atrioventricular rhythms has also been noted when oxytocin was used concomitantly with cyclopropane anesthesia. Therefore, subjects should be cautioned if undergoing elective surgery or emergency surgery to discuss the use of anesthetics with the prescribing physician and possible drug-drug interactions with oxytocin.

7. Study Procedures

7.1. Recruitment of Subjects

Subject recruitment methods at each site will be based on their local population; however, standard tactics will be used (i.e., flyers, newspaper advertisements, radio advertisements, and television advertisements). Advarra Investigational Review Board (IRB) and NIAAA will approve all advertising materials used for subject recruitment. Interested candidates responding to recruitment materials by telephone will be asked to complete a standardized telephone interview that includes questions about their drinking behavior, health status, interest in participation, and availability for the entire 13 weeks of the trial post randomization. Study staff will ask these questions without revealing the entry criteria for the study. Candidates who report drinking and other information consistent with the entry criteria and appear to be available and interested in the study will meet with the investigator or designated investigational staff ideally within 14 days after the initial inquiry to start the informed consent and assessment process.

7.2. Informed Consent

At the first screening visit, candidates will meet with either the site PI or his/her designee and receive an explanation of the study purpose and requirements. If still interested after receiving an explanation of the study, the candidate will be given an opportunity to review, inquire about, and sign the study informed consent form approved by the IRB. Subjects must have blood alcohol content (BAC) of 0.000 measured by breathalyzer when signing the informed consent document (tested shortly before or just after providing consent). Repeat measurements of BAC are permitted at the discretion of the investigator. Subjects will be given a copy of the signed informed consent form. All subjects who sign informed consent must be registered in the electronic data management system (EDMS) as enrolled in the study. The EDMS will assign the subject a unique subject ID number that will be used on all of the data collection forms and electronic system entries.

7.3. Selection and Withdrawal of Subjects

7.3.1. Inclusion Criteria

Subjects must meet each one of the following inclusion criteria in order to be eligible for participation in the study:

1. Be at least 21 years of age.
2. Have a current (past 12 months) DSM-5 diagnosis of AUD (4 or more symptoms) assessed using the MINI neuropsychiatric interview version 7.0.2 (at least moderate severity, ICD-10-CM Code F10.20 alcohol dependence, uncomplicated).
3. If male, report drinking an average of at least 28 drinks per week or if female report drinking an average of at least 21 drinks per week and at least one heavy drinking day per week for the 28-day period prior to consent and at least one heavy drinking day in the 7- day period prior to randomization.

4. Have a BAC by breathalyzer equal to 0.000 when s/he signed the informed consent document (either just prior to or immediately after signing consent).
5. Be seeking treatment for problems with alcohol and express a goal of abstinence or a reduction in drinking.
6. Be able to verbalize an understanding of the consent form, able to provide written informed consent, verbalize willingness to complete study procedures, able to understand written and oral instructions in English and able to complete the questionnaires required by the protocol.
7. Agree (if the subject is female and of childbearing potential) to use at least one of the following methods of birth control, unless she is surgically sterile, partner is surgically sterile or she is postmenopausal:
    - oral contraceptives,
    - contraceptive sponge,
    - patch,
    - double barrier (diaphragm/spermicidal or condom/spermicidal),
    - intrauterine contraceptive system,
    - etonogestrel implant,
    - medroxyprogesterone acetate contraceptive injection,
    - complete abstinence from sexual intercourse, and/or
    - hormonal vaginal contraceptive ring.
8. Be able to take intranasal investigational products and be willing to adhere to the investigational product regimen.
9. Complete all assessments required at screening and baseline.
10. Have a place to live in the 2 weeks prior to randomization and not be at risk that s/he will lose his/her housing by Study Week 15.
11. Not anticipate any significant problems with transportation arrangements or available time to travel to the study site by Study Week 15.
12. Not have any plans to move within Study Week 15 to a location which would make continued participation in the study impractical.
13. Not have any unresolved legal problems that could jeopardize continuation or completion of the study.
14. Provide contact information of someone, such as a family member, spouse, or significant other, who may be able to contact the subject in case of a missed clinic appointment.
15. Be someone who in the opinion of the investigator would be expected to complete the study protocol.
16. Agree to the schedule of visits, verbally acknowledge that s/he will be able to attend each scheduled visit, participate in phone visits and that s/he does not have any already scheduled events or a job that may substantially interfere with study participation.

17. If taking a medication for depression or anxiety, must have been taking a stable dose in the 2-months prior to randomization and plan to continue during the study. This includes drugs such as the following:
    - SSRIs
    - Dual uptake inhibitors
    - SNRIs
    - Tricyclic antidepressants
    - MAOIs
    - Bupropion
18. Not currently taking oxytocin and agree not to take non-study oxytocin for the duration of the study.
19. Agree to not use nicotine nasal spray for the duration of the study. Note; other forms of nicotine replacement therapy (gum, patch, etc., are permitted).

7.3.2. Exclusion Criteria

Subjects will not be eligible to participate in this study if any one of the following exclusion criteria is met:

1. Have current substance use disorder for any psychoactive substance (including sedatives and hypnotics) other than alcohol, nicotine or mild marijuana use disorder as defined by DSM-5 criteria.
2. Have a urine toxicology screen positive during screening or baseline for any of the following substances:
    - benzodiazepines,
    - cocaine,
    - opioids,
    - amphetamines,
    - buprenorphine,
    - methadone,
    - methamphetamines
    - oxycodone,
    - MDMA, and/or
    - barbiturates.

    Note: Testing for tetrahydrocannabinol (THC) will be included in the urine drug test; however, subjects who test positive for THC are still eligible to participate in the study unless they endorse moderate or severe substance use disorder for marijuana as indicated by DSM-5 criteria. The results for THC will be recorded for information only. If positive for opioids but recent opiate use for acute pain is reported by the subject, then the subject can be re-screened.

3. Have been hospitalized for alcohol intoxication delirium, alcohol withdrawal delirium, alcohol-induced persisting dementia or amnestic disorder, or have had an alcohol withdrawal seizure, alcohol-induced psychotic disorder with a primary diagnosis of alcohol use disorder or a history of any seizure disorder.
4. Have participated (received treatment) in any behavioral and/or pharmacological intervention research study for the treatment of alcohol problems in the past 7 years.
5. Be mandated by the court to obtain treatment for problems with alcohol.
6. Be anyone who in the opinion of the investigator could not be safely withdrawn from alcohol without medical detoxification.
7. Be currently undergoing psychotherapy by a licensed therapist or psychiatrist for alcohol problems

   NOTE: Current psychotherapy should be considered on a case-by-case basis. Psychotherapy for a disorder that may be related to the subject's use of alcohol should be exclusionary. However, shorter term focused behavioral therapy for defined, non-alcohol related problems may be acceptable.
8. Have undergone medical detoxification (e.g., reports using a benzodiazepine) during the screening phase (prior to randomization).
9. Have known allergy to oxytocin.
10. Have significant medical conditions (e.g., chronic rhinitis during the past year) that may preclude successful insufflations of the intranasal investigational products or other medical conditions (e.g., nasal lesions) that could be affected by repeated intranasal administrations.
11. Have been treated with a pharmacotherapy for alcohol problems within 6 months prior to randomization.
12. Have taken any anti-convulsants, hypnotics, barbiturates, antipsychotics, psychomotor stimulants (such as methylphenidate), or benzodiazepines within 5-half lives days prior to the date of randomization.
13. Have any of the following, based on DSM-5 criteria as assessed using the MINI:

    - Current or lifetime diagnosis of psychotic disorders
    - Current bipolar disorder

    Note: Subjects diagnosed with psychiatric disorders not specifically excluded above may be excluded at the discretion of the PI if the comorbid psychiatric condition compromises the study integrity by virtue of its type, duration, or intensity.
14. Have any of the following:

    - attempted suicide past year,
    - current (past year) suicide behavior disorder in accordance with DSM-5 criteria as assessed using the MINI (see note below about assessment of subjects diagnosed at low risk), or

- current (since screening MINI) suicidality risk as indicated during the conduct of the C-SSRS with concurrence after a study physician's evaluation if the response to C-SSRS questions 1 or 2 is "yes").

Note: The MINI suicidality module rates scores of 1 to 8 as a diagnosis of low risk of suicidality. As the MINI questions that could result in a low risk score are considered inadequate to fully determine the potential suicidal risk of an individual (e.g., "Feel hopeless" and "Think that you would be better off dead or wish you were dead?" responses of "yes" dictates a score of 1 for each question), any subject who scores in the low risk category should be evaluated further by a study physician or psychologist who should document whether the subject is appropriate for study inclusion based on his/her clinical judgment of the potential suicide risk of the subject. Likewise, if the subject responds "yes" to either of the first two questions on the screening C-SSRS performed on the day of randomization as a final eligibility check, the subject should also evaluated by a study physician for current suicidality risk, who should document the subject's suitability for study inclusion.

15. Have moderate or serious dementia as assessed by clinical exam.
16. Be pregnant or breast-feeding or have plans to become pregnant at any time during the study.
17. Have clinically significant abnormal liver enzyme levels defined as AST or ALT 5-fold above the upper limit of normal (ULN), or bilirubin greater than 2 times the ULN.

    Note: If the subject has values of liver enzyme that are 3.0-to-4.9 fold above the ULN and bilirubin that is 1.5-to-1.9 fold above the ULN of normal, these assessments should be repeated at least a week apart and if still in this range or higher, the subject should be excluded from the study and referred to their physician for further follow-up.
18. Have sodium < 132 mmol/L or > 150 mmol/L or potassium < 3.2 mmol/L or > 5.5 mmol/L or abnormal calculated creatinine clearance (<60 mL/min), as calculated by Cockcroft and Gault formula.
19. Have a serious or unstable medical illness or any potentially life-threatening or progressive medical condition other than addiction that may compromise subject safety or study conduct.
20. Have clinically significant gastrointestinal, neurological, renal, or cardiovascular disease such cardiac arrhythmia, uncontrolled hypertension, congestive heart failure, or any other ECG abnormality considered clinically significant by the PI.
21. Have data suggesting cirrhosis of the liver.
22. Have taken oxytocin during the 6-month period prior to randomization for treatment of any disorder or if ever treated with oxytocin for AUD.
23. Have UPSIT score ≤ 30 in men and ≤31 in women at screening.

7.4. Eligibility Screening

After the subject undergoes alcohol breathalyzer testing and signs informed consent, screening may begin. During the first screening visit (additional visits are permitted if needed), subjects will undergo the following assessments:

- Demographics and locator form
- Urine drug screen
- Medical history
- Physical examination and body weight
- MINI
- Clinical chemistry
- Pregnancy test for females of childbearing potential and birth control methods, if female
- Vital signs
- ECG
- TLFB for the previous 28 days
- Prior medication use
- CIWA-AR
- Drinking goal
- UPSIT

Subjects will be instructed that if they are taking a medication for depression or anxiety that they should continue to do so throughout the study. They will also be instructed that they should report any new medications they are taking at each visit or telephone contract.

The above assessments can be performed in any order except that it is recommended to perform physical examinations including vital signs prior to blood draws. If any of these assessments reveal that the subject is not eligible for the study, screening can be immediately terminated and no further data be collected. Clinical chemistry tests may be repeated at the discretion of the investigator if the first assessment yields values outside normal laboratory limits. The eligibility checklist will be reviewed, and if the subject is still eligible after the assessments are completed at the first screening visit (or additional screening visits), the subject will be scheduled for the final eligibility check and baseline visit. It is recommended that hypertensive subjects be referred to their primary care physician for additional assessment and possible treatment, and then be further evaluated for study inclusion.

7.5. Baseline and Final Eligibility Assessments

If the subject is eligible after performing all the initial screening assessments, s/he will be scheduled to start the study and will come to the clinic for a final eligibility check including the following assessments:

- Alcohol breathalyzer
- Urine drug screen
- Update medical history
- Update physical exam – new symptoms directed exam
- Vital signs
- CIWA-AR
- C-SSRS*
- Pregnancy test for females of childbearing potential (must be completed within 2 days prior to the subject being randomized and dispensed investigational product)
- Birth control methods, if female
- Prior medications update
- TLFB since screening visit

*Note that the MINI will be used to rule out subjects who attempted suicide in the past year and current (past year) suicidal ideation at initial screening, with the C-SSRS providing an update on current suicidal ideation since screening.

An eligibility checklist will be completed and reviewed by a study investigator and, if the subject is still eligible, he/she will complete the following baseline assessments:

- Other Services for Alcohol Use Problems

The subject will complete the following questionnaires electronically:

- Hyperkatifeia Scale
- Barrett Impulsivity Scale
- Cigarette Smoking Quantity-Frequency and Other Nicotine Use
- Buss-Perry Aggression Questionnaire – Short Form
- Experiences in Close Relationships-Relationship Structures
- PROMIS – Alcohol Negative Consequences
- PROMIS – Sleep Disturbances
- PROMIS – Pain Interference
- POMS
- IDS-30
- Spielberger Trait Anxiety Scale
- Urge to Drink Questionnaire
- SNAQ

The screening period will not be extended. If the investigator determines that the subject could be a viable participant who could not for extenuating circumstances complete the screening period in 14-days, the subject can be re-screened by completing all of the screening assessments again. In this case, the subject will be assigned a new subject number.

7.6. Measures Taken to Minimize/Avoid Bias

7.6.1. Randomization

If eligible for the study, subjects will be randomized in an approximate 1:1 ratio to receive either oxytocin or placebo using a stratified permuted block randomization procedure with "clinical site" as the stratification variable. Clinical site was chosen because both local study populations, and the investigative staff influence on the subject's drinking behaviors may differentially influence endpoints.

Centralized randomization will be performed using an interactive web response system (IWRS). The IWRS is available for randomization of subjects 24 hours/day, 7 days/week from any computer using a web browser.

If the subject is determined to be eligible, site personnel who are authorized to randomize subjects and who have completed training for the IWRS, will log onto the system and provide the EDMS pre-assigned unique subject number. The IWRS provides the randomized group kit number and assigns the subject to one of the two interventions. If the subject is randomized and is never dispensed study drug, then the subject will be considered a randomization failure and an additional subject will be randomized with the next randomization sequence at the time he/she is randomized at that site. Likewise, if the subject was randomized and then is determined to not be eligible for the study, and never received study drug, then another subject will be randomized such that the total numbers of subjects who were eligible, randomized, and dispensed study drug meet the enrollment goals. In the case of a subject who was eligible, randomized, and dispensed study drug but did not return for follow-up visits, this subject will not be replaced. Any subject who received study drug but was later determined to be ineligible will likewise not be replaced. The reason(s) that a subject was considered a randomization failure or screen failure will be documented in source documents and eCRFs.

7.6.2. Blinding

Oxytocin and placebo bottles will be identically matched in appearance and the bottle labels will not reveal the drug identity. The site investigator or designated approved study physician will make the decision to un-blind the identity of the investigational product in the event that the study blind needs to be broken to make medical decisions regarding subject treatment. If it is determined that unblinding is necessary to assess AEs or SAEs for expedited reporting, NIAAA may decide to request unblinding of a subject. Site staff or Sponsor's designee approved to un-blind the study drug will log into the IWRS to obtain the name of the investigational product to which the subject was randomized. The IWRS will automatically notify the un-blinded staff member at the Data Coordinating Center who will notify the Medical Monitor. NIAAA will be notified that an unblinding has occurred if unblinding has been performed by the investigator or medical monitor. Prior experience with oxytocin nasal spray compared with placebo nasal spray has not indicated that any differences would be noticed between formulations.

7.7. Interventions on Day 1

At study Day 1, after the subject is randomized, he/she will receive the first vial of intranasal investigational product, and an instruction sheet. The vial(s) being dispensed will be weighed prior to providing it to the subject. Site staff will explain the dosing plan to the subject and how to complete a Diary Card to track when doses are taken. The subject will administer the first dose while in the clinic on Day 1 under observation by site staff to make sure that the subject is using the nasal spray appropriately. The subject will watch the first module of Take Control, will be given the schedule of visits, and the Week 2 visit will be scheduled.

Every study subject will be provided with a wallet card and instructed to carry this card that identifies the potential investigational products that s/he could be taking during the study. The card will provide the name and 24-hour phone number of the investigator (physician) at the site who can be contacted in the event of an emergency. The card will also instruct the non-study physician rendering emergency care to contact the study physician and inform him/her about the care. Prior to release from the clinic, the subject will be asked if they are experiencing any side effects from the first dose of investigational product.

If possible, clinic visits should be scheduled on the same day of the week that the subject received the first dose of study drug, but a 3-day window is allowed for conducting visits within the scheduled study week. Visits may be scheduled and conducted on any day of the week. Visits can be conducted outside of the scheduled study week, but only based upon subject request (e.g., for reasons related to patient non-compliance with the study schedule). Each subject will receive a visit schedule to take home for future reference.

7.8. Treatment Phase

During Study Weeks 1 through 12 of the treatment phase of the study, subjects will be seen in person at the clinical site 7 times and assessed by telephone 5 times. There is a final clinic visit at Week 13 at the end of the 12-week treatment period.

An alcohol breathalyzer will be administered at each visit, prior to any study assessments, to determine if the subject meets the BAC requirement of a BAC ≤ 0.020 before proceeding with assessments. A urine drug test will be performed at each visit. At each of the clinic visits, subjects will meet with one or more study staff members who will systematically assess AEs since the last visit, take vital signs, collect concomitant medication use, administer questionnaires (CIWA-AR and C-SSRS), inquire about other medication use and assess drinking by TLFB. The UPSIT sniff card test and clinical chemistry, pregnancy test for women of child-bearing potential and birth control methods (if female) will be collected in accordance with the schedule in Table 3. An examination of the nasal mucosa will also be performed. In addition, subjects will electronically complete the questionnaires according to the schedule in Table 3. If the subject is returning a used study drug vial, the vial will be weighed and the diary card will be reviewed for compliance.

Brief telephone assessments will occur at Weeks 5, 7, 9, 11, and 12. The final in-clinic assessment will occur during Week 13. This visit should be scheduled after the subject takes the last dose of study drug.

Subjects will view a single module of Take Control at all clinic visits up to and including Week 10 that is expected to have a run-time of 10-15 minutes at clinic visits. The subject will be

instructed to bring the nasal spray vials and drug with them to the next visit so the site staff can perform drug accountability. The subject will also be instructed to contact site staff if they are experiencing any intolerable AEs and are contemplating drug discontinuation.

Additional in-clinic visits are permitted under the protocol, if needed, due to the following circumstances: (1) the subject has concerns either about the medication or their drinking and wishes to be seen at a time other than their next scheduled in-clinic visit, or, (2) the subject has missed a visit and wishes to resume regular participation before their next scheduled visit, (3) the subject has reported some change in health, functioning, or circumstances which necessitate a visit to conduct safety assessments and evaluate the risk of continued participation in the trial, or (4) clinical laboratory measurements need to be repeated.

Subjects desiring additional counseling or professional therapy for non-crisis psychiatric matters (e.g., marital problems, work issues) should be encouraged if within reason to postpone such activity until their study participation is concluded. Attendance at self-help support groups (i.e., Alcoholics Anonymous) will neither be encouraged or discouraged. Any attendance at self-help support groups will be recorded at the randomization visit and at the end of study visit in the Other Services Used for Alcohol Use Problems electronic case report form (eCRF).

7.9. Telephone Assessments

The brief telephone interview (approximately 10 minutes) will occur in accordance with the schedule in Table 3 to assess AEs, concomitant medication use and to encourage the subject to continue taking investigational products, to verify that the subject is taking the prescribed dose, and to remind the subject of the next scheduled visit. A summary of the telephone script follows:

1. AEs: An open-ended question will be asked as follows: “How have you been feeling since your last clinic visit or phone contact?” If the subject reports a new AE, the resolution of an AE, or a change in the severity of an AE, ask additional questions to determine the severity and dates of occurrence or resolution.
2. Concomitant Medications: Ask the following question: “Have you taken any new medications since you were last seen in the clinic or since our last call? If the subject responds affirmatively, record the name of the medication, the daily dose, route of administration, and reason used. If the medication is contraindicated for the study, notify a study physician, nurse practitioner, or physician assistant for follow-up with the subject.
3. Drug Compliance: Ask the subject is s/he has been taking the nasal spray and check on the number of actuations they report using and if they have had any issues with the device. Review procedures with the subject to ensure that the subject is taking the prescribed dose.
4. Reminders: Remind the subject of their next scheduled clinic visit, and adjust the date within the visit week if they have a conflict.

7.10. Final Clinic Visit

The final clinic visit occurs during Week 13; at which time the subject has completed taking investigational product. In addition to the assessments previously stated, subjects will have an Exit Interview assessment. The subject will be asked about their impression of whether they were receiving active drug or placebo, if they felt that the medication helped drinking, how they

would describe their experience taking the medication, if they would recommend it to a friend, if they would take it again if they needed further treatment in the future. The subject will be provided with a referral to a treatment program for their alcohol dependence. If a subject withdraws from the study early for any reason, the subject should be asked to return to the clinic for the conduct of all of the final clinic visit assessments.

7.11. Telephone Follow-up

Subjects will be contacted by telephone for a follow-up interview 1 to 2 weeks after the final in-clinic visit. During the telephone follow-up interview, the subject will be asked about any ongoing AEs that they may have been experiencing at the last clinic visit and any newly emerged medical conditions/AEs since that visit. To prompt reporting of new AEs, the subject will also be asked about any ongoing or new medication use.

7.12. Duration of Subject Participation

The total time period that each individual subject will participate is 17 weeks including up to 2 weeks for screening, 12 weeks of study interventions, the final safety and efficacy follow-up to one week after completing treatment (Week 13), and a final safety follow-up telephone contact 1- to 2-weeks after completion of study drug dosing (Weeks 14/15).

7.13. Safety Criteria for Stopping Doses

The PI or sub-investigator will follow the protocol to identify and intervene with subjects experiencing clinical deterioration during study participation. Criteria to determine when a subject requires a higher level of care and discontinuation from the trial intervention are detailed below.

7.14. Investigational Product Discontinuation

If the investigator determines that any study subject should discontinue taking investigational products, the subject should be told to discontinue taking the investigational products immediately. No dose reduction is necessary in this case. Subjects should continue in the study, even if withdrawn from investigational products, and complete all assessments. Dose reductions for intolerance are permitted (see section 6.7).

Pregnancy. Females who become pregnant during the course of the study will be instructed to discontinue use of the investigational product immediately. The investigator must report a pregnancy within 1 working day of the site being aware to the NIAAA Study Manager and the Medical Monitor.

Physical Illness. Subjects will need to be removed from investigational products if they have a serious illness or a disabling condition that precludes them from taking the investigational product.

Adverse Events. If the subject experiences any AEs that are considered study drug related and for which the investigator has determined that continuation of the study drug could be detrimental to the health of the subject, then drug will be immediately discontinued as described above.

UPSIT Score. If UPSIT score decreases to ≤ 26, study drug will be discontinued but the subject should continue in the study and be monitored using the UPSIT to determine if this AE is reversible.

7.15. Subject Withdrawal or Discontinuation Procedures

Each subject has the right to withdraw consent and withdraw from the study at any time. In addition, the investigator may find it necessary to discontinue a subject although it is recommended to continue to perform all study assessments, if possible.

In the event that a subject withdraws or is discontinued from the study, the reason(s) for the discontinuation from the study will be recorded and the final end of study assessments shown in Table 3 will be completed.

7.16. Situations Requiring Discontinuation from the Study as well as from Investigational Product

It is possible that there will be some subjects who cannot be safely managed in the clinical study even though investigational products have been discontinued. Examples are given below.

1. **Increased Drinking.** Subjects whose alcohol problem worsens, and, in the opinion of the site medical staff, require a more intense level of care than provided in the study may have investigational product suspended, and referred to more appropriate care.
2. **Psychiatric Crises.** Examples of psychiatric crises include but are not limited to the following:

   a. Acute psychosis (hallucinations, impaired reality testing, paranoid ideation, etc.) requiring medication and/or hospitalization or intensive outpatient intervention;
   b. Suicidal or homicidal ideation that results in a credible threat of violence directed at oneself or others;
   c. Hospitalization for psychiatric symptoms

   Subjects requiring more intensive treatment resulting from acute psychosis or suicidal/homicidal behavior will be referred to local treatment centers, emergency departments, or hospitalization as appropriate, but will not be provided with medication or psychotherapy by study staff.
3. **Absence from the Protocol due to Confinement in a Controlled Environment.** If a subject is confined to a controlled environment (such a hospital or jail where access to alcohol is presumably restricted) for less than 2 weeks, they can resume full participation in the trial if in the judgment of the investigator, the subject is still a good candidate for the study and continues to meet eligibility requirements. Before resuming investigational products the subject should be assessed by the study physician for appropriateness to resume the trial (e.g., any new medications or symptoms, pregnancy test, etc.). The decision to restart study drug at the full dose will be made in the judgment of the investigator based on the subject's time off study drug and past experience with side effects with the study drug.

If a subject is in a controlled environment (such a hospital or jail where access to alcohol is presumably restricted) for 2 weeks or more, the subject will be discontinued from the study.

7.17. Study Suspension and/or Termination Criteria

Enrollment will be halted if more than 2 treatment-related SAEs occur. In the event that this occurs, a DSMB will be convened and a decision in conjunction with the study Sponsor will be made regarding stopping the study.

NIAAA may terminate this study prematurely, either in its entirety or at any site, for reasonable cause provided that written notice is submitted in advance of the intended termination. The investigator may also terminate the study at his/her site for reasonable cause, after providing written notice to NIAAA in advance of the intended termination. Advance notice is not required by either party if the study is stopped due to safety concerns. If NIAAA terminates the study for safety reasons, NIAAA will immediately notify the investigators by telephone and subsequently provide written instructions for study termination. The FDA may stop the study at any time as well. If the FDA notifies NIAAA to stop the study, then NIAAA will notify the sites of this action.

8. Study Endpoints

8.1. Efficacy Endpoints

8.1.1. Primary Efficacy Endpoint

The primary efficacy endpoint is the weekly percentage of heavy drinking days during the 10- week treatment period. A "heavy drinking day" is 4 or more drinks per drinking day for women and 5 or more drinks per drinking day for men.

8.1.2. Secondary Efficacy Endpoints

Secondary endpoints will be analyzed over the 12 weeks of treatment including:

1. Percentage of subjects with no heavy drinking days during treatment
2. Percentage of subjects abstinent from alcohol during treatment
3. Percentage of subjects with a WHO drinking risk category decrease during treatment of:
    a. 1-level
    b. 2-levels
4. Endpoints analyzed monthly and with 1- and 2-month grace periods:
    a. Percentage of subjects with no heavy drinking days during the period
    b. Percentage of subjects abstinent from alcohol during the period
    c. Percentage of subjects with WHO drinking risk category decrease during the period of:
        – at least 1 level
        – at least 2 levels
5. Percentage of days abstinent per week
6. Weekly mean number of drinks per week
7. Weekly mean drinks per drinking day
8. MINI AUD Score at end of study
9. Cigarettes smoked per week among smokers
10. Abstinence from cigarette smoking
11. Other nicotine product use per week among other nicotine product users
12. Experiences in Close Relationships—Relationship Structures Questionnaire (ECR-RS) (attachment related anxiety)
13. PROMIS – alcohol-related negative consequences
14. PROMIS – sleep disturbances
15. PROMIS – pain interference
16. Profile of Moods States (POMS) scores (including subscale scores)

17. Urge to Drink Scores

8.2. Exploratory Endpoint

Hyperkatifeia Scale score at EOS

8.3. Safety Endpoints

Safety endpoints will be analyzed over the entire treatment and follow-up period.

1. Vital signs
2. Physical examination of the nasal mucosa
3. Smell testing with the UPSIT
4. Blood chemistries
5. Urine drug screen results
6. Blood alcohol concentration (BAC) by breathalyzer
7. AEs/SAEs
8. Electrocardiogram (ECG)
9. Clinical Institute of Withdrawal - Alcohol Revised (CIWA-AR) scores
10. Frequency of subjects with suicidal ideation at any time during the treatment period - Columbia-Suicide Severity Rating Scale (C-SSRS)
11. Change in appetite - Simplified Nutritional Appetite Questionnaire (SNAQ) scores and percentage of subjects with scores ≤14.
12. Buss-Perry Aggression score

8.4. Compliance

Compliance will be assessed using the Subject Diary and by weighing used study drug vials prior to and after use to calculate the amount dispensed.

9. Safety Monitoring Plan

Safety monitoring will be conducted throughout the study; therefore safety concerns will be identified by continuous review of the data by the PI, clinic staff, clinical monitor, medical monitor, and NIAAA.

Study Safety Management: The IRB, Medical Monitor, PI, clinical monitors, and NIAAA will review any safety concerns throughout the trial. In addition, a data and safety monitoring board (DSMB) will participate in this study. The roles of these individuals/committee are described below.

Medical Monitor: A Medical Monitor and Alternate Medical Monitor have been appointed by NIAAA for the study. The Medical Monitor will be available for making recommendations to the investigator and NIAAA on the severity of any SAEs, and the relatedness to the study interventions. The Medical Monitor will also be responsible for tracking and assessing trends in the AEs reported.

Clinical Monitors: All investigators will allow representatives of the Data Coordinating Center (Fast-Track Drugs and Biologics, LLC) staff to periodically monitor, at mutually convenient times during and after the study, all study data. These monitoring visits provide the Data Coordinating Center and NIAAA with the opportunity to evaluate the progress of the study and to obtain information about potential problems. The monitors will assure that submitted data are accurate and in agreement with any paper source documentation used; verify that investigational products are properly stored and accounted for, verify that subjects' consent for study participation has been properly obtained and documented, confirm that research subjects entered into the study meet inclusion and exclusion criteria, and assure that all essential documentation required by Good Clinical Practices (GCP) guidelines are appropriately filed.

Monitors will conduct a site initiation visit prior to the start of the study. At this visit, they will assure that proper study-related documentation exists, assist in training investigators and other site personnel in study procedures and GCP guidelines, confirm receipt of study supplies, and assure that acceptable facilities are available to conduct the study.

Routine monitoring visits by the Data Coordinating Center and NIAAA's representatives will be scheduled at appropriate intervals but more frequently at the beginning of the study. A monitoring visit soon after the first two subjects have been randomized is planned. At these visits, the monitors will verify that study procedures are being conducted according to the protocol guidelines, monitor eCRFs against source documents, review AEs and SAEs, and perform drug accountability. At the end of the study, they will confirm that the site has the appropriate essential documents on file, advise on storage of study records, and inspect the return and destruction records for unused investigational products.

Sponsor Site Visits: All investigators will allow NIAAA full access to study records during periodic site visits by NIAAA. Visits by NIAAA will be made at a mutually convenient time and will be scheduled in advance.

DSMB: An independent DSMB of external advisors will meet prior to the start of the study, quarterly during enrollment and follow-up and at trial end to review safety data. The Board will be blinded to subjects' actual randomized group assignments but may request at any time that the

blind be broken by the data center, if concerns arise from the blinded data. *Ad hoc* meetings will be convened if SAEs occur that are considered at least possibly related to the investigational product.

10. Assessment Methods

All study assessments will be performed at the visits and time points outlined in the Schedule of Assessments (Table 3); the following sections outline the details and procedures associated with the assessments. All assessments will be recorded on a source document with the exception of staff or subject completed questionnaires that will be completed electronically within the EDMS. If the EDMS is not accessible, then subject questionnaires can be completed using paper forms provided by the Data Management Center and transcribed into eCRFs.

10.1. Alcohol Breathalyzer

An alcohol breathalyzer will be administered at consent, at screening, and at every in-clinic visit as a safety measure. Acceptable BAC level at consent is equal to 0.000 and ≤ 0.020 for all other in-clinic visits prior to performing other assessments.

10.2. Adverse Events and Serious Adverse Events

The investigator and study site staff are responsible for the detection, documentation, classification, reporting, and follow-up of events meeting the definition of an AE or SAE.

10.2.1. Adverse Event Definition

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and may not necessarily have a causal relationship with the administered treatment. An AE can therefore be any unfavorable and unintended sign (including a clinically significant laboratory abnormality), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product. Pre-existing conditions, diseases, or disorders are not considered AEs unless there is a change in severity or frequency.

10.2.2. Serious Adverse Events and Serious Unexpected Adverse Events Definition

An SAE is any untoward medical occurrence that meets one of the following:

- Results in death
- Is life-threatening (at the time of the event)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect

A serious and unexpected AE is an SAE that is not identified in nature, intensity, or frequency in the risk information included in the Product Label for the drug.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the study subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition.

10.2.3. Methods/Timing for Assessing, Recording, and Analyzing Safety Endpoints

AEs will be assessed at study visits starting after the first administration of investigational product until the final follow-up visit. However, SAEs will be collected from the time of informed consent onward. General symptoms will be collected via an open-ended question: "How have you been feeling since your last visit or the last time we spoke?"

AEs will be documented in the source records and recorded on the eCRFs using accepted medical terms and/or the diagnoses that accurately characterize the event. When a diagnosis is known, the AE term recorded on the eCRF will be the diagnosis rather than a constellation of symptoms. The investigator will assess all AEs for seriousness, relationship to investigational product, and severity. When an event has not resolved by study closure, it will be documented on the AE eCRF as "ongoing".

If a woman has a positive pregnancy test after enrollment, the NIAAA Medical Monitor will be contacted and the pregnancy will be recorded as an AE. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been terminated or completed. The outcome of the pregnancy will be reported to the NIAAA Medical Monitor without delay within 24 hours of knowledge of the event if the outcome is a SAE (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed by study physicians until satisfactory resolution (the event either resolved or stabilized and is not expected to resolve in the near term). AEs must be reported up to 2 weeks following completion of, or termination from investigational product administration. At the follow-up telephone contact, AEs will be recorded and followed to resolution only if they are serious, or if the study physician assesses them to be clinically significant.

10.2.4. Clinical Laboratory Abnormalities and Other Abnormal Assessments

Abnormal clinical laboratory findings (e.g., clinical chemistry) will be reported as an AE unless the value was outside normal laboratory limits at baseline and did not increase by a severity grade level at the follow-up assessment. Grade 2 or greater hypokalemia and Grade 3 or greater results for any other laboratory test will be considered clinically significant. Likewise if blood pressure was elevated at baseline [pre-hypertension (systolic BP 120 - 139 mmHg or diastolic BP 80 - 89 mmHg)] and increased to a level indicative of an increase in the severity grade, hypertension will be reported as an AE.

10.2.5. Classification of Adverse Event Intensity and Relationship to Investigational Product

The severity of AEs or SAEs will be in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE version 5.0). A physician-investigator must make an assessment of severity. For those not listed in the CTCAE, the following criteria will be used:

Mild: An event that is usually transient, requiring no special treatment, and does not generally interfere with the subject's daily activities.

Moderate: An event that interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject. The event is usually ameliorated with additional specific therapeutic intervention.

Severe: An event that interrupts usual activities of daily living or significantly affects clinical status. The event poses a significant risk of harm to the subject and hospitalization may be required, and typically requires intensive therapeutic intervention.

The investigator must make an assessment of relationship to the investigational product based on the following criteria:

Unrelated: The subject did not receive the investigational product, the temporal sequence of the AE/SAE onset relative to administration of the investigational product is not reasonable, or there is another obvious cause of the AE/SAE.

Unlikely: There is evidence of exposure to the investigational product but there is another more likely cause of the AE/SAE.

Possible: There is evidence of exposure to the investigational product, the temporal sequence of the AE/SAE onset relative to administration of the investigational product is reasonable, but the AE/SAE could have been due to another equally likely cause.

Probable: There is evidence of exposure to the investigational product, the temporal sequence of the AE/SAE onset relative to administration of the investigational product is reasonable, and the AE/SAE is more likely explained by the investigational product than by any other cause.

Definite There is evidence of exposure to the investigational product, the temporal sequence of the AE/SAE onset relative to administration of the investigational product is reasonable, the AE/SAE is more likely explained by the investigational product than by any other cause, and the AE/SAE shows a pattern consistent with previous knowledge of the investigational product or investigational product class.

10.2.6. Outcomes and Actions Taken

All unresolved AEs will be followed for a minimum of 7 days (unless the AE is an ongoing pregnancy which must be followed to conclusion) after the subject's final study visit, unless the investigator's judgment dictates otherwise, the event has resolved or stabilized prior to the 7-day period, or the subject is lost to follow-up.

Investigators are not obligated to actively seek AEs or SAEs in former study subjects that occur following the follow-up period.

For each recorded AE or SAE, the investigator must make an assessment of outcome at the time of last observation, as follows:

Fatal:	The subject died.
Resolved without Sequelae:	The AE or SAE has ended.
Resolved with Sequelae:	The AE or SAE has ended but changes are noted from baseline.
Unresolved – Ongoing:	The AE has not ended and is ongoing at the end of the reporting period (i.e., 7 days after the final Follow-up visit) and the investigator deems that further follow up is not medically required
Unknown – Lost to Follow-up:	Lost to follow-up after repeated unsuccessful attempts to contact the subject.

Actions taken with respect to investigational agents (discontinuation or not) will also be recorded. In addition, if the AE was treated (medications or other physical measures), this will also be recorded.

10.2.7. Reporting Serious Adverse Events

10.2.7.1. 24 hour Reporting Requirements (Initial Report)

Any SAE, including death due to any cause, which occurs to any subject from the time of signing consent through the final follow-up visit whether or not related to the investigational product, must be reported ***within 24 hours*** of knowledge of the event by completing the AE/SAE eCRF. This will trigger an automatic notification of the SAE via an email communication to NIAAA and Fast-Track. Fast-Track will notify the Medical Monitors upon receipt of the notification and coordinate communications with the Medical Monitors.

10.2.7.2. 3-Day Supporting Documentation Requirements (Follow-up Report)

Written documentation for all SAEs must be received by the NIAAA Medical Monitor/Alternate within 3 days of reporting the event. Required eCRF that must be completed include the following:

- AE/SAE eCRF (revised if additional information is available)
- Concomitant Medication eCRF

In addition, paper copies of the following may be requested

- Copies of source documents pertinent to the event (laboratory reports, ECG tracings, medical chart notes, etc.). These should be identified only by Subject number and not include any subject identification information prohibited by Health Insurance Portability Accountability Act (HIPAA).
- Any other relevant information necessary to support the investigator's judgment regarding the SAE's relatedness severity to the investigational product OR by request of the Medical Monitor/Alternate.

These paper documents may be submitted by facsimile, as email attachments, or by attaching them to the subject's eCRF casebook.

10.2.7.3. Reporting to Advarra IRB

Reporting requirements for Advarra IRB include:

Immediately Reportable:

- Findings detected in the monitoring process when those findings could affect the safety of participants or their willingness to continue participation, influence the conduct of the study, or alter Advarra's approval to continue the study.
- Changes in research that were initiated without IRB review and approval to eliminate apparent immediate hazards to the human subjects to ensure the continued safety and welfare of subjects.
- Modifications to previously approved documents.
- Safety information that may help to provide additional protections for subject's safety and well-being, throughout the course of the study and after study completion.
- Communication of results from a research study to subjects when those results directly affect their safety or medical care.
- DSMB Reports

Report within 10 calendar days of discovery:

- Revisions to the Investigator's Brochure, as applicable.
- Revisions to the report of prior investigations, as applicable.
- Non-compliance – Failure by an investigator and/or sponsor to follow Advarra's requirements, applicable regulations or to protect human research subjects, including but not limited to the principles of the Belmont Report 'Serious non-compliance issues – non-compliance as defined as above and as determined to be serious in a way that adversely affects the rights and welfare of human subjects following the investigation and review by the IRB Continuing non-compliance issues – A pattern of repeated non-compliance or serious non-compliance as determined by the IRB.
- Significant deviations – Significant deviations are those that deviate from the approved protocol, informed consent process and affect or potentially affect the safety of subjects. Advarra does not consider protocol deviations to be different from protocol violations.
- Unanticipated adverse device effects.
- Unanticipated problems should be reported regardless of whether they occur during the study, after the study completion, or after participant withdrawal or completion. Any unanticipated problems involving risks to human subjects or others that are (1) unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied; (2) related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the

procedures in the research); and (3) suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. Examples of problems or events that may meet the definition of unanticipated problems involving risk to subjects or others may include, but are not limited to the following:

- Imminent threat of a reportable event that has not yet occurred
- Information indicating a change to the risk/benefit ratio of the research
- Death
- Breach of confidentiality, including lost or stolen study documents/data

10.3. Barrett Impulsiveness Scale

The Barratt Impulsiveness Scale (BIS-11; *Patton-1995*) is a questionnaire designed to assess the personality/behavioral construct of impulsiveness. It is a 30-item scale with items scored on a 4-point scale (1=rarely/never; 2= occasionally; 3=often; 4=almost always/always). Items 1, 7, 8, 9, 10, 12, 13, 15, 20, 29, and 30 are reverse scored. The total score is the sum of the individual items. This questionnaire will be completed by the subject electronically and will be both the source and eCRF.

10.4. Buss Perry Aggression Questionnaire – Short Form (BPAQ-SF)

The BPAQ-SF is a 12-item self-report measure that includes four subscales: physical aggression (4 items), verbal aggression (3 items), anger (2 items), and hostility (3 items) validated by *Diamond and Magaletta (2006)* that was developed from the 29-item scale developed by *Buss and Perry (1992)*. There is no time frame specified, and items are rated using a five-point scale from 1 “very unlike me” to 5 “very like me” with the score as the sum of the scores obtained by a respondent for the individual factors. This questionnaire will be completed by the subject electronically and will be both the source and eCRF.

10.5. Cigarette Smoking Quantity-Frequency and Other Nicotine Use

A smoking quantity frequency and nicotine use interview will include 3 questions to assess nicotine use via cigarette smoking or via other products during the study: 1) Over the past week, on how many days did you smoke cigarettes?; 2) On the days you smoked during the past week, how many cigarettes did you smoke on average?; and 3) Over the past week, on how many days did you use other nicotine products (ex. chew, cigars, cigarellos, e-cigarettes, vape, gum, patch, etc...)? This questionnaire will be completed by the subject electronically and will be both the source and eCRF.

10.6. Clinical Institute Withdrawal Assessment for Alcohol-revised (CIWA-AR)

The CIWA-AR modified telephone version is an adaptation for telephone administration of the CIWA-AR a brief 10-item measure used to provide a quantitative index of the severity of the alcohol withdrawal syndrome (*Sullivan-1989*). The CIWA-AR has been used both in clinical and research applications and has demonstrated both reliability and validity (*Sellers-1992, Stuppaeck-1994*). This questionnaire will be administered by a clinical staff member and subject responses will be recorded electronically and will be both the source and eCRF.

10.7. Clinical Chemistry

Clinical laboratory tests will be performed at the clinical site's local clinical laboratory. Laboratories performing these assessments should be directly regulated by the College of American Pathologists (CAP) or Clinical Laboratory Improvement Act (CLIA) guidelines. The laboratory will need to provide a copy of current certification. Additional laboratory samples may be taken at the discretion of the investigator if the results of any tests fall outside reference ranges or clinical symptoms necessitate testing to ensure safety. Clinical chemistry tests are listed below.

- Creatinine
- Total bilirubin
- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
- Sodium
- Potassium

Serum creatinine levels will be used to calculate creatinine clearance (CrCl) according to the Cockroft-Gault (1976) formula as follows:

Males $\quad CrCl\ (mL/min) = \dfrac{(140 - \text{age in years}) \times \text{body weight in kg}}{72 \times \text{serum creatinine mg/dL}}$

Females $\quad CrCl\ (mL/min) = \dfrac{0.85 \times (140 - \text{age in years}) \times \text{body weight in kg}}{72 \times \text{serum creatinine mg/dL}}$

For any laboratory test value outside the reference range that the investigator considers clinically significant:

- The investigator will repeat the test to verify the out-of-range value
- The investigator will follow the out-of-range value to a satisfactory clinical resolution
- A laboratory test value will be reported as an AE with the severity score assigned in accordance with the CTCAE version 5.0

10.8. Columbia Suicide Severity Rating Scale (C-SSRS)

The C-SSRS is a 4-page form asking questions about suicidal ideation, intensity of ideation, and suicidal behavior developed by Posner and collaborators at the New York State Psychiatric Institute (*Oquendo-2003*). This scale is intended for use by trained administrators. The questions contained in the C-SSRS are suggested probes. Ultimately, the determination of the presence of suicidality depends on clinical judgment. Training is required before administering the C-SSRS through a 30-minute interactive slide presentation followed by a question-answer session through the Columbia University Medical Center. Those completing the training are certified to administer the C-SSRS, and will receive a training certificate. As the MINI will be used to establish subject initial eligibility with respect to suicidality, the "Since Last Visit" version of the C-SSRS will be used at each clinic visit starting at Week 1. At Week 1, this scale will be used to assess current suicidal ideation since the MINI interview. This questionnaire will be

administered by a clinical staff member and subject responses will be recorded electronically and will be both the source and eCRF.

10.9. Demographics

Demographics data include the subject's age, gender, race/ethnicity, marital status, education, employment pattern, occupation, and income level. These data will be collected by site staff on a source document and entered into an eCRF.

10.10. Device Issues/Failures

The subject will be asked to report any issues using the nasal spray and record the issue on their Drug Diary. If a nasal spray pump failure occurs (e.g., no spray is coming out), then the subject will be asked to call the number on the wallet card to make arrangements to obtain a replacement nasal spray vial. The site will notify Fast-Track of any device problems within 24-hours after learning of the problem. A eCRF will be provided for reporting problems.

10.11. Drinking Goal

A subject's drinking goal will be assessed to determine the desire and motivation to completely abstain from drinking alcohol or reduce drinking without abstinence.

The Drinking Goal Questionnaire includes 4-items that will be administered by a member of the study staff. The questions include:

1. What goal have you chosen for yourself about drinking by the end of the study?
    a. To stop drinking
    b. Reduce drinking but not stop
2. Subjects who respond to question 1 with "Reduce drinking but not stop" will be asked to estimate, having achieved their drinking goal, the number of drinks they might consume on each day of a typical week.

All subjects will be asked questions capturing:

3. The level of motivation to reach this goal. Responses are a 1 to 10 scale with 1=not motivated and 10 = extremely motivated.
4. The level of confidence to reach this goal. Responses are a 1 to 10 scale with 1=not confident and 10 = extremely confident.

10.12. Brief Drinking Questionnaire

If a subject is withdrawn from the study early and is no longer participating in clinic visits or providing TLFB drinking data but is willing to be contacted by phone at the week most proximal to dropout, then they will be administered the Brief Drinking Questionnaire. The questions are taken from the list recommended by the NIAAA Task Force on Recommended Alcohol Questions (Oct 15-16, 2003) and ascertain: if there was any drinking during the target period; if so, how many a day on average; the largest number of drinks consumed on any one day; and, the number of times reaching the heavy drinking limit (4 or more for women and 5 or more for men). This data will be recorded on a source document and eCRF. This does not apply to subjects who are willing to supply daily drinking data by the TLFB method.

10.13. ECG

A 12-lead resting ECG will be obtained. Any abnormalities will be noted and an assessment of clinical significance will be done by a study physician.

10.14. Exit Interview

At the final in-clinic visit (or by phone if the subject withdraws early), the subject will complete a questionnaire for his/her impression of whether he/she was receiving active drug or placebo, if they felt that the medication helped drinking, how they would describe their experience taking the medication, if they would recommend it to a friend, if they would take it again if they needed further treatment in the future, and a question about desire to please people. This questionnaire will be completed by the subject electronically and will be both the source and eCRF.

10.15. Experiences in Close Relationships—Relationship Structures Questionnaire (ECR-RS)

The Experiences in Close Relationships-Relationship Structures scale (ECR-RS; *Fraleyl-2011*) is a questionnaire assessing two-dimensional relationship-specific attachment structures in adults beyond the traditional focus on romantic relationships. This is a 36-item questionnaire of which the first 18-item attachment-related anxiety scale will be assessed and evaluated. The presentation of the items will be randomized on the form for subject completion. Each item is rated on a 7-point scale where 1 = strongly disagree and 7 = strongly agree. The attachment-related anxiety is determined by averaging the scores of these 18 items. The ECR-RS questionnaire is completed by the subject electronically and will be both the source and eCRF

10.16. Family History of Alcohol Problems

Information on family history of alcohol problems will also be collected using the Family History of Alcohol Problems. The questionnaire provides subjects with a consistent set of cues for identifying blood relatives with alcohol problems by using a family tree listing for relatives (*Mann-1985*). This questionnaire will be restricted to subject's parents' alcohol history. The questionnaire will be completed by the subject electronically and will be both the source and eCRF.

10.17. Hyperkatifeia Questionnaire

Hyperkatifeia is referred to as an exacerbated negative emotional state during drug and alcohol withdrawal characterized as emotional distress and pain. Hyperkatifeia falls within the negative affect stage of the addiction cycle where individuals experiencing hyperkatifeia are vulnerable to relapse to alcohol or drug use motivated by alleviation of such negative affective symptoms (Koob-1997; Koob-2019). Negative reinforcement becomes the source of motivation to a person to prevent hyperkatifeia of alcohol and drug withdrawal (Koob-2021). Koob (2021) postulates: "Although all drugs of abuse have positive reinforcing properties, the termination of drug taking inevitably leads to negative emotional states of acute and protracted withdrawal in the withdrawal/negative affect stage, which generates a second motivational drive from negative reinforcement. Negative reinforcement can be defined as an increase in the probability of a response that is produced by the removal of an aversive event. Here, negative reinforcement becomes the source of motivation for drug seeking as the individual works to reduce, terminate, or prevent the negative emotional state or hyperkatifeia of drug withdrawal."

Negative emotionality has been shown to be particularly important in predicting alcohol treatment outcomes (Votaw-2020). Individuals with comorbid diagnoses of depression or anxiety tend to have higher AUD severity entering treatment and greater drinking and functional impairment following treatment (Burns-2005). Furthermore, changes in negative affect have been shown to play a mediating role in alcohol treatment outcomes (Wilcox-2018).

NIAAA has developed a 24-item self-report Hyperkatifeia Scale to capture negative emotionality after stopping alcohol drinking that will be assessed for the first time in this study of heavy drinkers with a diagnosis of moderate to severe AUD. The items address four measures associated with negative emotional state including stress/anxiety, depression, stress, irritable and pain and is provided in Appendix C. It takes less than two minutes to complete.

One major distinction between the Hyperkatifeia Scale and other negative emotional state scales is the Hyperkatifeia Scale directly links negative emotional state to the absence of alcohol. Another key difference between this scale and other negative emotional state scales is questions directly related to pain. Pain may be particularly important in AUD and hyperkatifeia as there is a positive association between pain severity and a higher risk for AUD (Lawton- 2009; Edlund-2013). Also, physical pain appears to be a significant predictor of alcohol use and heavy alcohol use and relapse to drinking after a period of abstinence (Larson-2007; Caldeiro-2008; Witkiewitz-2015).

This questionnaire will be completed by the subject electronically and will be both the source and eCRF.

10.18. Inventory of Drinking Situations (IDS-30)

The IDS-30 is a 30-item patient completed questionnaire of reward and relief drinking that asks subjects to rate the frequency (0= never, 3 = almost always) of heavy drinking in various situations over the past year. Fifteen items measure reward drinking tendencies and 15 items measure relief drinking tendencies (*Mann-2017*). This questionnaire will be completed by the subject electronically and will be both the source and eCRF.

10.19. Locator Form

After signing informed consent, subjects will be asked to provide names, addresses, and phone numbers of several friends and/or family members who can be contacted if the subject cannot be located (Locator Form). This locator form will be used to assist in contacting subjects between visits and at follow-up. This form asks subjects his/her name, address, and phone number and to provide names, addresses, and phone numbers of several friends and family members who can be contacted if the subject cannot be located. This information is essential and will be collected during screening and will be updated throughout the study as necessary. This information will remain exclusively at the site.

10.20. Medical/Surgical History

A medical history will be taken for all potential study subjects to assure medical fitness during screening. Current and past year chronic rhinitis will be specifically queried as this is exclusionary. Chronic rhinitis is defined as symptoms of rhinitis lasting longer than 6 weeks. Signs and symptoms of rhinitis may include stuffy or runny nose, sneezing, phlegm in the throat (postnasal drip) and cough. The age at which the subject started drinking alcohol regularly at

least 3 times per month (age of onset) will also be collected as part of the medical history. Dates of prior surgeries will be recorded.

The medical/surgical history will be updated on the day planned for randomization by asking the subject if anything has changed since the initial screening interview.

10.21. MINI

The MINI (paper version 7.0.2) is a short structured diagnostic interview, developed jointly by psychiatrists and clinicians in the United States and Europe, for DSM-5 and ICD-10 psychiatric disorders (*Sheehan-1998*). With an administration time of approximately 15 minutes, it was designed to meet the need for a short but accurate structured psychiatric interview for multicenter clinical trials and epidemiology studies and to be used as a first step in outcome tracking in non-research clinical settings. Diagnoses recorded on the MINI will be recorded on the interview form and entered into an eCRF. The individual items of the Alcohol Use Disorders Module will be also collected on an eCRF in addition to all other diagnoses. At the last study visit, the Alcohol Use Disorders Module will be assessed again, but the initial drinking question will not be asked and the time period for which the questions apply has been revised to "the past month" instead of the past 12 months.

10.22. Other Services Used for Alcohol Use Problems

Prior (lifetime) treatment for alcohol problems and drinking will be collected at baseline. The answers to the following questions will be collected:

1. Number of lifetime inpatient hospitalizations (i.e. at least one overnight stay) to get help with reducing or quitting drinking.

2. Number of lifetime inpatient hospitalizations (i.e. at least one overnight stay) for illnesses, injuries, or accidents due to drinking.

3. Number of times in lifetime underwent alcohol detoxification using medication.

4. Number of lifetime outpatient visits (i.e. no overnight stay) with a health professional to get help with reducing or quitting drinking.

5. In the past year, how many alcoholics anonymous (AA), 12 Step, Save Ourselves, or similar group meetings attended for alcohol problems or drinking.

At the end of the study, the subject will be interviewed about attendance at group meetings [AA, 12-step programs, secular organizations for sobriety (SOS), or similar group meetings] and visits with health professionals for assistance in reducing or stopping drinking during the 12-week treatment period. Use of additional counseling or professional therapy for non-crisis psychiatric matters or any additional pharmacologic or non-pharmacologic treatments received will be documented. This questionnaire will be administered by a clinical staff member and subject's responses will be recorded electronically and will be both the source and eCRF.

10.23. Profile of Mood State (POMS)

The POMS measures dimensions of affect or mood (*McNair and Heuchert-2005*). It consists of 65 adjectives to which the subject responds according to a 5-point scale ranging from "not at all" to "extremely." Six subscale scores will be computed for items grouped as follows: Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor-Activity, Fatigue-Inertia, and Confusion-Bewilderment. A total mood disturbance score will also be computed which consists of the sum of the 5 of the six subscale scores (the Vigor-Activity score is not included). This questionnaire will be completed by the subject electronically and will be both the source and eCRF.

10.24. Pregnancy Test and Birth Control Record

An FDA approved rapid result urine pregnancy test will be used (i.e., dipstick test). If applicable, subjects will be asked to sign a release of information form for study personnel to access medical records to obtain information regarding the outcome of a pregnancy that occurred during the study.

The Birth Control Assessment is designed to determine a female subject's compliance with the birth control specifications detailed in the inclusion criteria.

10.25. Prior and Concomitant Medications

All medications taken by the subject 2-months prior to the start of screening, during the screening period, and through the final follow-up contact will be recorded on a source document and eCRF.

10.26. PROMIS Questionnaires

PROMIS® (Patient-Reported Outcomes Measurement Information System) is a set of person-centered measures that evaluates and monitors physical, mental, and social health in adults and children that were developed through the support of U. S. Department of Health and Human Services. See http://www.healthmeasures.net/explore-measurement-systems/promis for a description of the development of these assessments. Three of these assessments, described below, will be utilized in this study.

10.26.1. Patient-Reported Outcomes Measurement Information System (PROMIS) – Alcohol Use Negative Consequences – Long Form

For negative consequences for alcohol use, the long form of the PROMIS Alcohol Negative Consequences questionnaire will be used to assess outcomes of alcohol use over the past 30 days. This 31-item questionnaire assesses drinking patterns (e.g., quantity and frequency of consumption, time spent drinking, episodes of heavy drinking), cue-based drinking (internal states and external contexts), cravings to drink (e.g., urgency, compulsivity), and efforts to control drinking (e.g., difficulty in limiting drinking) that indicate problematic drinking, particularly at the high end of the severity continuum. The PROMIS Alcohol Negative Consequences questionnaire is completed by the subject electronically and will be both the source and eCRF.

10.26.2. PROMIS – Sleep Disturbances – Short From 8b

For assessment of subject's sleep quality, the PROMIS Sleep Disturbance Short Form 8b will be used. The questionnaire consists of 8 questions regarding the quality of sleep over the past 7 days. Each item on the measure is rated on a 5-point scale with a range of scores from 8 to 40 with higher scores indicating greater severity of sleep disturbance. The American Psychiatric Association has established levels of severity (none to slight, mild, moderate or severe based on the T-score derived from a conversion table). The PROMIS Sleep Disturbance questionnaire completed by the subject electronically and will be both the source and eCRF.

10.26.3. PROMIS – Pain Interference – Short Form 8a

For the assessment of pain interference, the shortened 8a version of the PROMIS for pain interference will be used to measure the self-reported consequences of pain over the past 7 days. This 8-item questionnaire assesses how pain hinders daily activities. Each item on the measure is rated on a 5-point scale with a range of scores from 8 to 40. The PROMIS pain interference questionnaire completed by the subject electronically and will be both the source and eCRF.

10.27. Physical Examination

A physical examination of the oral cavity, head, eyes, ears, nose, and throat, cardiovascular system, lungs, abdomen, extremities, skin, neuropsychiatric mental status and sensory/motor status, musculoskeletal system and general appearance will be performed during screening. The nasal exam should specifically assess any nasal lesions that could be affected by repeated intranasal administrations, as this may be exclusionary. The physical exam will be updated on the day planned for randomization by querying the subject about any physical changes since the screening examination. An examination of the nasal mucosa will be performed again at Week 1 and at all clinic visits after the initiation of investigational products to assess local application site reactions. In addition, a limited nasal and oral mucosal inspection and any symptom directed physical examination will be performed, as applicable, at other visits if the subject reports any problems. Weight (pounds) will be collected per the schedule in Table 3. Abnormal findings will be reported as AEs, if appropriate.

10.28. Screen Failures Documentation

To document the reason that a subject who consented to the study was not randomized, the Reasons the Subject Was Not Eligible eCRF will be completed for these subjects.

10.29. Spielberger Trait Anxiety Inventory (STAI)

The Spielberger State-Trait Anxiety Inventory (STAI) has been used extensively in research and clinical practice (*Spielberger-1983*). The fundamental qualities evaluated by the STAI are feelings of apprehension, tension, nervousness, and worry. The assessment includes separate self-report scales for measuring state and trait anxiety. Only the trait anxiety scale will be collected in this study. The T-Anxiety scale (STAI Form Y-2) named on the form as "Self Evaluation Spielberger Trait Scale" consists of 20 statements that assess how people generally feel. This is a subject completed questionnaire that takes approximately 6 to 10 minutes to complete. This questionnaire will be completed by the subject electronically and will be both the source and eCRF.

10.30. Simplified Nutritional Appetite Questionnaire (SNAQ)

Oxytocin reduces appetite, body weight, reward and hunger driven eating. The 8-item Council on Nutrition appetite questionnaire scale and its 4-item derivative The Simplified Nutritional Appetite Questionnaire (SNAQ) were developed to assess appetite and predict weight loss. The changes in appetite during the study will be evaluated using the clinically simpler version of the two scales, the SNAQ (*Wilson-2005*). The 4 item SNAQ asks: 1) My appetite is; 2) When I eat; 3: Food tastes; and 4) Normally I eat; with 5 possible responses that are scored numerically from 1 to 5. The sum of the responses is the total scale score. A SNAQ score ≤14 indicates significant risk of at least 5% weight loss within six months. This questionnaire will be completed by the subject electronically and will be both the source and eCRF.

10.31. Subject Disposition

A subject disposition eCRF will be completed for all subjects who are randomized to the study and who are dispensed investigational product. This eCRF will be used to record the following data as applicable: 1) completion status of the subject at the end of their participation and if they were discontinued early, and reason for early discontinuation.

Completion status is as follows:

1. Subject completed full study (i.e., telephone contact was made at the Week 14/15 follow-up).
2. Subject completed the full intervention phase of the study (i.e., subject came to Week 13 clinic visit – during Week 13).
3. Subject was withdrawn prior to the Week 13 visit, but completed the end of study assessments scheduled at Week 13 (reasons for early withdrawal are to be specified).
4. Subject was withdrawn prior to the Week 13 visit and did not complete the end of study assessments (reasons for early withdrawal are to be specified).
5. Subject discontinued investigational products and the reason(s).

Even if the subject had investigational product suspended for any reason but attended clinic visits, the above definitions still apply.

In addition, if the subject was confined and/or incarcerated at any time during the study, the dates of confinement and/or incarceration will be collected.

10.32. TLFB Interview

Drinking behavior will be assessed using the TLFB methodology (*Sobell & Sobell-1992*). The TLFB is a semi-structured interview that provides estimates of the daily quantity of alcohol consumption during specified time periods. It uses a calendar prompt and a number of other memory aids (e.g., holidays, payday, and other personally relevant dates) to facilitate accurate recall of drinking or other drug use during the target period. The procedure has been widely used in clinical and research contexts. It has demonstrated adequate levels of reliability and validity when administered as an in-person interview, over the telephone, and when administered via computer (*Carey-1997, Sobell-1988, Sobell-1996*). After consent is signed, the TLFB interview will be performed for the 28-day period prior to signing consent. Thereafter, the interview will

be for the previous days between the last assessment and the day prior to the day of the assessment. It is estimated that a 28-day TLFB assessment will take 20 minutes to complete. In the event of missed visits, collection of missed drinking data at the following visit is required.

If a subject requests to withdraw from the study but agrees to continued telephone contact to assess drinking, the TLFB will be performed over the phone for the duration of the study at a frequency acceptable to the study subject and site staff.

An Excel spreadsheet customized for use in this study will be used for double data entry by clinical site staff to collect the TLFB drinking data. This spreadsheet contains a calculator to determine standard drink units (SDUs). This spreadsheet will be reviewed, compared with source documents and collected by study monitors for upload into the main study database.

Drinking days are defined as the number of days in which the subject reported any alcohol consumption (i.e., > 0 standard drinking units [SDUs]). A standard drink contains approximately 0.6 fluid ounces (oz) of pure alcohol. The data given by the subjects on amount and type of alcoholic beverage(s) consumed will be converted to SDUs. Standard drink unit definitions are provided in Table 5.

Table 5: Standard Drink Unit Definitions

For beer (~ 5% alcohol), the approximate number of SDUs in: • 12 oz = 1.0 • 16 oz = 1.3 • 24 oz = 2.0 • 40 oz = 3.3 For malt liquor (~ 7% alcohol), the approximate number of SDUs in**:** • 12 oz = 1.4 • 16 oz = 1.9 • 22 oz = 2.6 • 40 oz = 4.7
For table wine (~ 12% alcohol), the approximate number of SDUs in: • 750 mL bottle = 25oz = 5.0 • 5 oz glass = 1.0 • 10 oz glass = 2.0
For 80 proof spirits (~ 40% alcohol), or hard liquor, the approximate number of SDUs in: • 1.5 oz (mixed drink) = 1.0 • 12.7 oz (pint) = 8.5 • 25 oz (a fifth) = 17.0 • 1.75 L (59 oz) = 39.0

10.33. World Health Organization Drinking Risk Categorical Scale

The WHO has developed a drinking risk categorical scale that can be used in a responder analysis approach to assess clinically relevant decreases in alcohol consumption (*Aubin-2015*). Two dichotomous endpoints will be analyzed: WHO 1-level and WHO 2-level decrease in alcohol consumption. The WHO 1- and 2-level decrease endpoints are the percentage of subjects

experiencing at least a 1- and 2-level decrease in WHO levels of alcohol consumption, respectively, from the level at baseline (the period including the 28 days before screening) to the level during the treatment phase (e.g., Study Weeks 9-12).

The WHO levels of average alcohol consumption per day are as follows:

	Males	**Females**
Low Risk	1 to 40g	1 to 20g
Medium Risk	41 to 60g	21 to 40g
High Risk	61 to 100g	41 to 60g
Very High Risk	101+g	61+g

where 14g = 1 SDU (*WHO-2000*). In computing the WHO alcohol consumption level, average drinks per day will be used, computed as the sum of all drinks in the 28 day period divided by the number of days with non-missing drinking data in that period. Abstinent subjects will be included in a separate "Abstinent" category.

10.34. University of Pennsylvania Smell Identification Test

The UPSIT will be assessed at screening and at clinic visits shown in Table 3 to determine if intranasal investigational products cause any changes in sensory discrimination. It consists of 4 booklets each containing 10 microencapsulated (scratch and sniff) odors. The UPSIT test has been studied in individuals with alcohol addiction and has shown that this population generally had generally lower UPSIT scores that non-drinking controls. *Ditralgilia (1991)* reported that: "Thirty-two percent of the alcoholics' UPSIT scores, in comparison to five percent of the controls' scores, fell into the clinically impaired range. Although current smoking patterns correlated significantly with UPSIT indices, comparisons limited to nonsmokers still indicated that the alcoholics were significantly impaired on this olfactory task". Anosmia (loss of sense of smell) and microsmia (reduced ability to smell) will be reported as AEs when the following scores are reported: anosmia: score of 6 - 18, severe microsmia: score between 19 and 25, moderate microsmia: 26 - 30 in women and 26 - 29 in men, mild microsmia: 31 - 34 in women and 30 - 33 in men Normosmia (not an AE) is a score of more than 34 in women and 33 in men. If a subject presents with clinically impaired values at baseline, numeric decreases in the score will be reported as an AE when the score decreases to a more severe level of microsmia or anosmia.

10.35. Urge-to-Drink Questionnaire

The Urge to Drink Scale is a modified version of an assessment that was developed and validated by researchers at the University of Pennsylvania's Center for Studies of Addiction (*Flannery-1999*). This measure has been shown to be a valid and reliable instrument for assessing an individual's urge to drink alcohol. Total scores of 10 or higher during treatment have been reported to be associated with increased risk for relapse (*Flannery-1999*); *Flannery-2003*). This scale has 5 items with 7 different responses to each item scored as 0 to 7. The questions pertain to the past week. The total score is the sum of the point scores for the individual items.

10.36. Urine Drug Screen

An FDA cleared, CLIA waived urine drug test card will be used to assess candidates for recent use of opiates (i.e., morphine test), cocaine, amphetamines, methamphetamine, THC, buprenorphine, methadone, benzodiazepines, oxycodone, barbiturates, 3,4-methylenedioxy-methamphetamine (MDMA – also known as ecstasy). During screening subjects must be negative for all substances except THC and in special cases opioids. If positive for opioids but recent opioid use for acute pain is reported by the subject, then the subject can be re-screened. If positive for these drugs at other times during the study, the subject will not be removed from the study but should be asked about medication use and possibly have re-evaluation of their medical history for substance abuse.

10.37. Vital Signs

Vital signs to be assessed include sitting blood pressure and pulse rate (after sitting for at least 3 minutes).

11. Statistical Methods and Determination of Sample Size

11.1. Statistical and Analytical Plans

Complete details of the statistical analyses to be performed will be documented in a SAP, which will be completed prior to locking and unblinding the study data. This document will include more detail of planned statistical analyses and additional exploratory analyses. Any changes to the final SAP will be outlined in the final study report.

11.2. Statistical Hypotheses

Primary Efficacy Endpoint: It is hypothesized that oxytocin, as compared to placebo will reduce the weekly percentage of heavy drinking days over the 10-week maintenance treatment period. This hypothesis will be tested using other time periods during treatment.

Secondary Efficacy Drinking and Smoking Endpoints:

Over the 10-week maintenance treatment period, it is hypothesized that the oxytocin group, as compared to the placebo group, will:

1. Increase the percentage of subjects with no heavy drinking days
2. Increase the percentage of subjects abstinent from alcohol
3. Increase the percentage of subjects with a WHO drinking risk category decrease of at least:
    a. 1-level
    b. 2-levels
4. Increase the percentage of days abstinent per week
5. Decease the weekly mean number of drinks per week
6. Decrease the weekly mean drinks per drinking day
7. Decrease the cigarettes smoked per week among smokers
8. Increase the smoking abstinence rate among smokers
9. Decrease the weekly number of days of nicotine use among nicotine users
10. Decrease in attachment related anxiety scores of the ECR-RS
11. Decrease in PROMIS sleep disturbance, alcohol negative consequences, and pain interference scores
12. Decrease in POMS subscale tension-anxiety and depression scores
13. Decrease in the Urge to Drink scale scores

11.3. Analysis Populations

The study analysis populations will consist of the following:

Full Analysis Set: The full set is defined as subjects randomized to participate in the study who took at least one dose of investigational product. The full analysis set will be used to evaluate all efficacy and safety endpoints.

11.4. Description of Statistical Methods

11.4.1. General Approach

For descriptive purposes, dichotomous and categorical variables will be presented as number of observations and percentages; continuous variables will be given as means, standard deviations (SD), median, minimum (min) and maximum (max). Statistical tests will be two-tailed at a 0.05 Type I error rate. P-values for the primary and secondary endpoints of < 0.05 will be considered statistically significant. The SAP will define the covariate selection process. Endpoint data will also be screened for outliers and skewness. Appropriate non-parametric tests will be used to compare treatment groups on continuous baseline characteristics that are not normally distributed. Continuous endpoint data that are not normally distributed will be transformed. Cohen's d will be used to calculate the effect size for means and Cohen's h will be used to calculate the effect size for proportions. Odds ratios will be provided for all dichotomous outcomes and least squares means will be provided for all continuous endpoints. Descriptive statistics – mean, SD, median, min and max – of all endpoint data will be provided for each assessment point or summarized at each week for drinking endpoints. All data will be presented in listings.

11.4.2. Analysis Addressing the Primary Efficacy Endpoint

The analysis of the primary endpoint will be a mixed-effect model for repeated measures. Treatment group, study week, treatment group by study week interaction, clinical site and baseline percentage of heavy drinking days will be covariates in the mixed effects model. Additional covariates may be selected as specified in the SAP. The primary endpoint will be performed on available data in the Full Analysis Set with no imputation for missing data. A sensitivity analysis will be performed using multiple imputation for missing drinking data (*Jakobsen-2017*). Multiple imputation replaces each missing value with a set of "m" plausible values that represent the uncertainty about the right value to impute. The imputation model will include a limited set of variables likely to be associated with missingness on the primary endpoint, excluding treatment assignment. The variables may include but are not limited to site, time and baseline alcohol use. Specification of the variables used in the multiple imputation models will be provided in the SAP.

11.4.3. Secondary Efficacy Endpoints Analysis

Analysis of the continuous secondary endpoints (weekly mean drinks per week, weekly mean drinks per drinking day, percentage of days abstinent, number of cigarettes smoked per week, number of days of other nicotine product use per week, POMS scores, ECR-RS scores, PROMIS scores, and Urge to Drink scores will be analyzed in a similar fashion to that of the primary outcome (except substituting baseline equivalent of the outcome). Analysis of the dichotomous secondary outcomes (percentage of subjects with no heavy drinking days, percentage subjects abstinent from alcohol, percentage of subjects with 1- and 2-level decreases in WHO drinking categories, and percentage of smokers abstinent from smoking) will be conducted via logistic

regression of the endpoint measured across the entire treatment period (monthly and with grace periods, as specified). Secondary analyses to satisfy statistical hypotheses will be performed on data collected during the 10-week maintenance period; however, as appropriate descriptive statistics will be used to present these data during the initial 2-week period as well. Covariate selection beyond treatment group, clinical site, and baseline equivalent of the endpoint will be specified in the SAP. These analyses will be performed using the Full Analysis Set subjects with no imputation for missing data.

11.4.4. Safety Analyses

AEs will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA) and will be grouped by system organ class (SOC) and preferred term (PT) designation. The severity, frequency, and relationship of AEs to investigational product will be presented by SOC and PT groupings. Listings of each individual AE including start date, stop date, severity, relationship, outcome, and duration will be provided. Each AE (based on PT) will be counted once only for a given study subject. If the same AE occurred on multiple occasions, the highest severity will be assumed. Thus, study subjects are not counted multiple times in a given numerator in the calculation of frequencies for a specific AE. C-SSRS reports of suicidality or suicidal ideation will be reported as AEs and analyzed as AEs if the investigator determines after an interview with the subject that the responses are consistent with suicidal ideation or attempt.

Clinical chemistry, urine drug screen results, pregnancy test results, BAC results, and SNAQ scores will be reported as summary statistics for each assessment time point. The percentages of subjects in each group with a SNAQ score ≤14 at each visit will be presented. Vital signs will be presented as summary statistics and change from baseline. The proportions of ECG results considered clinically significant will also be provided. In addition, change from baseline (shift tables) will also be presented for clinical chemistry data. The numbers and percentages of subjects who had urine tests positive for THC at each time point for all subjects, for subjects who were positive at baseline, and who were negative at baseline (representing new use) will be summarized. The numbers and proportion of subjects who reported CIWA-AR scores ≥ 10 at any time after the start of dosing will be presented. Buss-Perry Aggression scores, SNAQ scores and body weight changes will be presented for each visit and will be compared between groups using repeated measures ANOVA. The numbers and proportions of subjects mild, moderate, severe microsomia or any smell loss (anosmia) compared with screening assessment using the UPSIT will also be presented.

11.4.5. Compliance and Retention Analyses

Medication compliance, defined as the amount of investigational product taken as a proportion of the total amount prescribed per protocol, will be evaluated for the oxytocin and placebo groups based on the Subject Diary. Average amounts of investigational product taken (volume determined from weighing the vials prior to and after dispensing and subtracting the difference) overall and weekly will be reported for the oxytocin and placebo groups. The research participation rate, defined as percentage of subjects with complete drinking data, will be compared between treatment groups. In addition, the percentage of subjects discontinuing medication or early withdrawal from the study and a listing of these reasons for discontinuation will be provided.

11.4.6. Baseline Descriptive Statistics

Summaries of the characteristics of the subjects in each of the study groups at baseline will be prepared. Baseline characteristics will be compared between the oxytocin and placebo groups using appropriate statistical methods. A summary will be prepared to show dropouts/retention over time in each group, along with the reason for early termination. The number of missing observations will be presented between groups.

11.4.7. Exploratory Analyses

A number of variables will be tested as potential moderators of the medication treatment effect on the PHDD primary endpoint. Weeks 3-12 will be the period of interest. Continuous moderator variables will be dichotomized. The potential moderator variables that will be examined include measures of anxiety and depression (ECR-RS subscales of attachment and anxiety, POMS subscales of depression and anxiety, STAI, and BIS), those suggestive of alcohol withdrawal (i.e., withdrawal question on the MINI for alcohol use disorder), and alcohol-related treatment goal.

There is interest in exploring the use of a new Hyperkatifeia Scale as a predictor and moderator of drug response. Exploratory analyses utilizing Hyperkatifeia Scale scores will include: 1) conducting moderator analyses using the total score; and 2) comparison of change from baseline between treatment groups for total score and subscales scores.

11.4.8. *Ad Hoc* Analyses

Ad hoc analyses may be performed on the Hyperkatifeia Scale as a planned part of exploring the utility of this scale including total scores, subscales, and individual items. *Ad hoc* analyses utilizing Hyperkatifeia Scale scores may include: 1) identification of baseline subsets and relationship to drinking outcomes; 2) conducting mediator analyses; 3) factor analysis to better understand the underlying dimensions of hyperkatifeia; and, 4) validity of the Hyperkatifeia Scale by comparing to other measures (e.g., POMS and PROMIS scales or subscale scores). The data from this study could be used for meta-analyses with results of other studies to expand data availability for the proposed *ad hoc* analyses.

11.4.9. Adjustment for Covariates

Covariates used in logistic regression and mixed effects models are described in section 11.4.2 and 11.4.3.

11.4.10. Interim Analyses and Data Monitoring

No interim analyses are planned for this study.

11.4.11. Multiple Comparison/Multiplicity

No adjustments will be made in comparisons of multiple secondary endpoints as this is a pilot study designed to explore the potential of oxytocin for the treatment of AUD.

11.4.12. Tabulation of Individual Response Data

Individual subject data will be listed by measure and time point as appropriate.

11.5. Determination of Sample Size

With an intake sample of 100 subjects (50 subjects per arm) and 12% attrition, it is projected that the sample size for primary endpoint analyses will be 88 (44 subjects per arm). The alpha level for the primary analyses will be 0.05, two-tailed. An estimate of the effect size (Cohen's d) for the investigational products is 0.60. Equal variances in both groups are assumed. These assumptions lead to a projected power of 0.80.

12. Quality Control and Quality Assurance

This study will be conducted under International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines and all applicable regulatory requirements.

Actions to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study centers; the review of protocol procedures with the investigator and study personnel prior to study start; the design of suitable source documents with appropriate instructions for use; the internal audit of source data according to GCP and internal procedures to ensure their accuracy, completeness, and verifiability; as well as the periodic site monitoring by the Sponsor's representatives (clinical monitors of Fast-Track Drugs & Biologics, LLC). Written instructions will be provided for collection, preparation, and shipment of blood samples. Clinical monitors will review source documents and eCRFs for accuracy and completeness during on-site monitoring visits; any discrepancies will be resolved with the investigator, as appropriate.

Significant and/or repeated noncompliance will be investigated and remedial action instituted when appropriate. Failure to comply with remedial actions may result in study site termination and regulatory authority notification.

12.1. Study Monitoring

Study monitoring will be the responsibility of designated clinical monitors of Fast-Track. Monitors will assure compliance with the clinical protocol and ICH GCPs, human subject's protection, drug accountability, maintenance of the site regulatory file, and conformance of eCRF data with source documents. Monitoring visits by clinical monitors will be scheduled to take place at the initiation of the study, during the study at appropriate intervals, and after the last subject has completed the study. A report of monitoring observations will be provided to the PI (for corrective actions) and the Sponsor.

12.2. Audits and Inspections

Authorized representatives of the Sponsor, the FDA, and the IRB may visit the site to perform audits or inspections, including source data verification. The purpose of the audit or inspection is to systematically and independently examine all study related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines, and any applicable regulatory requirements.

The PI should contact the Sponsor's representative and Fast-Track if contacted by a regulatory agency about an inspection.

13. Ethics

13.1. Central Institutional Review Board

NIH has issued a policy requiring that NIH-funded, multi-site research use a single IRB of record for the ethical review of human subjects research protocols funded by the NIH that are carried out at more than one site in the United States. Advarra IRB will be the central IRB for this study. Advarra IRB will review the protocol, informed consent, advertisements and any other materials given to subject, as well as progress reports on a continuing basis in accordance with all applicable regulations, including Title 21, Code of Federal Regulations (CFR), Parts 50 and 56.

13.2. Ethics Review

The study will be conducted under a protocol reviewed by the central IRB; the study is to be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study will ensure that the hazards do not outweigh the potential benefits; the results to be reported will be accurate; subjects will give their informed consent and will be competent to do so and not under duress; and all study staff will comply with the ethical principles in 21 Code of Federal Regulations (CFR) Part 50 and the Belmont Principles.

13.2.1. Review/Approval of Study Protocol

The study may not begin until the IND has been submitted to the FDA and the 30-day waiting period has expired without notification by FDA to the Sponsor of any clinical hold issues. NIAAA will be the study Sponsor. The site must obtain written approval from the IRB to conduct the study before study initiation. NIAAA will issue a formal authorization letter for the study to be initiated at the site. Progress reports will be submitted to the IRB by the Investigator at the frequency requested by the IRB.

13.2.2. Protocol Modifications

All necessary protocol changes will be submitted in writing as protocol amendments to the IRB by Fast-Track for approval prior to implementation. NIAAA will submit all protocol amendments to the FDA.

13.2.3. Protocol Deviation Reporting Procedures

All subject-specific deviations from the protocol are to be documented. The PI or designee will be responsible for identifying and reporting all deviations, which are occurrences involving a procedure that did not follow the study protocol. Any protocol deviation that adversely affects the safety or rights of a subject or scientific integrity of the study is considered a major deviation and will be reported immediately to the NIAAA Project Manager and the IRB.

13.3. Ethical Conduct of the Study

This study will be conducted in accordance with all applicable Federal human research protections requirements and the Belmont Principles of respect for persons, beneficence, and justice.

The procedures set out in this study are designed to ensure that the sponsor's representative and all study personnel abide by the principles of the ICH GCP Guideline and the Code of Federal Regulations (CFR). The PI confirms this by signing this study protocol and Form FDA 1572.

13.3.1. Confidentiality

13.3.1.1. Confidentiality of Data

Particular attention is drawn to the regulations promulgated by the FDA under the Freedom of Information Act providing, in part, that proprietary information furnished to clinical investigators and IRB will be kept confidential by the FDA only if maintained in confidence by the clinical investigator and IRB.

By signing this protocol the investigator affirms to NIAAA that information furnished to the investigator by NIAAA will be maintained in confidence and such information will be divulged to the IRB or similar or expert committee; affiliated institution; and employees only under an appropriate understanding of confidentiality with such board or committee, affiliated institution and employees.

13.3.1.2. Confidentiality of Subject Records

To maintain subject confidentiality, all laboratory specimens, eCRFs, reports and other records will be identified by a subject number only. Research and clinical records will be stored in a locked cabinet. Only research staff, NIAAA program officials, and Fast-Track Drugs & Biologics clinical monitors will have access to the records. Subject information will not be released without written permission, except as necessary for monitoring by representatives of NIAAA. Upon approval of the study by an IRB, an application will be filed with NIAAA for a Certificate of Confidentiality.

By signing the protocol the investigator agrees that within local regulatory restrictions and ethical considerations, NIAAA or any regulatory agency may consult and/or copy study documents in order to verify eCRF data.

13.3.2. Compensation for Participation

Subjects will be compensated for travel expenses and for time contributed to this research study in the form of cash or vouchers. Compensation will be provided in increasing amounts with each subject visit and is detailed in the informed consent form.

13.3.3. Written Informed Consent

The informed consent process and document will be reviewed and approved by the IRB and sponsor's representative prior to initiation of the study. The consent document contains a full explanation of the possible risks, advantages, and alternate treatment options, and availability of treatment in the case of injury, in accordance with 21 CFR Part 50. The consent document indicates that by signature, the subject, permits access to relevant medical records by the sponsor's representative and by representatives of the FDA. The sponsor's representative will submit a copy of the initial IRB- and sponsor's representative-approved consent form to the FDA and will maintain copies of revised consent documents that have been reviewed and approved by the IRB.

A written informed consent document, in compliance with 21 CFR Part 50, 32 CFR Part 219, and the Belmont Principles, and HIPAA Authorization will be signed by the subject before any study-related procedures are initiated for each subject.

All potential subjects for the study will be given a current copy of the Informed Consent Form to read. All aspects of the study and informed consent will be explained in lay language to the subject by either the investigator, or a medically trained designee. Any subject who is unable to demonstrate understanding of the information contained in the informed consent will be excluded from study participation.

All study subjects will be given a copy of the signed informed consent.

13.3.4. Delegation of Responsibilities and Adequate Resources

The PI should have adequate time to conduct the study properly and should have an adequate number of qualified staff to assist with the conduct of the study.

The term “investigator” used throughout this protocol refers to the PI and/or qualified subinvestigators. The PI may delegate responsibilities to other study site personnel. The PI shall delegate tasks only to individuals qualified by education, training, and experience to perform the delegated tasks. The PI shall have direct oversight of all delegated activities and shall document delegation of responsibilities. The PI is responsible for ensuring all delegated staff has been properly trained on the protocol and their assigned study responsibilities. A delegation log identifying all delegated duties and the individual to whom they have been delegated will be maintained at the study site.

13.3.5. Financial Disclosure

Clinical investigators are required to provide financial disclosure information for the submission of certification or disclosure statements required under 21 CFR § 54. As defined in 21 CFR § 54.2, a clinical investigator is a listed or identified investigator or subinvestigator who is directly involved in the treatment or evaluation of research subjects. The term also includes the spouse and each dependent child of the investigator. In addition, investigators must promptly update financial disclosure information if any relevant changes occur during the course of the investigation and for 1 year following completion of the study.

14. Data Handling and Record Keeping

Source documents include but are not limited to original documents, data and records such as hospital/medical records (including electronic health records), clinic charts, laboratory results, data recorded in automated instruments, and pharmacy records, etc. This study will use an electronic data management system (EDMS) (IBM Clinical Development) and eCRFs. Data will be transcribed from source documentation into web-based eCRFs. Only questionnaire data will be entered directly into eCRF (i.e., without prior written or electronic record of data). Paper copies of the eCRFs will be provided in the event that the site cannot access the EDMS at the time the questionnaire is being completed. The transcribed data will be consistent with the source documents or the discrepancies will be explained.

Clinical monitors will review all source records and compare them to the data entered into the eCRF. All entries, corrections, and alterations will be made by the investigator or other authorized study personnel. Any errors identified during monitoring will have a query posted by monitor for site staff to address. The EDMS system maintains a full audit trail of data entry, data corrections, and data queries.

14.1. Subject Identification and Confidentiality

Subjects will be identified on eCRFs by a unique subject number. No personal identifier will be used in any publication or communication used to support this research study. The subject number will be used if it becomes necessary to identify data specific to a single subject. The Sponsor's representative and designated clinical monitors of Fast-Track, the IRB, and the FDA are eligible to review medical and research records related to this study as a part of their responsibility to protect human subjects in clinical research. Personal identifiers will be removed from photocopied or electronic medical and research records.

14.2. Inspection of Records

The sponsor's representative or designee will be allowed to visit the investigation facilities for the purpose of monitoring any aspect of the study. The investigator agrees to allow the monitor to inspect the drug storage area, investigational product stocks, drug accountability records, subject charts, study source documents, and other records relative to study conduct.

Subjects' health information is used to report results of research to the sponsor's representative and Federal regulators and may be reviewed during study audits for compliance with study plans, regulations, and research policies. The consent document indicates that by signature, the subject permits access to relevant medical records by the sponsor's representative and by representatives of the FDA.

Upon a subject's termination from the trial, completed eCRFs will be ready and available for on-site review by the sponsor's representative at scheduled monitoring visits.

14.3. Retention of Records

The investigator is responsible for creating and/or maintaining all study documentation required by Title 21 Code of Federal Regulations (21CFR) Parts 50, 54, 56, and 312, ICH E6 section 8, as well as any other documentation defined in the protocol. The investigator must provide key

documents to the Sponsor prior to start of the study. A complete list of required regulatory documents will be provided in the study Manual of Procedures.

Federal and local regulations require that the investigator retain a copy of all regulatory documents and records that support the data for this study for whichever of the following is the longest period of time:

- A period of 2 years following the final date of approval by the FDA or other regulatory agency of the study drug for the purposes that were the subject of the investigation; or
- A period of 5 years following the date on which the results of the investigation were submitted to the FDA or other regulatory agency in support of, or as part of, an application for a research or marketing permit for the study drug for the purposes that were the subject of the investigation.

The Sponsor will notify investigators once one of the above 2 timeframes has been satisfied.

If the investigation does not result in the submission of the data in support of, or as part of, an application for a research or marketing permit, records must be retained for a period of 2 years following notification by the Sponsor that the entire clinical investigation (not merely the investigator's portion) is completed, terminated, or discontinued or 2 years following withdrawal of the Investigational New Drug application/Clinical Trial Authorization or request for marketing approval (New Drug Application/Marketing Authorization Application).

If the investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The Sponsor must be notified in writing of the name and address of the new custodian. Study records should not be destroyed without consultation with the Sponsor.

14.4. Trial Registration

As the IND holder and Sponsor, NIAAA will register the trial on the National Library of Medicine's Clinical Trials Registry on the world wide web at http://www.clinicaltrials.gov.

15. Publication Policy

Review of manuscripts resulting from this study or from data generated during this study must occur according to the NIAAA Publications Policy prior to submission for publication. Authorship shall be consistent with NIAAA policies.

16. Protocol Signature Page

NIAAA REPRESENTATIVES

Typed Name	Signature	Date
Raye Z. Litten, Ph.D.	_____	_____
Daniel E. Falk, Ph.D.	_____	_____
Jenica Patterson, Ph.D.	_____	_____
Megan Ryan, MBA, CCRP	_____	_____

INVESTIGATORS

I agree to conduct this clinical study in accordance with the design and specific provisions of this protocol; deviations from the protocol are acceptable only with a mutually agreed upon protocol amendment with the IRB approval. I also agree to report all information or data in accordance with the protocol, and in particular I agree to report any serious adverse experiences as defined in section 10.2.7 of this protocol.

Typed Name	Signature	Date
_____	_____	_____
_____	_____	_____

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18. Appendices

Appendix A. Example Drug Diary Card

Subject ID Number: _____ Bottle # 1

Administer one dose (5 sprays in alternating nostrils) each morning.

Days	Date	# sprays morning
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		

Subject ID Number: _____ Bottle # 2 - 6

Administer one dose (5 sprays in alternating nostrils) each morning and each evening.

Bottle #	Days	Date	# sprays morning	# sprays evening
	15			
	16			
	17			
	18			
	19			
	20			
	21			
	22			
	23			
	24			
	25			
	26			
	27			
	28			

Appendix B. Common Terminology Criteria for Adverse Events for Clinical Laboratory Adverse Events and Elevated Blood Pressure

The following are the toxicity grades for which grading criteria are provided by the CTCAE (Version 4.03) for chemistry tests performed in this study and for elevated blood pressure (hypertension).

Table 6: CTCAE Criteria for Clinical Laboratory Adverse Events and Blood Pressure Elevations

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4
ALT elevated	>ULN – 3.0 x ULN	>3.0 – 5.0 x ULN	>5.0 – 20.0 x ULN	>20.0 x ULN
AST elevated	>ULN – 3.0 x ULN	>3.0 – 5.0 x ULN	>5.0 – 20.0 x ULN	>20.0 x ULN
Total bilirubin elevated (bilirubinemia)	>ULN – 1.5 x ULN	>1.5 – 3.0 x ULN	>3.0 – 10.0 x ULN	>10.0 x ULN
Hypernatremia (elevated sodium)	>ULN - 150 mmol/L	>150 - 155 mmol/L	>155 - 160 mmol/L; hospitalization indicated	>160 mmol/L; life-threatening consequences
Hyponatremia (low sodium)	<LLN - 130 mmol/L	-	<130 - 120 mmol/L	<120 mmol/L; life-threatening consequences
Hyperkalemia (elevated potassium)	>ULN - 5.5 mmol/L	>5.5 - 6.0 mmol/L	>6.0 - 7.0 mmol/L; hospitalization indicated	>7.0 mmol/L; life-threatening consequences
Hypokalemia (low potassium)	<LLN - 3.0 mmol/L	<LLN - 3.0 mmol/L; symptomatic; intervention indicated	<3.0 - 2.5 mmol/L; hospitalization indicated	<2.5 mmol/L; life-threatening consequences
Creatinine elevated	>ULN - 1.5 x ULN	>1.5 - 3.0 x ULN	>3.0 - 6.0 x ULN	>6.0 x ULN
Hypertension	Pre-hypertension (systolic BP 120 - 139 mmHg or diastolic BP 80 - 89 mmHg)	Stage 1 hypertension (systolic BP 140 - 159 mmHg or diastolic BP 90 - 99 mmHg); medical intervention indicated; recurrent or persistent (24 hrs); symptomatic increase by >20 mmHg (diastolic) or to >140/90 mmHg if previously WNL; monotherapy indicated	Stage 2 hypertension (systolic BP≥160 mmHg or diastolic BP ≥100 mmHg); medical intervention indicated; more than one drug or more intensive therapy than previously used indicated	Life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis); urgent intervention indicated

Appendix C. Hyperkatifia Scale

People sometimes experience uncomfortable feelings or sensations when they don't drink alcohol for some time. These feelings or sensations could happen when waking up after overnight sleep, after a long day at work, or not drinking for several days or weeks.

At baseline: Take a few minutes to think back to before you decided to enter this study and change your drinking. Think about how you felt during the time(s) you couldn't have a drink. Please read each phrase and circle a number 0, 1, 2, 3, or 4 that indicates how intensely you felt these feelings and sensations when you were not drinking alcohol.

At end of study: During this study, think about how you felt during the time(s) you didn't drink. Please read each phrase and circle a number 0, 1, 2, 3, or 4 that indicates how intensely you felt these feelings and sensations when you were not drinking alcohol.

The rating scale is as follows:
0 Not at all
1 A little
2 Moderately
3 Quite a lot
4 Extremely

1	Unhappy	0 1 2 3 4
2	Low energy	0 1 2 3 4
3	Distressed	0 1 2 3 4
4	Grouchy	0 1 2 3 4
5	Uneasy	0 1 2 3 4
6	Unable to concentrate	0 1 2 3 4
7	Fatigued	0 1 2 3 4
8	Anxious	0 1 2 3 4
9	Depressed	0 1 2 3 4
10	Uncomfortable	0 1 2 3 4
11	Nervous	0 1 2 3 4
12	Restless	0 1 2 3 4
13	Irritable	0 1 2 3 4
14	Discontented	0 1 2 3 4
15	On edge	0 1 2 3 4
16	Annoyed	0 1 2 3 4
17	Bad tempered	0 1 2 3 4
18	Tense	0 1 2 3 4
19	Revved up	0 1 2 3 4
20	Light touches are painful	0 1 2 3 4
21	Unable to sleep	0 1 2 3 4
22	Angry	0 1 2 3 4

23	Fearful	0 1 2 3 4
24	More sensitive to pain	0 1 2 3 4

When completing this checklist, I was thinking about a time when I didn't drink alcohol for:

- A few hours
- Overnight or sleeping hours
- 1-2 days
- 3-5 days
- 6-7 days
- More than a week

Scale and subscale scoring:

The total score is the sum of the individual items.

Subscale scores are the sum of the individual items for each subscale as follows:

Stress/Anxiety = items 3 + 5 + 6 + 8 + 11 + 15 + 18 + 19 + 21 + 23

Depression = items 1 + 2 + 7 + 9

Irritable = items 4 + 12 + 13 + 14 + 16 + 17 + 22

Pain = items 10 + 20 + 24