1. Protocol Synopsis

Name of Sponsor: National Institute on Alcohol Abuse and Alcoholism (NIAAA)	
Name of Investigational Product: ANS-6637	
Name of Active Ingredient: ANS-6637	
Protocol Number: HLAB-002	
Study Title: Human Laboratory Study of ANS-6637 for Alcohol Use Disorder	
NIAAA Principal Investigator (PI): Raye Litten, Ph.D.	
Clinical Centers/PIs: Robert Miranda, Ph.D., Brown University; Stephanie O'Malley, Ph.D., Yale University; Joseph P. Schacht, Ph.D., Medical University of South Carolina	
Study Period: Approximately 1 year	**Phase of Development:** 2
Objectives: **Primary:** The primary objective of this study is to evaluate the effects of two different doses of ANS-6637, 200 mg (given as 2 x 100 mg tablet) and 600 mg (given as 2 x 300 mg tablet) once a day, and matched placebo, on alcohol cue-elicited alcohol craving during a human laboratory paradigm after 1 week of daily dosing among subjects with moderate to severe alcohol use disorder (AUD) as confirmed by the Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5™). **Secondary:** Secondary objectives include evaluation of ANS-6637 200 mg, ANS-6637 600 mg, and matched placebo on reduction of alcohol consumption, alcohol craving, cigarette smoking (among smokers) and nicotine use (among nicotine users), mood, sleep, alcohol use negative consequences, study retention, and safety and tolerability throughout the last 4 weeks of the treatment phase of the study.	
Methodology: This study is a 3-arm, double-blind, randomized, placebo-controlled, parallel group, 3-site study designed to assess the effects of ANS-6637 as compared with placebo on responses to *in vivo* alcohol cue exposure in the human laboratory setting. After signing informed consent, subjects will be screened for eligibility and have other baseline assessments. Screening is permitted over a 14-day period and most baseline assessments will be performed on the day of randomization. Assessments include alcohol breathalyzer test (before signing consent), medical history, physical examination, vital signs, electrocardiogram (ECG), drinking history by the timeline follow-back (TLFB) method, Clinical Institute Withdrawal Assessment for Alcohol-revised (CIWA-AR), prior medication use, MINI neuropsychiatric interview, urine drug test, smoking quantity frequency and nicotine use interview, clinical laboratory tests including chemistry, hematology, thyroid function tests, and medical urinalysis, craving responses during cue reactivity session, Columbia Suicide Severity Rating Scale (CSSR-S), drinking goal, Penn Alcohol Craving Scale (PACS), Pittsburg Sleep Quality Index (PSQI), PROMIS Alcohol Negative Consequences short form, Profile of Moods State (POMS), blood sample for aldehyde dehydrogenase 2 (ALDH2) deficiency genetic test and confirmation that subjects are treatment seeking and desire a reduction or cessation of drinking. Women of child-bearing potential will have a pregnancy test. If eligible for the study, 81 subjects will be randomized using a stratified permuted block randomization procedure in an approximate 1:1:1 ratio (targeting 27 subjects per group and 27 subjects per each of 3 clinical sites) to receive either ANS-6637 200 mg once daily, ANS-6637 600 mg once daily, or matched placebo for 5 weeks. Clinical site will be used as the stratification variable. Subjects will be seen in the clinic at screening, at randomization and 5 other times during the study. A final follow-up telephone interview will occur 2 weeks after the end of study in-clinic visit. After the first week of investigational product administration at Study Week 2, subjects will undergo a cue reactivity session including 4 individual visual analog scale (VAS) items assessing alcohol craving and 1 item assessing beverage liking. Other assessments during the treatment period include TLFB, clinical laboratory tests, blood for serum levels of study drug for determining medication compliance, vital signs, ECG, concomitant medications, CIWA-AR, C-SSRS, pregnancy test, male and female birth control methods, adverse events (AEs), PACS, smoking quantity/frequency, PSQI, and POMS. The end of study visit will include all of the weekly assessments and a	

final ECG, Exit Interview, PROMIS questionnaire, and a treatment referral.
Number of Subjects (Planned): 81
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 (moderate to severe AUD). They must also be seeking treatment for AUD and if male, report drinking an average of at least 35 drinks per week or if female report drinking an average of at least 28 drinks per week prior to consent, and have at least 1 heavy drinking day (4 or more drinks per day for women/5 or more drinks per day for men) during the 7-day period prior to randomization.
Investigational Product, Dosage and Mode of Administration: The target doses are 200 mg (2 x 100 mg tablets) and 600 mg (2 x 300 mg tablets) of ANS-6637 by oral administration once daily for 5 weeks.
Reference Therapy, Dosage and Mode of Administration: Subjects in the placebo group will take an equivalent number of identically matched placebo tablets (2 per day) by oral administration once daily for 5 weeks.
Duration of Study: Each subject will participate in the study for up to 10 weeks, including up to 2 weeks of screening, 5 weeks of treatment, one end-of-study visit during the week following the last treatment dose, and a final telephone contact 2 weeks after completing treatment for a safety follow-up.
Criteria for Evaluation: **Primary:** The primary efficacy endpoint is the "strength" of alcohol craving Visual Analog Scale (VAS) score (item 1 below) upon presentation of the first alcohol cue at Week 2 – after one week of investigational product treatment. Confirmatory secondary endpoints include the VAS scores for the other 3 VAS scales (items 2 through 4 below) for the first alcohol cue and the average score of the 4 VAS craving items; and the difference score (alcohol cue VAS score minus water cue VAS score) for all 4 of the individual VAS craving items and their average score. The beverage liking VAS item is also a confirmatory secondary endpoint. The 4 VAS craving items in order of appearance are: 1. How strong is your craving to drink alcohol? - note this is the primary efficacy endpoint 2. Having a drink would make things just perfect. 3. If I could drink alcohol now, I would drink it. 4. It would be hard to turn down a drink right now. The beverage liking item is: How much did you like the beverage just shown to you? **Secondary:** Secondary efficacy endpoints will be analyzed over the last 4 weeks of the treatment period. 1. Percentage of subjects with no heavy drinking days. A "heavy drinking day" is 4 or more drinks per drinking day for women and 5 or more drinks per drinking day for men. 2. Percentage of subjects abstinent from alcohol. 3. Percentage of subjects with at least a World Health Organization (WHO) 2-level decrease in alcohol consumption. 4. Percentage of subjects with at least a WHO 1-level decrease in alcohol consumption. 5. Percentage of days abstinent per week. 6. Percentage of heavy drinking days per week. 7. Percentage of very heavy drinking days per week. A "very heavy drinking day" is 8 or more drinks per drinking day for women and 10 or more drinks per drinking day for men. 8. Weekly mean number of drinks per week. 9. Weekly mean drinks per drinking day. 10. Cigarettes smoked per week among smokers. 11. Percentage of subjects with no nicotine use among those reporting nicotine use at baseline. 12. Alcohol craving score (PACS). 13. Sleep quality (PSQI) score. 14. Profile of Mood States (POMS) score (and subscale scores).

15. PROMIS Alcohol Negative Consequences score.

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

1. Vital signs
2. Body weight
3. Clinical laboratory parameters
4. BAC by breathalyzer
5. Urine drug tests
6. AEs (included elicited AEs and AEs in subjects taking a CYP 3A4 sensitive substrate)
7. ECG results
8. CIWA-AR scores
9. Frequency of subjects with suicidal ideation at any time during the treatment period (C-SSRS)
10. Concomitant medication use
11. Alcohol Craving Questionnaire – Short Form – Revised (ACQ-SF-SR) score (pre- and post-cue response sessions)

Compliance: Self report of compliance with study drug self-administration is confirmed by digital photography (AiCure software) with investigational products and GS-548351plasma levels. GS-548351 is the active moiety of the prodrug ANS-6637.

Statistical Methods (Data Analysis):

Analysis Populations:

Modified intention-to-treat (mITT) Analysis Set: The mITT set is defined as subjects randomized to participate in the study that took at least one dose of investigational product and had a non-missing VAS craving primary endpoint.

Evaluable Analysis Set: The evaluable analysis set for the secondary endpoints is defined as those subjects randomized to the study who took at least 2 tablets per day for at least 80% of days in Weeks 1-5.

Safety Analysis Set: The safety analysis set includes all subjects who took at least one dose of investigational product.

Analysis of the Primary Efficacy Endpoint: Each subject will have an initial alcohol cue for "strength" of alcohol craving score from the VAS that is the primary endpoint. Analysis of covariance (ANCOVA) with the "strength" of alcohol craving value as the dependent variable and the pretreatment "strength' of alcohol craving score from the first alcohol cue as an independent fixed effect. Clinical site will also be included as an independent factor. There are 3 comparisons (ANS-6637 600 mg vs placebo; ANS-6637 200 mg vs. placebo; and ANS-6637 600 mg vs. ANS-6637 200 mg) and Tukey's method will be used to adjust for multiple comparisons. .No imputation for missing endpoint data will be performed.

Analysis of the Secondary Endpoints: There are 3 additional questions asked during the cue session for each beverage cue. Each of these questions will be analyzed in the same manner as the primary endpoint. Tukey's method will be used within question and not across questions. An overall mean of the 4 questions will also be analyzed in the same manner. The difference between the first alcohol cue and water cue for each VAS item will be computed at both the pre and post treatment time points. The difference values for each VAS item and the average difference will be analyzed similarly to the primary endpoint. Continuous secondary endpoints (percent heavy drinking days, percent very heavy drinking days, percent days abstinent, drinks per week, drinks per drinking day, number of cigarettes smoked per week, PACS, POMS, and PROMIS, and PSQI score) during the last 4 weeks of treatment period will be analyzed using a mixed-effects model with site, assessment time, and baseline drinking as fixed factors. Models will also include time by treatment group interaction term. Additional covariates may be included that are significantly correlated with outcome and/or if there are differences across the treatment groups.

Analysis of the dichotomous secondary endpoints (percentage subjects with no heavy drinking days, percentage subjects abstinent from alcohol, percentage of subjects achieving at least a one and two-level shift in WHO alcohol consumption, and percentage of subjects with no nicotine use among subjects with any use during the week before randomization) during the last 4 weeks of treatment period will be conducted via logistic regression. Covariates may be included provided there are a sufficient number of events.

No imputation for missing endpoint data will be performed for secondary endpoints.

Safety Analyses: AEs will be coded using the most recent version of the Medical Dictionary of Regulatory Activities (MedDRA) preferred terms and will be grouped by system, organ, and class (SOC) designation. The severity, frequency, and relationship of AEs to investigational product will be presented by preferred term by SOC grouping. Listings of each individual AE including start date, stop date, severity, relationship, outcome, and duration will be provided. Each AE (based on preferred terminology) will be counted once only for a given study subject. If the same AE occurred on multiple occasions, the highest severity and relationship to investigational product will be assumed. Thus, study participants are not counted multiple times in a given numerator in the calculation of frequencies for a specific AE. The elicited AEs of flushing or heart rate increases (palpitations) will be presented as counts and frequencies over all subjects and separately over the subjects who reported any of these as counts and frequencies of reported events by treatment group and severity score for ALDH2 deficient and ALDH2 non-deficient subjects. Laboratory data, vital signs, ECG results, alcohol breathalyzer results, urine drug test results, and CIWA scores will be reported as summary statistics. The numbers and proportion of subjects who reported CIWA scores ≥ 10 at any time after the start of dosing will be presented. Changes in clinical laboratory tests and vital signs will also be presented as summary statistics of change from baseline. The number and percentage of subjects with a higher ACQ-SF-R score post cue session will be provided by treatment group.

Compliance and Participation Outcomes: Medication compliance is defined as the amount of investigational products taken as a proportion of the total amount prescribed. Compliance will also be evaluated by determining the proportion of subjects who were prescribed ANS-6637, reported taking ANS-6637 (by AiCure assessment), and had a plasma sample with detectable GS-548351, the active moiety of ANS-6637. The participation rate is the percentage of subjects with complete drinking data. Compliance and participation rates will be reported on a weekly basis and across the entire trial duration. Compliance by GS-548351 plasma levels will be reported as number and percentage of subjects with a level above the limit of detection at each time point among subjects who were prescribed ANS-6637 and pill ingestion was confirmed by self-report or AiCure assessment. Descriptive statistics will also be provided.

Baseline Descriptive Statistics: Summaries of the characteristics of the subjects in each of the study groups at baseline will be prepared for the mITT, evaluable, and safety analysis sets. Baseline characteristics will be compared between the ANS-6637 groups and placebo group using appropriate statistical methods.

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2. List of Abbreviations and Definition of Terms

Table 1: List of Abbreviations and Definition of Terms

Abbreviation	Definition
4-HNE	4-hydroxy-2E-nonel
ACQ-SF-R	Alcohol Craving Questionnaire – Short Form – Revised
AE	adverse event
ALDH2	aldehyde dehydrogenase 2
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
AUD	Alcohol Use Disorder
BAC	blood alcohol concentration
CAP	College of American Pathologists
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Act
CIWA-AR	Clinical Institute Withdrawal Assessment for Alcohol-revised
CrCl	creatinine clearance
C-SSRS	Columbia-Suicide Severity Rating Scale
CTCAE	common terminology criteria for adverse events
CV	coefficient of variation
CYP	cytochrome P450
DA	dopamine
dL	deciliter
DOPAL	dopamine aldehyde
DSM-5	Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition
DSMB	Data and Safety Monitoring Board
ECG	electrocardiogram
eCRF	electronic case report form
EDTA	ethylenediaminetetraacetic acid
eGFR	estimated glomerular filtration rate
EOS	end of study
EtG	ethylglucuronide
EtOH	ethanol
FDA	Food and Drug Administration
g	gram
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
HIPAA	Health Insurance Portability Accountability Act

Abbreviation	Definition
hr	hour
ICH	International Conference on Harmonization
IRB	Institutional Review Board
L	liter
LS	least squares
MAOI	monoamine oxidase inhibitors
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
µg	microgram
MDMA	3,4-methylenedioxy-methamphetamine
min	minutes
MINI	MINI Neuropsychiatric Interview
mITT	modified intention-to-treat
mL	milliliter
mm	millimeter
NIAAA	National Institutes on Alcohol Abuse and Alcoholism
OTC	over-the-counter
oz	ounce
PACS	Penn Alcohol Craving Scale
PI	principal investigator
PK	pharmacokinetic
POMS	Profile of Moods State
PSQI	Pittsburg Sleep Quality Index
PT	preferred term
pTH	tyrosine hydroxylase
SAE	serious adverse event
SAP	statistical analysis plan
SE	standard error
SER	severe ethanol reaction
SD	standard deviation
SDU	standard drinking unit
SOC	System, organ, class
SNRI	serotonin-norepinephrine reuptake inhibitors
SSRI	selective serotonin reuptake inhibitors
$t_{½}$	half-life
TEAE	treatment emergent adverse events
T3	triiodothyronine
T4	thyroxine
THC	tetrahydrocannabinol
THP	tetrahydropapaveroline
TLFB	timeline followback
Tmax	time to maximum plasma concentration
TSH	thyroid-stimulating hormone
ULN	upper limit of normal

Abbreviation	Definition
US	United States
VAS	visual analog scale
VTA	ventral tegmental area
WHO	World Health Organization

3. Introduction

Alcohol use disorder (AUD) (alcohol dependence and abuse) affects 76 million adults world-wide, including 18 million Americans, and 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 $224 billion each year in the United States (US) (NIAAA-2014).

3.1. Human Laboratory Studies

Human laboratory studies of acute responses to alcohol, alcohol cues, or other pharmacological and/or experimental manipulations have progressed in important ways, and have the potential to greatly advance our understanding neurobehavioral mechanisms of alcohol effects on behavior (Plebani-2014). These studies may help identify important individual difference factors affecting alcohol response, such as personal traits, drinking characteristics, and genotype, and aid in our understanding of the variability in cue and craving states, and provide additional information relevant to the design of clinical studies to assess the effectiveness of pharmacological agents. The study to be conducted in this protocol utilizes an alcohol cue reactivity model to attempt to recreate in the laboratory risk conditions for relapse similar to those experienced by alcoholics in their natural environment (Niaura-1988; Litt-1999).

Human laboratory studies have been conducted that examine the effect of positive and negative affective stimuli and beverage cues on craving in non-treatment-seeking subjects with alcohol dependence. In a study reported by Mason (2008), the moderating effects of a priming exposure to both positive and negative affective stimuli on beverage cue-induced craving were examined. Subjects were exposed to a standardized set of pleasant, neutral, or unpleasant visual stimuli followed by alcohol or water cues and psychophysiological cue reactivity measures were obtained during beverage presentation and subjective reactivity measures were taken following beverage presentation. Psychophysiological measures included heart rate, skin conductance, and facial electromyogram which were monitored throughout each experimental trial as confirmatory measures of the primary subjective measures of craving and emotion. Alcohol craving in response to each affect-beverage condition was assessed using 4 individual Visual Analog Scales (VAS). In addition, the Self-Assessment Manikin (Bradley-1994) was utilized as a non-verbal pictorial assessment technique to measure emotional reactivity (valence, arousal, and dominance) associated with a person's affective reaction to stimuli.

Results of mixed-effect analyses of subjective outcome measures in the Mason (2008) study showed statistically significant main effects of alcoholic beverage cue on three of four individual craving questions, and on the mean of the four items. The effect size for alcoholic beverage cue on subjective craving measures ranged from 0.58 (strength of craving), to 0.22 (difficult to turn down); the composite effect size was 0.45. Positive affect independent of beverage cue significantly increased craving strength and showed a trend for a significant increase in the composite craving scale. Effect sizes for positive affect ranged from 0.55 (difficult to turn down) to nearly zero (make things perfect). For negative affect, the largest effect size was 0.18 (strength of craving), and none of the effects of negative affective stimuli on measure of craving were

statistically significant. No interaction effects were detected between alcoholic beverage cue and affective stimuli on any outcome measure of craving. An important finding of this study is that positive affective stimuli commonly associated with drinking situations can induce craving in the absence of alcohol cues.

Another human laboratory proof-of-concept study was conducted by Mason (2009) to evaluate the effectiveness of gabapentin to attenuate some of the symptoms of protracted abstinence from alcohol. The laboratory study design was intended to model and predict the critical first week on medication during a clinical trial, when participants' sleep and mood disturbances, or other side effects, could dramatically affect medication compliance and increase the risk for subject discontinuation. The experimental design mirrored the human lab study model where positive affective stimuli induced induce craving (Mason-2008). This study design employed a 4-hour cue reactivity protocol which included a baseline evaluation, followed by the cue reactivity procedures, and subsequent debriefing. As in the previous human lab study, 3 affective stimuli (positive, neutral, negative) and 2 beverage cues (alcohol and water) were deployed within-subjects, in a block-factorial design of 6 repeated measures. All six mood-beverage cue combinations were presented to each subject (with order varying systematically across subjects) during the course of a single afternoon. Alcohol craving was assessed using four separate VAS measurements and psychophysiological measures of heart rate, skin conductance, and electromyogram were recorded.

This study results showed that subjects randomized to receive gabapentin had significantly attenuated craving responses to three of the four subjective craving measures (how strong is your urge to drink, I would drink now if I could, it would be difficult to turn down a drink now) Mason (2009). In contrast, gabapentin reduced arousal induced by all three types of cues (beverage, positive affect, and negative affect) as measured by SAM emotional reactions. Given that subjects randomized to receive gabapentin had reduced alcohol craving, and analysis of the secondary study endpoints demonstrated significantly improved measures of sleep quality, the hypothesis was supported that gabapentin may be effective for treating the protracted abstinence phase in alcohol dependence.

3.2. Rationale for Studying ANS-6637

Alcohol has been shown to stimulate an increase in dopamine (DA) levels predominately in the nucleus accumbens, which is believed to play a central role in the reward or reinforcement process in the brain (Di Chiara-1988, Boileau-2003, Tizabi-2002). Aldehyde dehydrogenase 2 (ALDH2) inhibition has been shown to attenuate the alcohol-induced surge in DA, without affecting basal levels of DA, prevent operant self-administration of alcohol, and eliminate cue-induced reinstatement of alcohol seeking even when alcohol is not available (Arolfo-2006). To further demonstrate a central effect of ALDH2 inhibition on alcohol seeking behavior, ALDH2 antisense injected into the ventral tegmental area (VTA) of inbred alcohol preferring (iP) rats was shown to decrease alcohol drinking in a time course that correlates with the expression of antisense. These observations suggest that ANS-6637 inhibiting ALDH2 in the VTA appears to attenuate alcohol seeking behavior and support the hypothesis that administration of selective, reversible inhibitor of ALDH2 may be of therapeutic potential in reducing the rewarding effects of ethanol.

3.3. Pharmacology of ANS-6637

ANS-6637 is the orally bioavailable prodrug of the selective, potent, reversible inhibitor of ALDH2, GS-548351 is being developed by Amygdala Neurosciences. Nonclinical studies have demonstrated that selective reversible ALDH2 inhibitors, such as GS-455534 (a.k.a. CVT-10216), can reduce consumption of alcohol (Arolfo-2009, Feltenstein-2008), cocaine, nicotine, heroin, and methamphetamine, and abnormal cravings for carbohydrate and fatty acids in animal models. ALDH2 inhibition during drug or cue stimulated activation of DA synthesis, which leads to drug seeking behavior, is thought to restore DA homeostasis and attenuate craving (Arolfo-2009, Yao-2010). Importantly, inhibition of ALDH2 only interferes with addictive drug-stimulated increases in DA signaling and does not change basal levels of DA in vitro (Yao-2010) and in an animal model of binge eating (Bocarsly-2014). It is believed that ALDH2 inhibition interferes with the metabolic clearance of dopamine aldehyde (DOPAL) allowing for DOPAL to condense with DA to form tetrahydropapaveroline (THP). THP is a selective inhibitor of activated phosphorylated tyrosine hydroxylase (p-TH), which is the rate-limiting step in DA synthesis. In a rat model of alcohol self-administration exposure to an ALDH2 inhibitor did not affect baseline levels of dopamine since THP does not inhibit activities of other enzymes involved in DA metabolism, but rather a targeted suppression of dopaminergic surges (Feltenstein-2008; Diamond-2015). The initial Phase 1 trial demonstrated that single and multiple doses ANS-6637 (100 mg, 300 mg, 600 mg, and 900 mg) was well-tolerated with only mild AEs. The Phase 1b study, completed in 2017 (NCT03203499), showed that ANS-6637 (25 mg, 50 mg, 100 mg, 200 mg, 400 mg, and 600 mg) was safe and well-tolerated even during excessive alcohol drinking, demonstrating that ALDH2 inhibition with oral ANS-6637 is suitable even for patients with comorbid alcohol use.

Plasma pharmacokinetic (PK) parameters of the active metabolite GS-548351 for single and multiple oral doses of ANS-6637 (300 and 600 mg) are shown in Table 2. When ANS-6637 was administered as single ascending doses in the fasted state, GS-548351 exhibited approximately dose-proportional PK over the dose range of 100 to 600 mg and less than dose proportional PK over the dose range of 600 to 900 mg. The median GS-548351 t_{max} ranged from 2.00 to 3.01 hours after dosing and demonstrated low intersubject variability in PK parameters[(<30% coefficient of variation (CV)]. The median terminal half-life ($t_{½}$) of GS-548351 ranged from 16.49 to 19.48 hours. The PK parameters following a single dose of ANS-6637 at 100 mg in smokers (data available in the Investigators Brochure) and nonsmokers were comparable.

Mean steady-state PK parameters of GS-548351 following once per day dosing of ANS-6637 (300 and 600 mg/day) under fasted conditions for 10 days are presented in Table 2. Median plasma $t_{½}$ values ranged from 16.41 to 19.24 hours, which were comparable with median values observed after single doses. Upon multiple dosing of ANS-6637, GS-548351 exhibited slightly less than dose-proportional PK (C_{max} and $AUC_{(0\text{-}tau)}$). GS-548351 plasma concentrations accumulated upon multiple dosing in accordance with its half-life and dosing interval at a dose of ANS-6637 of 300 mg once daily. However, at the 600 mg once daily dose levels, there was less accumulation of GS-548351 from Days 1 to 10 of dosing compared with the lower dose level.

Plasma concentrations of GS-548351 following administration of ANS-6637 as a single, 300-mg oral dose under fed (high-fat meal; test treatment) or fasted (reference treatment) conditions in

healthy nonsmokers was determined. There was a slight decrease in C_{max} (23%) when ANS-6637 was administered in the fed state (high-fat meal) as compared with the fasted state, but GS-548351 exposure (AUC_{0-inf}) was comparable (7% decrease) in the fed state as compared with the fasted state. The slight decrease in C_{max} in the fed state is unlikely to be clinically relevant.

Table 2: Pharmacokinetics of GS-548351 after Single and Multiple Doses in Healthy Subjects (Study GS-US-272-0101)

	Single Dose ANS-6637		Multiple Dose ANS-6637 after 10 days of once daily dosing	
GS-548351 Mean (CV)[a] PK Parameter	**300 mg (N = 6)**	**600 mg (N = 6)**	**300 mg QD[b] (N = 5)**	**600 mg QD (N = 5)**
C_{max} (ng/mL)	5756.7 (28.6)	13,433.3 (13.9)	7534.0 (20.4)	13,080.0 (12.4)
T_{max} (h)	2.00 (2.00, 3.05)	2.00 (2.00, 3.00)	1.50 (1.50, 3.00)	2.00 (2.00, 4.00)
$t_{1/2}$ (h)	17.43 (16.04, 19.07)	18.12 (17.32, 18.57)	17.65 (16.70, 18.25)	16.87 (16.77, 17.46)
AUC_{0-last} (h•ng/mL)	90,688.0 (24.8)	184,251.0 (21.1)		
AUC_{0-inf} (h•ng/mL)[c]	106,699.8 (27.6)	211,984.2 (24.2)		
$AUC_{(0-tau)}$ (h•ng/mL)			85,038.0 (16.2)	141,653.2 (17.9)

[a] CV= coefficient of variation
[b] Once daily.
[c] AUC=area under the concentration curve.

3.4. Safety of ANS-6637

ALDH2 is the second enzyme of the major oxidative pathway of alcohol metabolism that converts acetaldehyde to acetic acid. Since it is known that ALDH2 deficiency may lead to increases in plasma acetaldehyde (see for example Bae-2012) levels, it was important to demonstrate the safety of ANS-6637 when given in combination with ethanol.

3.4.1. Interactions of ANS-6637 with Alcohol

A Phase 1b a single-center, randomized, double-blind, placebo-controlled, single-ascending-dose cohort study to evaluate the safety and tolerability of the co-administration of up to 6 dose levels of ANS-6637 and ethanol in 48 healthy male alcohol drinkers (Study ANS-A-C1-001). Dose levels included doses of 25 mg, 50 mg, 100 mg, 200 mg, 400 mg and 600 mg. Study drug administration occurred approximately 1 hour following ingestion of a standardized (moderate fat) meal. Approximately 2 hours after receiving the study drug, subjects began a session of repeat ethanol administration, during which they received up to 5 doses of ethanol (14 g each) every 30 minutes, or until a stopping criterion applied.

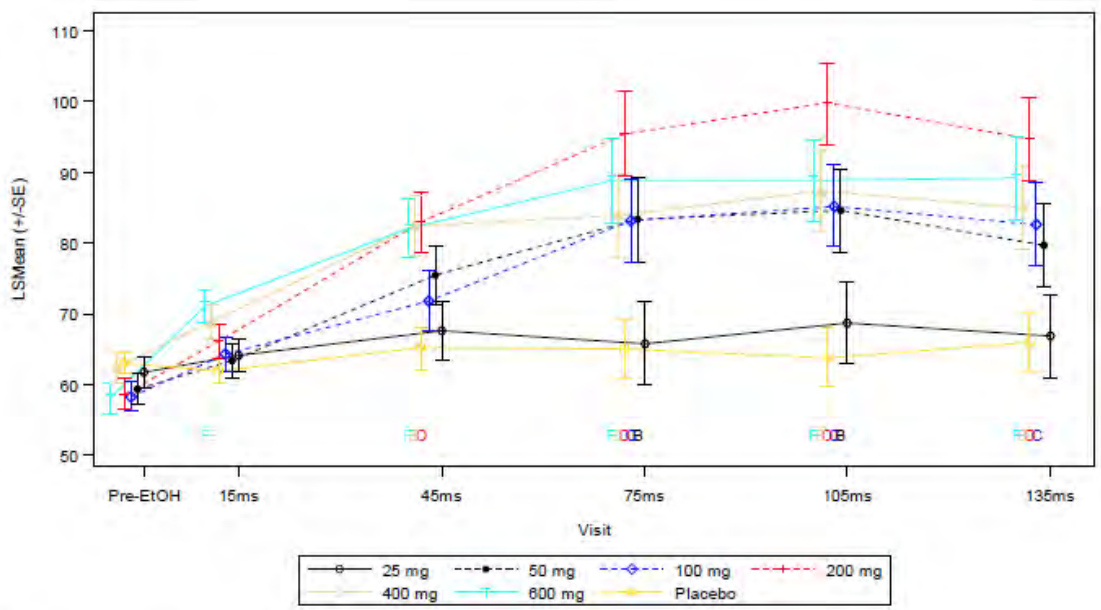
In general, all treatments were well tolerated in this study. There were no deaths or serious adverse events (SAEs) and no subject was discontinued due to an AE. Two (5.6%) subjects did not consume all 5 ethanol administrations due to treatment emergent adverse events (TEAE).

The majority of TEAEs were mild in severity (152 [92.7%] events); 1 (0.6%) severe TEAE was experienced by a subject administered ANS-6637 100 mg. The majority of TEAEs were judged to be related to study drug (i.e., ANS-6637 or ethanol) in both ANS-6637–treated (118 [88.7%] events) and placebo-treated (19 [61.3%] events) groups. The most commonly reported TEAEs (≥20%) were flushing, headache, feeling hot, and feeling drunk. The incidence of flushing and feeling hot was higher following administration of ANS-6637, while that of feeling drunk was higher following placebo; the incidence of headache was reported at a similar incidence with ANS-6637 and placebo. In general, there was no clear dose response for the most common TEAEs; however, flushing, headache and feeling drunk appeared to increase with increasing dose of ANS-6637, though feeling drunk was reported for only a small number of subjects.

All mean laboratory values were within normal ranges at baseline and follow-up, with the exception of an abnormal non-clinically significant increase in mean creatine kinase at follow-up in the ANS-6637 100 mg dose group, primarily driven by 1 subject who had an abnormal, but not clinically significant creatine kinase value of 1350 U/L (repeat 3 days later: 583 U/L). There were no TEAEs related to laboratory values.

Mean vital signs values were within normal ranges at the time points tested. Administration of ANS-6637 doses >25 mg resulted in increases in mean heart rate on Day 1 beginning 15 minutes after first ethanol administration; greater increases in heart rate were observed for higher ANS-6637 doses (i.e., 200 mg, 400 mg and 600 mg). Statistically significant increases in heart rate for ANS-6637–treated subjects compared with placebo-treated subjects began at 45 minutes after first ethanol administration until the last measured time point (135 minutes after first ethanol administration). The range of statistically significant differences from placebo was +15.3 to +35.9 beats per minute for ANS-6637 200 mg through 600 mg, and +14.7 to +19.4 beats per minute for ANS-6637 50 mg and 100 mg. Heart rates remained below the stopping criteria of 160 bpm (specifically agreed to by FDA) during the entire 5-drink alcohol challenge and generally plateaued after the third alcohol challenge. One subject (at 200 mg) registered a heart rate of 129 bpm. Increases in heart rate were commonly not associated with staff prompted, subject reported ‘palpitations’. There were no significant changes in diastolic or systolic blood pressure. The time course for heart rate increases is shown in Figure 1.

Figure 1: Mean (SE) Heart Rate Values over Time after Ethanol Administration by ANS-6637 Treatment



EtOH = ethanol; LS = least squares; SE = standard error

Five TEAEs of tachycardia were reported: 1 subject who received ANS-6637 50 mg had tachyarrhythmia, 3 subjects who received ANS-6637 200 mg had sinus tachycardia, and 1 subject who received ANS-6637 200 mg had tachycardia. All TEAEs began after consuming ethanol. The TEAEs were judged to be mild in severity and at least possibly related to study drug (i.e., ANS-6637 or ethanol). One subject in the ANS-6637 200 mg group also experienced a TEAE of tachypnea after consuming ethanol that was judged to be mild and possibly related to study drug (i.e., ANS-6637 or ethanol). Mean electrocardiogram (ECG) interval measures were similar between baseline and follow-up and there were no TEAEs related to ECG values.

A number of clinically significant findings related to general appearance on physical examinations were reported, the majority of which were related to flushing observed on Day 1. On the ethanol response subjective assessment, ANS-6637-treated subjects were more likely to report feeling heat sensation and feeling palpitations compared with placebo-treated subjects. Only a small number of subjects reported feeling breathless or headache, but these were also more commonly reported among ANS-6637-treated subjects. There were no differences between placebo- and ANS-6637-treated subjects on the nausea or vomiting subscales. There was no notable GS-548351 exposure-ethanol response relationship. Flushing was not observed in any subjects who received ANS-6637 25 mg. Flushing was observed for all other ANS-6637 doses and placebo, with the most reports of severe flushing observed between 45 minutes and 135 minutes after the first ethanol administration, corresponding to between 2 and 5 drinks consumed. Flushing was observed in far fewer subjects following placebo than with ANS-6637.

3.4.2. Heart Rate Changes in Study GS-US-272-1180

Study GS-US-272-1180 preceded the Phase 1b study and was a Phase 1, fixed-sequence, double-blind, placebo-controlled study, which evaluated the potential for a pharmacodynamic interaction upon co-administration of ANS-6637 and ethanol in healthy adult subjects. The only subject who received both ANS-6637 200 mg and ethanol 0.25 g/kg developed a protocol-defined severe ethanol reaction (SER) within 10 minutes of the start of ethanol consumption. This reaction was characterized by a maximum increase in heart rate of 65 bpm above baseline, with a heart rate of 80 and 145 bpm pre- and post-dose, respectively. AEs that occurred after ANS-6637 and ethanol co-administration included the following: mild feeling cold (reported as in the abdomen), feeling hot (reported as in the back and left arm and also in general), non-cardiac chest pain, oropharyngeal pain, paresthesia (reported as of the hands), decreased appetite, and asthenia; moderate AEs of flushing (reported as of the face and neck), palpitations, anxiety, tachycardia, dizziness, nausea, dyspnea, and headache (reported as temporal); and severe AEs of postural dizziness and presyncope. The AEs that occurred after ANS-6637 and ethanol coadministration began as early as 9 minutes after ingestion of ethanol (flushing [face]) and up to approximately 2 hours after ethanol ingestion (feeling hot [back]), with the exception of decreased appetite and headache. The decreased appetite was reported approximately 10 hours after the start of ethanol consumption on Day 4 and headache was reported 1 day later. The AEs associated with the SER resolved without sequelae. Intravenous fluids were administered approximately 2 hours after ingestion of ethanol to treat the pre-syncopal episode.

3.4.3. Clinical Laboratory Adverse Events with ANS-6637

In Study GS-US-272-0101, one subject, a nonsmoker who received ANS-6637, had SAEs [hyperbilirubinemia and abnormal alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)], which were assessed by the investigator as mild in severity and related to study drug. These laboratory findings were observed on Day 21, 11 days after completing study drug, and resolved by Day 68. Therefore, levels of ALT, AST, ALP, and total bilirubin will be monitored in study subjects in this study. Transient abnormal laboratory test results for thyroid stimulating hormone (TSH) and free T4 were observed in some subjects, the clinical importance of which is currently unknown. A thyroid panel is included in this study to monitor for changes in thyroid hormone levels. No other patterns of laboratory abnormalities indicating a relationship to study drug or dose were observed across cohorts.

3.4.4. Changes in Body Weight and Appetite

In repeat dose non-clinical studies in male and female rats, daily oral administration of ANS-6637 to at a dose of 1,000 mg/kg/day for 4 weeks or at doses of 1,000/600 mg/kg/day for 13 weeks produced decreases in body weight gain and food consumption. Therefore, body weights will be monitored weekly in this study and subjects will be queried about appetite changes.

3.5. Comparative Results of ANS-6637 with Another ALDH2 Inhibitor (Disulfiram)

The results of Study ANS-A-C1-001 are in contrast to results reported with disulfiram, a non-selective, irreversible, ALDH2 inhibitor and the slight reduction in diastolic blood pressure

observed in heterozygous carriers of the ALDH2*2 polymorphism after alcohol consumption (Roache-2011, Bae-2012). The combination of disulfiram with alcohol resulted in increased heart rate (to over 120 bpm), reductions in diastolic blood pressure (to below 50 mm Hg) and a significant QT interval prolongation (Roache-2011). The reported adverse reactions to disulfiram with alcohol have been attributed to acetaldehyde accumulation due to ALDH2 inhibition; however, the precise mechanism for these effects has not been determined. Disulfiram and its major metabolite diethylthiocarbonate (Johansson-1991, Koppaka-2012) are nonspecific irreversible inhibitors of ALDH1 (Koppaka-2012) and ALDH1B1 (Stagos-2010) and are widely toxic (Mathieu-2015). Indeed, disulfiram is a more potent inhibitor of ALDH1 than ALDH2, resulting in the accumulation of known toxic substrates, e.g. 4-hydroxy-2E-nonel (4-HNE), which may synergize with acetaldehyde to produce severe adverse reactions. 4-HNE is largely metabolized by ALDH1B1, not ALDH2 (Stagos-2010). Therefore, we do not anticipate increases in 4-HNE in the presence of ANS-6637 due to its selectivity for inhibition of ALDH2.

The physiological effects of ethanol ingestion in healthy male carriers of the mutant ALDH2 genotype (Bae-2012), which results in ALDH2 deficiency, were similar to that observed with oral ANS-6637 in combination with alcohol (that is an increase in heart rate and mild to moderate flushing) (Study ANS-A-C1-001). However, oral ANS-6637 in combination with alcohol showed no change in blood pressure in contrast to a slightly greater reduction of diastolic blood pressure in mutant ALDH2 genotype carriers at the highest dose of alcohol tested (mean decrease of 14 mmHg from baseline), which was greater than the change observed in wildtype ALDH2 genotype carriers (mean decrease of 9 mmHg) (Bae-2012).

3.6. Drug-Drug Interaction Study (SEARCHPK)

The effect of oral ANS-6637 on CYP3A4 metabolic activity was evaluated in healthy volunteers by studying the pharmacokinetics of midazolam, a CYP3A4 sensitive substrate (SEARCH PK). This open label, fixed sequence intra-subject drug-drug interaction study was designed to evaluate the effect of oral ANS-6637 at steady state (600 mg once daily for 5 days) on single dose pharmacokinetics of midazolam and its metabolite 1′-hydroxymidazolam, in healthy male and female volunteers (N=12). A clinically relevant effect was pre-specified to be if the 90% confidence interval (CI) for the geometric mean ratio area under the curve time 0-infinity ($AUC_{0\text{-}inf}$) was outside of the boundary of 70-143%. Preliminary pharmacokinetic results indicate that the $AUC_{0\text{-}inf}$ of midazolam and 1′-hydroxymidazolam in combination with oral ANS-6637 compared to midazolam alone are within the 70-143% pre-specified boundary, indicating there was no clinically meaningful effect of steady state oral ANS-6637 on overall exposure to midazolam.

In SEARCH PK, there were no deaths or SAEs, no subject was discontinued due to a treatment related adverse event and no subject received a concomitant medication to treat an AE. All subjects reported at least one AE, the majority of which were mild in severity and considered to be related to a study drug (ANS-6637 or MDZ). The most common AE was fatigue that occurred following midazolam administration, which is expected based on the known pharmacology of midazolam.

In one subject on the last day of oral ANS-6637 dosing, the same day midazolam was administered, ALT was elevated (94 IU/L). The following day after the last dose of oral ANS-

6637 and midazolam ALT value increased to 193 IU/L. At a physician follow-up visit 4 and 15 days later, the ALT value had decreased to 79 IU/L and 41 IU/L, respectively. The AE was judged to be severe and probably related to ANS-6637 and possibly related to midazolam.

All other mean clinical laboratory values were within normal ranges over time, with the exception of serum creatinine on the fifth and final day of oral ANS-6637 administration and 24-hrs after last dose of ANS-6637 resulting in decreases in calculated estimated glomerular filtration rate (eGFR). In five of 12 subjects, eGFR was calculated to be <90 mL/min/1.73m^2 (moderate severity). In these 5 subjects, the average increase in serum creatinine from baseline was 0.1 mg/dL and average decrease in eGFR was 6 mL/min/1.73m^2. Urinalysis was without evidence of hematuria, leukocyturia, casts, or other signs of renal tubular damage.

In four of the five subjects with moderate decrease in eGFR serum creatinine was not increased above the normal range (0.67-1.17 mg/dL). In one subject, with a pre-dose serum creatinine of 1.08 mg/dL (eGFR = 92 mL/min/1.73m^2), the serum creatinine was 1.35 mg/dL (eGFR = 69 mL/min/1.73m^2) on the fifth and final day of oral ANS-6637 administration and was 1.34 mg/dL (eGFR = 70 mL/min/1.73m2) 24-hrs after last dose of ANS-6637. At last study visit (7 days after the last dose of ANS-6637) the serum creatinine (1.08 mg/dL, eGFR = 91 mL/min/1.73m^2) was the same as baseline level. The subject had dry mucous membranes on physical examination, and urinalysis was unremarkable.

3.7. Discussion of the Study Design

The current study design uses paired evaluation of 2 beverage cues (water and alcohol). The difference between the cues on a single question of "strength" of craving from a VAS is the primary response. The average response within treatment group is compared using t-tests. This design has been utilized in other studies evaluating diminution of alcohol craving by candidate drugs being developed for AUD.

To verify a safe return to a baseline state following the cue exposure trials, the Alcohol Craving Questionnaire short form revised (ACQ-SF-R) (Singleton-1994) will be administered both prior to and following the cue reactivity procedure to ensure that the trials had not resulted in a prolonged subjective urge to drink.

The 4-week treatment period starting with the first visit of Week 2 and ending with the final assessments at the beginning of Week 6 is designed to obtain a preliminary assessment of the effects of ANS-6637 on drinking patterns, and other secondary endpoints including sleep, cigarette smoking and other nicotine use, craving, withdrawal, mood indicative of an effect on efficacy (reduction in drinking, reduction in craving, reduction in nicotine products use, improvement in sleep, and improvement in mood problems) and safety.

Other safety assessments, clinical laboratory measurements, vital signs, ECG, elicited adverse events, body weight and appetite changes are consistent with prior non-clinical and clinical study findings.

4. Study Objectives

4.1. Primary Objective

The primary objective of this study is to evaluate the effects of two different doses of ANS-6637, 200 mg (given as 2 x 100 mg tablet) and 600 mg (given as 2 x 300 mg tablet) once a day, and matched placebo, on alcohol cue-elicited alcohol craving during a human laboratory paradigm after 1 week of daily dosing among subjects with moderate to severe alcohol use disorder (AUD) as confirmed by the Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5™).

4.2. Secondary Objectives

Secondary objectives include evaluation of ANS-6637 200 mg once-daily, ANS-6637 600 mg once daily, and matched placebo on reduction of alcohol consumption, alcohol craving weekly (PACS), cigarette smoking (among smokers), nicotine use (among nicotine users), mood, sleep, study retention, alcohol negative consequences, and safety and tolerability throughout the last 4 weeks of the treatment period.

5. Investigational Plan

This is a 3-arm, double-blind, randomized, placebo-controlled, parallel group, 3-site study designed to assess the effects of ANS-6637 as compared with placebo on responses to *in vivo* alcohol cue exposure in the human laboratory setting. After signing informed consent, subjects will be screened for eligibility and have other baseline assessments. Screening is permitted over a 14-day period and most baseline assessments will be performed on the day of randomization. Assessments include alcohol breathalyzer test (before signing consent), medical history, physical examination, vital signs, ECG, drinking history by the timeline follow-back (TLFB) method, Clinical Institute Withdrawal Assessment for Alcohol-revised (CIWA-AR), prior medication use, MINI neuropsychiatric interview, urine drug test, smoking quantity frequency interview, clinical laboratory tests including chemistry, hematology, thyroid function tests, and medical urinalysis, response to cue reactivity, Columbia Suicide Severity Rating Scale (CSSR-S), drinking goal, PACS, Pittsburg Sleep Quality Index (PSQI), PROMIS Alcohol Negative Consequences short form, Profile of Moods State (POMS), blood sample for ALDH2 deficiency genetics test, and confirmation that subjects are treatment seeking and desire a reduction or cessation of drinking. Women of child-bearing potential will have a pregnancy test. If eligible for the study, 81 subjects will be randomized using a stratified permuted block randomization procedure in an approximate 1:1:1 ratio (targeting 27 subjects per group and 27 subjects per each of 3 clinical sites) to receive either ANS-6637 200 mg once daily, ANS-6637 600 mg once daily, or matched placebo for 5 weeks. Clinical site will be used as a stratification variable.

After the first week of investigational product administration (at the Week 2 visit), subjects will undergo a cue reactivity session including 4 individual VAS items assessing alcohol craving and 1 item assessing beverage liking. If the session cannot be conducted at that visit, it can be rescheduled for another session no more than 7 days thereafter. An ACQ-SF-R questionnaire will be administered before and after presentation of beverages during the cue reactivity session on the screening visit and study Week 2 visit to ensure that subjects have returned to levels at or below those prior to the session. If the participant's craving levels do not return to pre-session levels, then they will meet with a licensed psychologist or other mental health professional to address residual urges to drink. Other assessments at baseline (prior to the first dose of investigational product) and/or during the treatment period include clinical laboratory tests, blood for medication compliance, vital signs, ECG, concomitant medications, CIWA-AR, pregnancy test and birth control methods (males and females), drinking goal, AEs, PACS, smoking quantity/frequency, PSQI, and POMS.

Study assessments and procedures will be performed at the visits and time points outlined in the Schedule of Assessments (Table 3).

Table 3: Schedule of Assessments

Study Phase	**Screening**	**Treatment**					**End of Study**[a]	**Follow-up Call**
Clinic Visit #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
Study Week (Days)[b]	**-2 to -1 (-14 to -1)**	**1 (1 to 7)**	**2 (8 to 14)**	**3 (15 to 21)**	**4 (22 to 28)**	**5 (29 to 35)**	**6 (36 to 42)**	**8 (50 to 56)**
Informed Consent	X							
Alcohol Breathalyzer	X	X	X	X	X	X	X	
Urine Drug Test[c]	X	X	X	X	X	X	X	
Locator Form	X	Update	Update	Update	Update	Update	Update	
Demographics	X							
Medical History	X	Update						
Physical Exam	X	Update						
Body Weight	X	X	X	X	X	X	X	
MINI V 7.0	X							
C-SSRS		X	X	X	X	X	X	
Clinical Chemistry[d]	X		X	X	X	X	X	
Hematology[e]	X			X			X	
Thyroid Function Tests[f]	X		X	X	X	X	X	
Medical Urinalysis	X			X			X	
Pregnancy Test	X	X	X	X	X	X	X	
Birth control methods (all subjects)	X	X	X	X	X	X	X	
Vital Signs[g]	X	X	X	X	X	X	X	
Eligibility Checklist	X	X						
Drinking Goal		X						
ECG	X			X			X	
Prior and Concomitant Meds	X	X	X	X	X	X	X	X

Study Phase	**Screening**	**Treatment**					**End of Study**[a]	**Follow-up Call**
Clinic Visit #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
Study Week (Days)[b]	**-2 to -1 (-14 to -1)**	**1 (1 to 7)**	**2 (8 to 14)**	**3 (15 to 21)**	**4 (22 to 28)**	**5 (29 to 35)**	**6 (36 to 42)**	**8 (50 to 56)**
CIWA-AR	X	X	X	X	X	X	X	X
Screening Cue Reactivity Session: VAS Scales, typical alcoholic beverage	X							
Pharmacogenetic sampling (ALDH2)		X						
Randomization		X (Day 1)						
Blood for Drug Concentration			X		X			
Drug compliance/ accountability/ Review AiCure		Dispense Day 1	X	X	X	X	X	
AEs (open ended question) and elicited AE questions		X	X	X	X	X	X	X
Brief Telephone Interview[h]		2 times during the week	As needed if the subject misses a clinic visit					
Take Control		X	X	X[i]	X	X	X	
Treatment Cue Reactivity Session: VAS Scales, typical alcoholic beverage			X					
TLFB	X	X	X	X	X	X	X	
Brief Drinking Questionnaire	AS NEEDED							
Exit Interview							X	
ACQ-SF-R	2X pre/post cue session		2X pre/post cue session					
PACS		X	X	X	X	X	X	

Study Phase	**Screening**	**Treatment**					**End of Study**[a]	**Follow-up Call**
Clinic Visit #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
Study Week (Days)[b]	**-2 to -1 (-14 to -1)**	**1 (1 to 7)**	**2 (8 to 14)**	**3 (15 to 21)**	**4 (22 to 28)**	**5 (29 to 35)**	**6 (36 to 42)**	**8 (50 to 56)**
Smoking quantity/frequency and nicotine use		X	X	X	X	X	X	
PSQI		X					X	
POMS		X			X		X	
PROMIS Alcohol Negative Consequences		X					X	
Treatment Referral							X	
Follow-Up Telephone Interview								X
Final Subject Disposition								X

[a] EOS - end of study. These assessments are to be done at Week 6 or if the subject discontinues early and agrees to a final clinic visit.

[b] Within each study week, there should be a least two days elapsed since the visit in the prior week.

[c] Test for opiates (i.e., morphine test), cocaine, amphetamines, methamphetamine, THC, buprenorphine, methadone, benzodiazepines, oxycodone, barbiturates, 3,4-methylenedioxy-methamphetamine (MDMA – also known as ecstasy), and EtG.

[d] AST, ALT, alkaline phosphatase, total bilirubin, creatinine, gamma glutamyl transferase (GGT), and albumin. CrCl will also be calculated each time creatine is measured.

[e] Hematology tests include complete blood cell count with differential.

[f] The thyroid panel includes: thyroid-stimulating hormone (TSH), thyroxine (T4) and free thyroxine (free T4), and triiodothyronine (T3).

[g] Sitting blood pressure and heart rate.

[h] AEs, concomitant medications, CIWA-AR, and drug compliance reminder. Telephone calls during the first week, should be separated by at least one day between calls, and will include assessments for AEs, concomitant medications, CIWA-AR, and a drug compliance reminder. The second call should be several days before the cue session to remind the subject of the planned visit.

[i] Two Take Control modules will be viewed at this visit.

6. Study Interventions

6.1. Investigational Products: ANS-6637 and Placebo

ANS-6637 tablets in 100 mg and 300 mg strength will be supplied by Amygdala Neurosciences, Inc., along with identical matching placebo tablets. The 100 mg and 300 mg ANS-6637 are identical in appearance. Tablets will be supplied in bottles containing 30 tablets. Drug kits containing 3 bottles of tablets labeled with a randomization kit number will be supplied to clinical sites, by Catalent Inc., for dispensing to study subjects.

6.2. Investigational Product Storage

ANS-6637 tablets and matching placebo tablets should be stored at a controlled room temperature of 25°C (77°F); excursions are permitted between 15°C to 30°C (59°F to 86°F). Kits should be stored in a secure area at the clinical site.

6.3. Investigational Product Administration

ANS-6637 and placebo tablets will be self-administered by subjects once daily preferably in the morning. On the day of the cue reactive session at Week 2, the daily dose must be taken prior to arrival at the clinic; however, this cue session should be scheduled as late in the day as possible. The daily dose will be 2 x 100 mg tablets (200 mg total daily dose), or 2 x 300 mg tablets (600 mg total daily dose), or 2 placebo tablets. The first dose will be taken in the study clinic on the day of randomization regardless of the time of day. Subjects will be provided with water to take with first dose.

6.4. Investigational Product Dispensing

Each study drug kit will contain 3 bottles of investigational product with 30 tablets per bottle. This is sufficient for 15 days of dosing. Subjects are scheduled to come to the clinic each week and will be asked to bring the bottle(s) that were previously dispensed for accountability.

Dispensing and accountability (tablet counts) will be performed as shown in Table 4.

Table 4: Investigational Product Dispensing Plan

Study Week	Dispensing – Return Plan
Week 1	**Dispense Bottle 1** and administer the first dose to the subject in the clinic – Demonstrate the use of the AiCure application on the smart phone.
Week 2	Subject returns with Bottle 1, perform a tablet count and return Bottle 1 to the subject. **Dispense Bottle 2**. Subject leaves with Bottle 1 and 2. Check AiCure to complete drug compliance.
Week 3	Subject returns with Bottles 1 and 2. Perform a tablet count on both Bottles. Keep Bottle 1 in the clinic (should have 2 tablets if subject returns 14 days after the first visit). Return Bottle 2 to the subject and **dispense Bottle 3**. Check AiCure to complete drug compliance.
Week 4	Subject returns with Bottles 2 and 3. Perform a tablet count on both Bottles. Keep Bottle 2 and return Bottle 3 to the subject. Check AiCure to complete drug compliance.
Week 5	Subject returns Bottle 3. Perform a tablet count and return Bottle 3 to the subject. Check AiCure to complete drug compliance.
Week 6	Subject returns Bottle 3. Perform a tablet count for final accountability. Store bottles with leftover tablets until the end of the study. Check AiCure to complete drug compliance.

6.5. Investigational Product Accountability

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 tablets returned at each visit for each bottle that was dispensed and returned. Subject compliance will also be monitored using a Medication Adherence and Reminder System provided by AiCure application. A detailed description of this electronic platform is described in section 6.7. Comparison of the Medication Adherence and Reminder System records with the tablet counts will be used for drug compliance and drug accountability. If discrepancies are noted, subjects will also be asked to account for any missing tablets or other discrepancies.

Missed Doses: If a subject misses more than one dose of investigational product, he/she will be instructed to re-start taking the investigational product at the dosage level that s/he was taking before stopping (in case the dose was reduced). If one dose of investigational product is missed, he/she should take it at the usual time they take their next dose of investigational product. The subject should not double up doses.

Dose Reduction and Discontinuation. In the judgement of the investigator, if the subject experiences intolerable AEs that appear to be related to the investigational products, or the subject experiences significant loss of appetite or body weight (e.g. >5% of baseline body weight) not associated with a self-directed or prescribed weight loss regimen, or reports at least moderately severe palpitations requiring an intervention (CTCAE Grade 2) at any in clinic visit, the dose may be reduced to 1 tablet once daily. If the AE resolves at the lower dose, no attempt to increase the dose to the original target should be made. If the investigational product is still intolerable at the lower dose it should be discontinued, but the subject should continue in the study and return for all follow-up safety visits.

6.6. Medication Adherence and Reminder System

6.6.1. System Description

This Study will employ a medication adherence monitoring platform ("Platform") for all subjects in the study. The Platform uses artificial intelligence on smartphones to confirm medication ingestion. In addition, built-in reminders and a communication system allow real-time intervention in case of drug interruptions.

Use of this Platform will in no way supersede or replace the physician and/or prescribed medication protocol of the subjects. Because the Platform does not change the medication protocol of the patients, but rather encourages adherence to the predefined protocol, use of this Platform presents minimal risk to the subjects. Use of the Platform will be required for all subjects in the study.

The monitoring Platform requires that all subjects take each dose of the medication while using a smartphone. The Platform will be provided to subjects preloaded on a smartphone, or subjects will download the Platform onto their own mobile device during the first visit.

When at home, study subjects will receive a medication reminder at a time within a predefined window. This notification reminds subjects to take their medication dose while using the Platform. Subjects will follow a series of prescribed steps in front of the front-facing webcam to visually confirm their ingestion of the medication. The application on the smartphone will make an automated determination of whether the subject has properly taken their medication at the prescribed time. There is no need for a healthcare provider to review the administration, nor would a healthcare provider need to be available at the time the patient takes their medication. The amount of guidance that the device provides to the subject is automatically reduced as the subject becomes more proficient at using the application.

After the device confirms proper medication ingestion, video recordings will be encrypted and transmitted to a secure centralized location for further analysis, including testing for duplicate enrollment. The captured data and video is reviewable through a roles and rules restricted system ensuring privacy of the information. The system is compliant with the Health Insurance Portability and Accountability Act (HIPAA), which protects the privacy and security of healthcare information.

Phone numbers of the subjects may also be collected and stored in an encrypted manner. Storing the phone numbers will allow for direct communication with subjects, including automated messaging from the Platform device and contact by healthcare providers or other monitoring personnel. At no time is the phone number visible to healthcare providers or monitoring personnel. Individuals outside the clinical sites will not be provided with subject names, nor will they be given access to subject's medical records.

The Platform may provide significant benefits to Study subjects as well as to the other stakeholders in the trial. Subjects will benefit from rapid and tailored intervention in case of non-adherence (drug interruptions) without having to visit the clinic for unscheduled visits. Healthcare providers will have access to real-time and continuous adherence data without having to rely on self-reported data or frequent study visits by subjects. Subjects who regularly fail to take their medication will be contacted by healthcare providers or other Study monitoring personnel for retraining.

6.6.2. Subject Risk

The Platform provides no more than minimal risk to subjects. This protocol only introduces a smartphone-based monitoring application that prompts the user to take their medication, verifies ingestion, and stores encrypted data securely for analysis. Use of the Platform does not affect titration, dosage, route of administration or treatment duration.

All Study data, including any identifiable subject information, will be obtained and encrypted by the application. Subjects will be assigned a unique code number according to the protocol and their identity will not be stored with the study data obtained. After the subject has taken the medication and confirmation of proper ingestion has been completed, the encrypted data will be automatically forwarded to a secure server. The server is compliant with the HIPAA, which protects the privacy and security of healthcare information. The data will be securely stored and only accessible to healthcare providers and other authorized personnel through two-way authentication.

The data may also be retained in a secure manner beyond the term of the trial and utilized to improve the operation of the Platform, categorize adherence activity by disease state or other useful categories, and/or for regulatory filings by the Platform Provider to support future applications for the Platform Provider's product. Individuals who are not associated with the care and treatment of patients will not have access to patient identity or any medical records.

6.6.3. Subject Confidentiality

The Platform Provider will protect subjects' personal information to the full extent required by law. However, information from this Study, including de-identified video recording(s) of subject performance of various actions, may be submitted to the Study site, and potentially to the U.S. Food and Drug Administration (FDA). Both information obtained by the application, and information in the subject Informed Consent, may be examined by the study site or the study site's representatives, and may also be reviewed by the FDA and other regulatory agencies, or IRBs. All of these parties are bound to safeguard the rights, safety and well-being of all clinical trial subjects, and to maintain all information in confidence.

The results of this research project may be presented at meetings or in publications; however, specific subject will not be identified by name in these presentations and/or publications. Information from this Study may also be retained by the Platform Provider for the purpose of improving the Platform, to allow for future analysis of various facial and other parameters, the reporting of high level statistical analysis of the Platform, to improve the internal workings of the system running on the smartphone device, or for regulatory filings by the Platform Provider to support future applications for the Provider's product.

6.7. Take Control Behavioral Platform

The behavioral platform "Take Control" (Devine-2016) will consist of a series of 7 computerized modules viewed by subjects at 6 visits. Subjects will view a single module of "Take Control" at each clinic visit starting at the randomization visit on Week 1, Day 1 with the exception of the Week 3 visit, where two modules will be viewed. 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 clinic. 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 publically 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. Subject viewing of the Take Control Modules will be recorded on an eCRF.

6.8. Concomitant Medications

Concomitant medications must be approved by the investigator. 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.

Oral ANS-6637 is believed to suppress the surge in dopamine associated with substance use disorders and therefore, patients with attention-deficit/hyperactivity disorder treated with dopamine stimulants, or patients with restless legs syndrome, or Parkinson's disease treated with dopamine agonists will be excluded. In addition, there have been reports indicating an association of ALDH2*2 polymorphisms and pesticide (irreversible inhibitors of ALDH2) exposure and the incidence of Parkinson's and reduced cognitive function in patients with Parkinson's (Fitzmaurice-2014, Ritz-2016, Yu-216, Zhao-2015). Therefore, subjects with a family history of Parkinson's will also be excluded.

For study inclusion, 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)

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 approved for the treatment of alcoholism are prohibited at screening and during the study:

- Oral naltrexone
- Depot naltrexone (Vivitrol)
- Disulfiram (Antabuse)
- Acamprosate (Campral)

- Nalmefene (Selincro)
- Varenicline (Chantix)
- Topiramate
- Baclofen
- Prazosin
- Ondansetron
- Zonisamide

Investigators should also be aware of potential drug-drug interactions of ANS-6637. The dissolution rate and solubility of ANS-6637 from a solid formulation are driven primarily by the pH-dependent gastrointestinal tract environment. Since ANS-6637 is ionizable, there is a potential for drug-drug interactions with proton pump inhibitors and histamine-2 antagonists; however, these classes of medications are not specifically prohibited during the study. Substrate-dependent inhibition of CYP3A by GS-548351 has been observed in vitro, suggesting the potential for ANS-6637 administration to increase the exposure of CYP3A-sensitive substrates. Medications that are CYP3A-sensitive substrates with a narrow therapeutic index are prohibited during the study, but otherwise are not excluded. Examples include: alfentanil, cyclosporine, dihydroergotamine, ergotamine, everolimus, fentanyl, pimozide, quinidine, sirolimus, and tacrolimus. AEs will be monitored closely in any subjects taking a concomitant medication that is a CYP3A-sensitive substrate.

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 any questions regarding concurrent or prior medications.

The Study Manual of Procedures provides a list of prohibited medications and medications that are permitted with restrictions or that require close safety monitoring. Refer to the Manual or Procedures for guidance when making decisions about subject safety management and use of concomitant medications.

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 (including but not limited to: flyers, social media, newspaper advertisements, radio advertisements, and television advertisements). The central institutional review board (IRB) and NIAAA will approve all advertising materials used for subject recruitment. Advertising materials may also require local institutional review depending on the clinical site's policies. 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 trial. 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 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 central 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.

7.3. Selection and Withdrawal of Subjects

7.3.1. Inclusion Criteria

To be eligible, the subject must:

1. Be at least 21 years of age.
2. Meet the DSM-5 criteria for alcohol use disorder of at least moderate severity.
3. If male, report drinking a weekly average of at least 35 drinks per week or if female report drinking a weekly average of at least 28 drinks per week for the 28-day period prior to consent.
4. Have at least 1 heavy drinking day (4 or more drinks for women/5 or more drinks for men) during the 7-day period prior to randomization.
5. Be seeking treatment for AUD and desire a reduction or cessation of 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 child bearing potential) to use at least one of the following methods of birth control to at least 7 days post the last dose of study drug, unless she is surgically sterile, partner is surgically sterile or she is postmenopausal (one year):
    a. oral contraceptives,
    b. contraceptive sponge,
    c. patch,
    d. double barrier (diaphragm/spermicidal or condom/spermicidal),
    e. intrauterine contraceptive system,
    f. etonogestrel implant,
    g. medroxyprogesterone acetate contraceptive injection,
    h. complete abstinence from sexual intercourse, and/or
    hormonal vaginal contraceptive ring.
8. Agree (if male) to use acceptable methods of contraception if the male participant's partner could become pregnant from the time of the first administration of the study drug until 7 days following the final administration of the study drug. One of the following acceptable methods of contraception must be utilized:
    a. Surgical sterilization (vasectomy);
    b. The participant's female partner uses oral contraceptives (combination estrogen/progesterone pills), injectable progesterone or sub dermal implants (commenced at least 14 days prior to study drug administration to the male participant)
    c. The participant's female partner uses a medically prescribed topically applied transdermal contraceptive patch (commenced at least 14 days prior to study drug administration to the male participant);
    d. The participant's female partner has undergone tubal ligation (female sterilization) or is postmenopausal (one year);
    e. The participant's female partner has undergone placement of an intrauterine device or intrauterine system;
    f. True abstinence: when this is in line with the preferred and usual lifestyle of the participant.
9. Agree (if male) to refrain from sperm donation from the randomization visit to at least 7 days after the last dose of study drug.
10. Be able to take oral medication and be willing to adhere to the medication regimen.
11. Complete all assessments required at screening and baseline.
12. 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 in the next 2 months.
13. Not anticipate any significant problems with transportation arrangements or available time to travel to the study site over the next 2 months.

14. Not have any unresolved legal problems that could jeopardize continuation or completion of the study.
15. 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.
16. Have a BAC by breathalyzer equal to 0.000 when s/he signed the informed consent document.
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:
    a. Selective serotonin reuptake inhibitors (SSRIs)
    b. Dual uptake inhibitors
    c. Serotonin-norepinephrine reuptake inhibitors (SNRIs)
    d. Tricyclic antidepressants
    e. Monoamine oxidase inhibitors (MAOIs)
18. Be someone who in the opinion of the investigator would be expected to complete the study protocol.
19. 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.
20. Be willing to use a smartphone's video capability to record daily oral ingestion of tablets for the entire 5-week treatment period (subject's own smartphone or one provided by AiCure).
21. Have sitting (3 to 5 minutes) vital signs at the screening visit within the following limits:
    a. Systolic blood pressure 90 to 160 mmHg
    b. Diastolic blood pressure of 50 to 95 mmHg
    c. Heart rate of 40 to 90 beats per minute

Note: Vital signs may be repeated once if outside the limits above and if within the limits on a second evaluation, the subject may be included.

7.3.2. Exclusion Criteria

Subjects must not have any of the following:

1. Current (past 12 months) substance use disorder of at least moderate severity (4 or more criteria) for any psychoactive substance other than alcohol and nicotine, including sedatives and hypnotics, as defined by DSM-5 criteria.
2. Urine drug test positive performed during screening or baseline for any of the following substances:
    a. benzodiazepines,
    b. cocaine,
    c. opioids,
    d. amphetamines,

e. methamphetamine,
f. buprenorphine,
g. methadone,
h. barbiturates,
i. oxycodone,
j. and/or 3,4-methylenedioxy-methamphetamine (MDMA).

Note: Testing for 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 have moderate or greater severity for cannabis use disorder as indicated by DSM-5 criteria. The results for THC will be recorded for information only. If positive for opioids or oxycodone but recent opiate use for acute pain is reported by the subject, then the subject can be included at the discretion of the investigator.

3. VAS craving rating ("How strong is your craving to drink alcohol") during first presentation of alcohol cue <5 during the screening cue reactivity session.
4. 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 AUD or a history of any seizure disorder.
5. Have participated in any behavioral and/or pharmacological intervention research study for the treatment of alcoholism where the last intervention was within 3 years prior to signing the informed consent.
6. Be mandated by the court to obtain treatment for alcohol-dependence, or has probation or parole requirements that might interfere with study participation.
7. Be anyone who in the opinion of the investigator could not be safely withdrawn from alcohol without medical detoxification.
8. Have undergone medical detoxification (e.g., reports using a benzodiazepine) during the screening phase (prior to randomization).
9. Have been treated with a pharmacotherapy for alcohol use disorder within 6 months prior to randomization.
10. Have any of the following, based on DSM-5 criteria as assessed using the MINI:
    a. Current or lifetime diagnosis of psychotic disorders,
    b. Current bipolar disorder,
    c. Current major depressive episode,
    d. Current (past 3 months) eating disorder (anorexia or bulimia), or
    e. Within past year diagnosis of panic disorder with or without agoraphobia.

Note: Subjects diagnosed with psychiatric disorders not specifically excluded above may be included at the discretion of the PI as long as the concurrent treatment for the comorbid psychiatric condition does not compromise the study integrity by virtue of its type, duration, or intensity.

11. Have any of the following:

a. attempted suicide past year,
b. 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),
c. 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 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 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.

12. Have moderate or serious dementia as assessed by clinical exam.
13. Be pregnant or breast-feeding or have plans to become pregnant at any time during the study or within 7 days after the last dose of investigational product.
14. Have clinically significant abnormal laboratory values, including elevation of liver enzymes (AST or ALT > 2.5 x upper limit of normal or total bilirubin > 1.5 x the upper limit of normal).
15. Have abnormal calculated creatinine clearance defined as < 80 mL/min for subjects ≤ 55 years of age and < 65 mL/min for subjects > 55 years of age.
16. 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.
17. 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 problems for non-alcohol related problems may be acceptable.
18. Have data suggesting cirrhosis of the liver (albumin < 3.2 g/dL, or ascites by physical exam).
19. Have been previously treated with ANS-6637 for any reason.
20. Have had gastric bypass surgery.
21. Have had a severe reaction to disulfiram while drinking alcohol requiring medical attention.

22. Have a history of atherosclerotic cardiovascular disease including angina pectoris, myocardial infarction, stroke, transient ischemic attack, peripheral vascular disease or revascularization procedures or clinically significant ECG indicative of cardiovascular disease. Note: medically controlled hypertension is not exclusionary.
23. History of syncope, palpitations, or unexplained dizziness at screening.
24. Had a prior history of any severe adverse reactions to ethanol [e.g., flushing (noticeable redness of the neck or throat) and/or increased heart rate (subject reports sensation of increased heart rate or palpitations) after drinking alcohol].
25. Report heavy drinking of alcohol within 2 days on TLFB prior to screening and have a negative result on EtG urine test.
26. Have Parkinson's Disease or a family history of Parkinson's Disease.
27. Have restless legs syndrome and receiving dopamine agonist treatment.
28. Have attention-deficit disorder and receiving dopamine stimulant treatment.
29. Are taking a prohibited medication.

7.4. Eligibility Screening Assessments

After providing written informed consent, subjects complete the Screening Visit for initial evaluation of eligibility. A screening cue session will be conducted to familiarize subjects with the human lab setting and identify non-cue-reactive subjects for study exclusion (VAS alcohol craving rating upon first presentation of alcohol cue < score of 5 on the item, “How strong is your craving to drink alcohol?”).

During the first screening visit (additional visits are permitted if needed), subjects will undergo the following assessments (assessments may be performed in any order, except that consent and BAC must be completed first). However, it is recommended to perform physical examinations including vital signs prior to blood draws.

- Alcohol breathalyzer (must have a BAC of 0.000 to continue with assessments)
- Informed Consent
- Demographics and locator form
- Urine drug test
- Medical history
- Physical examination and weight
- Vital signs
- MINI Version 7.0.2
- Clinical chemistry, hematology, thyroid function tests, and medical urinalysis
- Pregnancy test for females of child bearing potential and birth control methods for males and females
- ECG

- Screening Cue Session (VAS craving scales and ACQ-SF-R)
- TLFB for the previous 28 days
- Prior medication use
- CIWA-AR
- Eligibility checklist initial screening questions

If any of these assessments reveal that the subject is not eligible for the study, screening can be immediately terminated.

Potential subjects must agree to adhere to the following prohibitions and restrictions during the course of the study to be eligible for participation and will be reminded of these restrictions during the telephone call prior to the cue reactive session at Week 2:

- Refrain from consuming caffeine after arriving for the lab session until study assessments are completed.
- Refrain from smoking during the cue-reactivity sessions (if the subject is a smoker, then smoking is permitted and encouraged approximately one hour before arriving at the cue reactivity session.

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 not to take ANS-6637 outside of the study during their time in the study and that they should report any new medications they are taking at each visit or telephone contract.

Clinical laboratory 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 baseline visit. It is recommended that hypertensive subjects be referred to their primary care physician for additional assessments 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 and randomization visit. The following assessments will be performed at this visit:

- Alcohol breathalyzer (must have a BAC of 0.02 or less to continue with assessments)
- Urine drug test
- Update medical history
- Update physical exam (if needed)
- Update locator form (if needed)
- Vital signs

- CIWA-AR
- C-SSRS
- Pregnancy test for females of child bearing potential
- Birth control methods (both males and females)
- Prior medications update
- TLFB

*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 assessments:

- PACS
- Drinking Goal
- Smoking Quantity Frequency and Nicotine Use Questionnaire
- PSQI
- POMS
- PROMIS Alcohol Consequences
- Blood sample for ALDH2 genetic test
- Body weight

7.6. Measures Taken to Minimize/Avoid Bias

7.6.1. Randomization (Day 1)

If eligible for the study, subjects will be randomized in an approximate 1:1:1 ratio to receive one of the doses of ANS-6637 or placebo using a stratified permuted block randomization procedure with clinical site as the stratification variable.

Randomizations will be performed via an interactive web-based randomization system (IWRS) provided by the data coordinating center.

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. The IWRS will handle this randomization assignment. Likewise, if the subject was randomized and then is determined to not be eligible for the study, and never took 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 took 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

ANS-6637 (both strengths) and placebo tablets will be identically matched in appearance. 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. The site investigator will automatically notify NIAAA that a subject was unblinded who will notify the Medical Monitor.

7.7. Interventions on Week 1, Day 1

Subjects will view a single module of Take Control during this clinic visit prior to dispensing study drug.

After the subject is randomized, he/she will receive the first bottle of study drug. Site staff will explain the dosing plan to the subject and how to use the AiCure application on their smartphone. The subject will be observed taking the first dose of study drug and the use the AiCure application for medication compliance.

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.

If possible, clinic visits should be scheduled on the same day of the week that the subject took the first dose of study drug; however, visits may be scheduled any day during the study week, but at least two days should have elapsed since the prior week's visit. 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. Week 1 Telephone Contact

During Week 1, subjects will be called two times for a safety assessment and will be given a reminder to continue taking their investigational product as prescribed. Telephone calls during the first week should be separated by at least one day between calls. The second call should be scheduled several days before the Cue Session at Week 2, to remind the subject of the pending visit and to complete all of the rest of the safety telephone assessments. The subject will also be instructed at this visit to take their investigational product tablets as usual in the morning. If the subject is a smoker, remind them that they will not be permitted to smoke or use other nicotine products after arrival at the clinic, until after the cue session is completed. However, smokers will be encouraged to smoke approximately 1 hour prior to coming to the clinic to help control urges to smoke during the cue session.

7.9. Treatment Period

During Study Weeks 1 through 5, 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. If the subject has a BAC > 0.02 and cannot wait for levels to return to ≤ 0.02, then the following safety assessments should be performed: vital signs, ECG if at Week 3 or 6, clinical laboratory tests (including pregnancy test if female), and urine drug test. Otherwise, the assessments listed in Table 3 will then be performed during these visits. If a subject arrives for a clinic visit who is too intoxicated to participate in study procedures, the site staff should follow their site specific standard operating procedure for managing these subjects.

The cue reactivity session will be performed at Week 2 (see section 10.11.2 for details).

The final in-clinic assessment will occur during Week 6. This visit should occur after the subject takes the last dose of study drug.

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 to postpone such activity until their study participation is concluded at the discretion of study physician.

7.10. Telephone Assessments

The brief telephone interview (approximately 10 minutes) will occur in accordance with the schedule in Table 3 or if a subject misses a clinic visit but agrees to check-in by telephone to assess AEs, concomitant medication use and the emergence of withdrawal symptoms, 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 and dose increases. If necessary, a TLFB interview can also be conducted. 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. Elicited AEs: Ask if the subject experienced about flushing reactions, noticeable heart rate increases or palpitations, and appetite changes.
3. CIWA-AR: Subjects will be assessed for the emergence of withdrawal symptoms using the CIWA-AR. The subject may be asked about changes in drinking status after responses to the CIWA-AR interview indicate significant withdrawal.

4. 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, then notify the PI or designee.
5. Drug Compliance: If the subject has not been using the AiCure smart phone application, provide retraining if necessary, verify that the subject is taking the prescribed dose, remind them to take investigational product, and remind them to return the drug bottles with remaining tablets at the next visit.

Additional reminders: Remind the subject of their next scheduled clinic visit, and adjust the date within the visit week if they have a conflict.

7.11. Final Clinic Visit

The final clinic visit occurs during Week 6; at which time the subject will have completed taking investigational product by the end of Week 5. The subject will complete study assessments in accordance with Table 3 and will be provided with a referral to a treatment program for their AUD. 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 the final clinic visit assessments.

7.12. Telephone Follow-up

Subjects will be contacted by telephone for a follow-up interview 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.

7.13. Duration of Subject Participation

The total time period that each individual subject will participate is up to 10 weeks including up to 2 weeks for screening, 5 weeks of study interventions, 1 week for an end-of-study visit, and a final safety follow-up telephone contact from 2 weeks after completion of study drug dosing.

7.14. Dose-adjustment Criteria

7.14.1. Safety Criteria for Dose Adjustment or 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.

7.14.2. Investigational Product Dose Reduction

The daily dosage of investigational product may be reduced by the study physician for any AE determined, by the study physician, to compromise the subject's ability to maintain activities of daily living or if the subject reports undue discomfort. The dose may be reduced to 1 tablet once daily.

7.14.3. Investigational Product Discontinuation

Subjects who are discontinued from investigational product after the Week 2 assessments should continue in the study and complete all assessments. If the subject discontinues before this time point, then they will not be eligible for the Week 2 Cue Reactivity assessment and will be discontinued from further study participation.

1. **Pregnancy.** Females who become pregnant during the course of the study interventions will be immediately discontinued from the investigational product. The investigator must report a pregnancy within 1 working day of the site being aware to the NIAAA Study Manager and the Medical Monitor. Pregnant females will be followed to determine the outcome of the pregnancy, fetus, and/or newborn child.
2. **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.
3. **Elevated Liver Enzymes.** Subjects whose ALT or AST is greater than 5X ULN should have these tests repeated as soon as possible and if still elevated should discontinue investigational products. If the repeat values are less than the criteria above, the subject should be monitored using clinical judgment. If ALT or AST levels are in the range of 3-4.9x ULN and total bilirubin is in the range of 1.5x-1.9x ULN, then the subject may be at risk for clinically significant hepatic dysfunction. The first action will be to have the laboratory test repeated as soon as possible to confirm the result. If the value was confirmed, then the subject will be withdrawn from receiving the study drug but will continue to be followed for the duration of the study as long as the subject agrees to continue in the study. Subjects discontinued from investigational products for elevated liver enzymes should be referred to their own physician for follow-up.
4. **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 immediately discontinued.

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 for any reason, including the occurrence of an AE or noncompliance with the protocol.

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 a pregnancy test (females only), vital signs, clinical laboratory, C-SSRS, TLFB and an assessment of AEs will be performed as soon as possible after discontinuation from the study. Other assessments scheduled for the end of study visit will be collected if possible (Table 3).

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; or,
    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 Termination Criteria

NIAAA may terminate this study prematurely, either in its entirety or at any sites, 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.

8. Study Endpoints

8.1. Efficacy Endpoints

8.1.1. Primary Efficacy Endpoint

The primary efficacy endpoint is the "strength" of alcohol craving VAS score (item 1 below) upon presentation of the first alcohol cue at Week 2 – after one week of investigational product treatment.

Confirmatory secondary endpoints include the VAS scores for the other 3 VAS scales (items 2 through 4 below) for the first alcohol cue and the average score of the 4 VAS craving items; and the difference score (alcohol craving VAS scores minus the water craving VAS score) for all 4 of the individual VAS craving items and their average score. The beverage liking VAS item is also a confirmatory secondary endpoint The 4 VAS craving items in the order of presentation are:

1. How strong is your craving to drink alcohol? - note this is the primary efficacy endpoint.
2. Having a drink would make things just perfect.
3. If I could drink alcohol now, I would drink it.
4. It would be hard to turn down a drink right now.

The beverage liking item is: How much did you like the beverage just given to you?

8.1.2. Secondary Efficacy Endpoints

Secondary efficacy endpoints will be analyzed over the last 4 weeks of the treatment period of treatment.

1. Percentage of subjects with no heavy drinking days. A "heavy drinking day" is 4 or more drinks per drinking day for women and 5 or more drinks per drinking day for men.
2. Percentage of subjects abstinent from alcohol
3. Percentage of subjects with at least a WHO 2-level decrease in alcohol consumption
4. Percentage of subjects with at least a WHO 1-level decrease in alcohol consumption
5. Percentage of days abstinent per week
6. Percentage of heavy drinking days per week
7. Percentage of very heavy drinking days per week. A "very heavy drinking day" is 8 or more drinks per drinking day for women and 10 or more drinks per drinking day for men.
8. Weekly mean number of drinks per week
9. Weekly mean drinks per drinking day
10. Cigarettes smoked per week among smokers
11. Percentage of subjects with no nicotine use among nicotine users
12. Alcohol craving score (PACS)

13. Sleep quality (PSQI) score
14. Profile of Mood States (POMS) score
15. PROMIS Alcohol Negative Consequences Score

8.2. Safety Endpoints

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

1. Vital signs
2. Body weight
3. Clinical laboratory parameters
4. BAC by breathalyzer
5. Urine drug tests
6. AEs and elicited AEs (flushing or heat sensation, heart rate increases or heart palpitations, and appetite change and AEs in subjects taking CYP3A-sensitive substrates)
7. ECG results
8. CIWA-AR scores
9. Frequency of subjects with suicidal ideation at any time during the treatment period (C-SSRS)
10. Concomitant medication use
11. ACQ-SF-SR score (pre- and post-cue response sessions)

8.3. Compliance

Compliance will be assessed using the AiCure smart phone application and by tablet counts of returned bottles at regular clinic visits. In addition, blood will be collected to determine plasma levels of GS-548351. Compliance will be calculated as the percentage of investigational products taken as prescribed and by the total amount of medication consumed. Participation in study visits will be evaluated as the percentage of subjects with complete drinking data. Compliance determined by GS-548351 plasma levels will be reported as number and percentage of subjects with a level above the limit of detection at each time point.

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.

The IRB, Medical Monitor, PI, Clinical Monitors and NIAAA (or its affiliates) 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 has 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 NIAAA to periodically monitor, at mutually convenient times during and after the study, all study data. These monitoring visits provide 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 NIAAA's representatives will be scheduled at appropriate intervals but more frequently at the beginning of the study. 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.

DSMB: An independent DSMB of external advisors will meet prior to the start of the study, and periodically 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.

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. However, if the BAC is > 0.02, then safety assessments will be performed as described in section 7.9.

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? Elicited AEs will be assessed as described in section 10.2.4.

AEs will be documented in the source records, and recorded on the eCRF using accepted medical terms and/or the diagnoses that accurately characterize the event. When a diagnosis is known, the AE term recorded on the CRF 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 CRF 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.

Besides routine monitoring of AEs during the conduct of the study, data presented to the DSMB will include an analysis of AEs in subjects taking a CYP3A-sensitive substrate as well as at the conclusion of the study.

10.2.4. Elicited Adverse Events

In addition to asked the open ended question regarding AEs, 3 specific questions about AEs will be asked at clinic visits and telephone calls:

1. Have you noticed any changes in your appetite?
2. Have you noticed a heat sensation (flushing or feeling hot) while you were drinking alcohol?
3. Have you noticed any increases in your heart rate or heart palpitations while you were drinking alcohol?

If the subject responds affirmatively to any of the above questions, then a designed clinician will interview the subject to determine the severity and relationship of the AE to the study drug, and if any actions should be taken (i.e., dose reduction or discontinuation of the study drug).

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

For each recorded AE or SAE, a physician-investigator must make an assessment of severity. For those AEs included in the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE version 5.0), these severity criteria will apply. 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.
Life-threatening	An event that puts the subject into imminent risk of death without intervention.

Abnormal clinical laboratory findings for which a severity grade is available in the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE version 5.0) 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. For laboratory tests not listed in the CTCAE that are outside of normal laboratory limits, the investigator must make a determination if test results are clinically significant. An abnormal lab value will be deemed clinically significant if either of the following conditions is met:

- The abnormality suggests a disease and/or organ toxicity that is new or has worsened from baseline.
- The abnormality is of a degree that requires additional active management, e.g., change of dose, discontinuation of the drug, close observation, more frequent follow-up assessments, or further diagnostic investigation.

Likewise if blood pressure (BP) was elevated at baseline (systolic BP 120 - 150 mmHg or diastolic BP 80 - 90 mmHg) and increased to a level indicative of an increase in the severity grade, hypertension will be reported as an AE and the severity grade will be in accordance with the criteria in the CTCAE version 5.0.

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 14 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 14-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., 14 days after the final Follow-up visit) or the investigator deems that further follow up is not medically required
Unknown – Lost to Follow-up:	Lost to follow-up at least 2 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 admission through discharge whether or not related to the investigational product, must be reported ***within 24 hours*** of knowledge of the event to the NIAAA Medical Monitor or alternate. Ms. Megan Ryan will coordinate communications to the Medical Monitors. Contact her as follows:

Megan Ryan: Tel: (301) 443-4225; **Email:** mryan1@mail.nih.gov

The following information must be provided with the initial report of an SAE or unexpected AE. A reporting form will be provided.

- Name of person reporting the SAE/unexpected AE
- Subject's I.D. number
- Name of the PI and institution
- Date the subject signed informed consent
- Date first dose of investigational product was ingested
- Description of the SAE/unexpected AE
- Date and time of Onset
- Date/time of administration of last dose of investigational product prior to the SAE/unexpected AE
- Severity of the SAE/unexpected AE
- Investigator's assessment of the relationship of the SAE/unexpected AE to investigational product (related, possibly related, probably related, unlikely related, not related)
- Any action taken with the investigational product, alteration to protocol defined schedule, diagnostics, and treatments secondary to the SAE/unexpected AE.

3-Day Supporting Documentation Requirements

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

- SAE Form
- Concomitant Medication eCRF pages
- AE eCRF pages
- Copies of source documents pertinent to the event (lab reports, ECG tracings, medical chart notes, etc.)

- Any other relevant information necessary to facilitate the investigator's judgment regarding the SAE's relatedness to the severity OR by request of the Medical Monitor/Alternate

These documents must be submitted by either email attachments or via overnight courier.

10.2.7.2. Reporting to the IRB

Unanticipated problems involving risk to subjects or others, SAEs related to participation in the study and all subject deaths should be promptly reported by phone, email, or fax to the central IRB.

Investigators are required to forward safety information provided by the sponsor's representative to the IRB.

10.3. ALDH2 Genetic Test

An approximately 2.5 mL sample of whole blood sample will be collected to assess ALDH2 status after the subject is determined to be eligible for the study at the randomization visit. If for some reason, the sample was not collected at baseline, it can be collected at any subsequent visit as this is a genetic test. Samples will be sent to QPS LLC Delaware Technology Park, 3 Innovation Way, Suite 240, Newark, DE 19711 for analysis at the end of the study. QPS will provide sample kits for blood collection. Additional details on sample collection, storage, and shipment for analysis will be provided in the Study Manual of Procedures.

10.4. Alcohol Craving Scale – Short Form – Revised (ACQ-SF-R)

The ACQ-SF-R contains 12-items adapted from the 47-item ACQ-NOW developed by Singleton (1994) to assess craving for alcohol among alcohol users in the current context (right now). There are 4 subscale scores for compulsivity, expectancy, purposefulness and emotionality and a total score. Each item has a 1 to 7 raw score (from strongly disagree to strongly agree). The sum of the raw scores for each factor is divided by 3 to yield a factor based score. Items 3, 8, and 11 are reverse keyed. A general craving index is derived by summing all items and dividing by 12. This form takes ~5 minutes to complete. This questionnaire will be completed by the subject and will be both the source and an eCRF.

10.5. Birth Control Record

Birth control methods for both males and females will be recorded on a source document and an eCRF.

10.6. Brief Drinking Questionnaire

If a subject has completed the human lab assessment and 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 asked a series of 6 questions that assess quantity and frequency of drinking, heavy drinking, and maximal drinking since the last day that non-missing TLFB data was provided. Phone calls will continue until the end of the treatment period, as deemed acceptable by the patient, to obtain data on the secondary drinking endpoints. The rules regarding standard drinking units (SDU) applies (see Table 6 for a

definition of SDU). These data will be recorded on a source document and an eCRF. This does not apply to subjects who are willing to supply daily drinking data by the TLFB method.

10.7. 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 and will be both the source and an eCRF.

10.8. Clinical Laboratory Tests

10.8.1. Chemistry Tests

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. All clinical laboratory data will be reviewed by the investigator for clinical significance. The total blood volume is approximately 76 mL. 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 include: creatinine, total bilirubin, ALT, AST, alkaline phosphatase, GGT, and albumin.

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

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

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

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 that requires a subject to be discontinued from the study or requires a subject to receive treatment will be recorded as an AE

10.8.2. Hematology Tests

Hematology tests include: complete blood cell count with differential.

10.8.3. Thyroid Panel

The thyroid panel includes: thyroid-stimulating hormone (TSH), thyroxine (T4) and free thyroxine (free T4), and triiodothyronine (T3).

10.8.4. Medical Urinalysis

A dipstick urinalysis will be performed that tests for specific gravity, ketones, pH, protein, blood, glucose, nitrites, bilirubin, and leukocyte esterase. If the dipstick is positive for blood, leukocyte esterase, or protein, then a microscopic analysis will be performed at the discretion of the investigator.

10.8.5. Pregnancy Test

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.

10.9. 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 Lighthouse Project web site (http://cssrs.columbia.edu). 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 with Visit 1 at Week 1. At Visit 1, 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 on an eCRF.

10.10. Cigarette Smoking Quantity-Frequency and Nicotine Use Questionnaire

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.

10.11. Cue Reactivity Sessions

10.11.1. Screening Session

Subjects will be familiarized with laboratory procedures during a practice cue reactivity session during screening. The methodology is presented in Mason (2009).

Subjects will be escorted to a comfortable chair in a lighting-controlled, sound-attenuated room for the screening alcohol cue reactivity session. Subjects will be given a 4 ounce (oz) glass of water to drink before starting the session. Following a 90 second rest period, a standardized glass of water will be placed on a small table after which subject will be instructed to pick it up and smell it, but not drink it, for duration of 3 minutes. Immediately after, the subject will complete a series of four VAS craving questions. The water will be removed and after a 90 second rest period, the subject's typical alcoholic beverage will be placed on a small table after which the subject will be instructed to pick it up and smell it, but not drink it, for duration of 3 minutes. Immediately after, the subject will complete the same 4 VAS craving questions and a beverage liking VAS item. The alcohol beverage will be removed and without any waiting period, the same alcoholic beverage will be placed on a small table after which subject will be instructed to pick it up and smell it, but not drink it, for duration of 3 minutes. Immediately after, the subject will complete the same 4 VAS craving questions and a beverage liking VAS item (see Section 10.11.2 for the beverage liking item).

Alcohol craving in response to a water and typical alcohol beverage cue is assessed using 4 individual VAS items adapted from the ACQ (Singleton-1994).

The VAS craving scale items include in the following order:

1. How strong is your craving to drink alcohol?
2. Having a drink would make things just perfect.
3. If I could drink alcohol now, I would drink it.
4. It would be hard to turn down a drink right now.

Anchors (scores) for items 1, 3, and 4 are: Strongly Disagree (0) to Strongly Agree (20).

Anchors (scores) for question 2 are: None (0) to Extremely Strong (20).

Subjects must have a VAS alcohol craving rating on the VAS item, "How strong is your craving to drink alcohol?" upon the first presentation of the alcohol cue ≥5 during this Mini Cue session at screening to be eligible for the study.

10.11.2. During Treatment Session

This assessment, conducted at Week 2, should be scheduled for as late in the afternoon as possible. Check subject compliance with investigational product using the AiCure smartphone application. If for some reason, the application is not working, check with the subject by self-report if they have taken at least 1 of the 2 prescribed tablets per day for the 3 days prior to the session and the on the morning of the session. Subjects must fulfill the following criteria before conducting the session:

1. Must have taken at least half the target dose per day during the 3 days prior to the session.
2. Must have taken at least half of the morning dose of investigational product on the day of the session.
3. Must have BAC ≤ 0.02 prior to starting the session.
4. Must have CIWA score < 8.

If any of the above conditions are not met, then the session cannot be conducted at that visit but can be rescheduled for another session no more than 7 days thereafter.

If the criteria above are met, then the session will be conducted as presented in Table 5. A detailed description of the study procedures is provided in the Study Manual of Procedures (MOP). All subjects will be required to drink 4oz of water and will be offered a restroom break prior to the session start.

Table 5: Schedule of Events for Cue Reactivity Session During Treatment

Visit Start	Subject arrives; all assessments for Study Week 2 are conducted (except Take Control) including: alcohol breathalyzer, urine drug test, C-SSRS, vital signs, concomitant medications, AEs, CIWA-AR, ACQ-SF-R, PK blood draw, drug accountability, TLFB, PACS, smoking quantity/frequency and nicotine use.
Preparation for Session	Prepare the subject for the session including a 90 second rest period prior to presenting the water cue to the subject
Session Start	**Step 1:** *In vivo* beverage cue: water is placed in front of subject for 3 minutes. **Step 2:** Ratings: subjects completes VAS craving and beverage liking VAS in presence of beverage cue. **Step 3:** Beverage removed from testing area after completion of ratings. **Step 4:** After a 90 second wait, repeat steps 1- 3 using presentation of the subject's typical alcohol beverage. **Step 5:** Without waiting, repeat steps 1-3 again using another preparation and presentation of the subject's typical alcohol beverage
Post Session	ACQ-SF-R is assessed to verify that craving has returned to pre-test levels or below. Take Control module will be viewed. Study Drug Bottle 1 is returned to the subject and Study Drug Bottle 2 is dispensed. The next appointment is scheduled.

After confirmation of eligibility to participate in the session and completion of clinical assessments, subjects will be escorted to a comfortable chair in a lighting-controlled, sound-attenuated room for the cue reactivity session.

During the cue session, subjects will be presented with a standardized glass of water (first session) then their typical alcoholic beverage (e.g., vodka, lager beer) (second session), followed by another preparation and presentation of the typical alcoholic beverage (third session). The water or alcohol beverage is presented in the subject's preferred mode of consumption (e.g., small tumbler for vodka, Pilsner glass for beer), and subject is instructed to hold and smell the

beverage for 3 minutes. Specific alcohol brand preferences are accommodated whenever possible, including choices of mixers (e.g., vodka will be poured into a glass along with orange juice if the typical drink were a screwdriver).

Following each beverage exposure period, subjects will complete all VAS scales (subjective craving and emotional response) by making selections from the computer screen (or paper form in the event that a computer or tablet is not available). The beverage will then be removed, then the procedure will be repeated for the remaining beverage(s).

The assessments to be conducted during the Cue Reactivity Sessions include:

1. Same 4 VAS scales that were conducted during the screening session (section 10.11.1).
2. Beverage liking question

The beverage liking question is a 20-point VAS item as follows:

"How much did you like the beverage just given to you?" (with an anchor of 0 on the left indicating "Strongly Dislike" and a 20 on the right indicating "Strongly Like").

All of the subjective measures will be recorded by the subject directly on an eCRF. In addition, the name (brand and type) of the typical beverage and the alcohol by volume (% alcohol) content will also be recorded on an eCRF by a member of the study staff. If a computer is not available, paper forms will be provided as a source document that will be transcribed into the eCRF.

10.12. 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 onto an eCRF.

10.13. 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.

10.14. 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. These data will be collected by site staff on a source document and onto an eCRF.

10.15. Eligibility Checklist

The Eligibility Checklist which includes all inclusion and exclusion criteria will be reviewed in stages as follows: 1) after screening measures are completed and before scheduling the Study Day 1 visit and 2) prior to randomization on Study Day 1. If the subject is not eligible for the study, the reasons for ineligibility will be recorded on an eCRF. These subjects will be considered screen failures.

10.16. Exit Interview

At the Week 6 visit, the subject will complete a questionnaire regarding their study experience. Questions will include:

1. Did you think you were receiving the study drug or the placebo?
2. What is your desire to please people?
3. If you had the opportunity in the future to take the study drug again, would you continue to take it for more than 5 weeks?
4. Did you limit your drinking because of flushing (a heat reaction or facial redness)?
5. Did you limit your drinking because of nausea or other effects?
6. Did your friends or family notice flushing?
7. If your friends or family noticed flushing, did this change your drinking?
8. Did you ever miss a dose of medication to avoid these effects?
9. Did you use any other services during the study to help you reduce drinking?

This eCRF will be used as the source document.

10.17. GS-548351 Plasma Levels

GS-548351 (the active moiety of the prodrug ANS-6637) will be measured plasma using a validated liquid chromatography – mass spectrometry assay. Venous blood will be collected twice during the study (see Table 3) to assess subject compliance with investigational products. Details of blood collection, sample preparation, storage and shipment to a central laboratory for testing will be described in the Study MOP. Briefly, samples for determining plasma levels of will be collected at Weeks 2 and 4. If the blood collection is missed at Weeks 2 or 4, then the sample will be collected at the next blood draw. Blood will be collected in 6 mL Vacutainer™ tubes containing K_2-EDTA anticoagulant. Plasma will be collected after centrifugation (at room temperature or refrigerated) and two aliquots of each subject's plasma will be stored at $< -20°C$ for up to one year, then at $\leq -70°C$, thereafter. One aliquot of each replicate plasma sample will

be shipped on dry ice to the testing lab at the end of the study for analysis. PPD Laboratories, 3230 Deming Way, Middleton, WI 53562 will be the testing laboratory. Collection tubes, labels, and associated shippers will be provided by PPD.

10.18. 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.

10.19. Medical History

A medical history will be taken for all potential study subjects to assure medical fitness during screening. The medical history will be updated on the day planned for randomization by asking the subject if anything has changed since the initial screening interview. Besides general medical history items, potential study subjects will be asked about family history of Parkinson's Disease and personal history of Parkinson's disease, history of severe reactions to alcohol consumption (flushing and heart rate increases/palpitations).

10.20. MINI

The MINI (paper version 7.0.3) 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 onto an eCRF. The individual items of the Alcohol Use Disorder Module will be also collected on an eCRF in addition to all other diagnoses.

10.21. Penn Alcohol Craving Scale

The PACS is a five-item self-administered instrument for assessing craving (Flannery-1999). Frequency, intensity, and duration of thoughts about drinking are assessed along with ability to resist drinking. The final item asks the responder to provide an average rating of his/her craving over the course of the past week. The questions on the PACS use descriptors coupled with numerical ratings ranging from 0 to 6. This questionnaire will be completed by the subject and will be both the source and an eCRF.

10.22. Pittsburg Sleep Quality Index

The PSQI is a 19-item questionnaire with 6 subscales (subjective sleep quality, sleep latency, sleep duration, habitual sleep disturbances, use of sleep medication and day time dysfunction) (Buysse-1989). Each subscale is rated from 0 to 3 with the higher scores reflecting more severe

sleep complaints. The addition of all the scores permits an analysis of the subject's overall sleep experience in the past 30 days. The lower the overall score, the better the person sleeps.

The tool has an adequate internal reliability, validity and consistency for clinical and community samples of the various populations. This questionnaire will be completed by the subject and will be both the source and an eCRF.

10.23. PROMIS Alcohol Negative Consequences

For negative consequences for alcohol use, the short form of the PROMIS Alcohol Negative Consequences questionnaire will be used to assess outcomes of alcohol use over the past 30 days (Pilkonis-2013).

The 7 PROMIS items include:

- Drinking created problems between me and others
- I disappointed others when I drank
- I was unreliable after I drank
- Others complained about my drinking
- I used poor judgment when I drank
- I said or did embarrassing things when I drank
- I had trouble getting things done after I drank

Each item is rated on a 5 point scale including: Never (1), Rarely (2), Sometimes (3), Often (4), and Almost Always (5) for the past 30 days.

10.24. 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. All medications reported by the subject will be recorded on a source document and an eCRF.

10.25. Profile of Mood State

The POMS measures dimensions of affect or mood (McNair-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 and will be both the source and an eCRF.

10.26. Physical Examination and Weight

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 physical exam will be updated on the day planned for randomization by querying the subject

about any physical changes since the screening examination. Weight (kg) will also be collected. These data will be recorded on a source document and an eCRF.

10.27. 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 CRF 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 (Week 6).
2. Subject completed the full intervention phase of the study (Week 5).
3. Subject was withdrawn prior to the Week 6 visit (reasons for early withdrawal are to be specified).
4. If the subject discontinued study medications, the date of discontinuation.

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.

10.28. TLFB Interview

Drinking behavior will be assessed using the TLFB methodology (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 6.

Table 6: 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 • 16 oz (pint) = 8.5 • 25 oz (a fifth) = 17.0 • 1.75 L (59 oz) = 39.0

10.29. Urine Drug Test

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), and EtG. If positive for opioids but recent opiate 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 re-evaluate their medical history for substance abuse. EtG is a biomarker of recent alcohol consumption that provides an objective measure of abstinence (Jatlow-2014). These data will be recorded on a source document and eCRF.

10.30. Vital Signs

Vital signs to be assessed include sitting blood pressure and pulse rate. Vital signs will be taken after resting seated in a chair for at least 3-5 min with both feet on the floor, legs not crossed with the arm used for the blood pressure measurement at approximately chest height with support. These data will be recorded on a source document and an eCRF.

10.31. 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.

11. Statistical Methods and Determination of Sample Size

A formal statistical analysis plan (SAP) will be finalized prior to locking the database and commencing with the analysis. The main study database can be locked prior to receipt of the plasma concentration data and the ALDH2 genetics testing data from the central laboratories.

11.1. Statistical Hypotheses

Primary Efficacy Endpoint: Subjects treated with ANS-6637 600 mg/day will report significantly lower VAS alcohol craving ratings in response to *in vivo* alcohol cues during human lab testing than ANS-6637 200 mg/day and placebo-treated subjects. Subjects treated with ANS-6637 200 mg/day will have significantly lower VAS alcohol craving ratings in response to *in vivo* alcohol cues than placebo-treated subjects.

The hypotheses for the confirmatory secondary endpoints for the cue reactivity sessions are the same as for the primary endpoint.

Secondary Efficacy Endpoints: In the same dose-response manner, it is hypothesized that, during the last 4 weeks of the treatment period, the ANS-6637 groups, as compared to the placebo group, will:

1. Increase the percentage of subjects with no heavy drinking days. A "heavy drinking day" is 4 or more drinks per drinking day for women and 5 or more drinks per drinking day for men.
2. Increase the percentage of subjects abstinent from alcohol
3. Increase the percentage of subjects with at least a WHO 2-level decrease in alcohol consumption
4. Increase the percentage of subjects with at least a WHO 1-level decrease in alcohol consumption
5. Increase the percentage of days abstinent per week
6. Decrease the percentage of heavy drinking days per week
7. Decrease the percentage of very heavy drinking days per week
8. Decrease the weekly mean number of drinks per week
9. Decrease the weekly mean drinks per drinking day
10. Decrease the weekly mean cigarettes smoked per week among smokers
11. Increase the percentage of subjects abstinent from nicotine use among subjects who used any nicotine products in the week before randomization
12. Decrease the mean alcohol craving score (PACS)
13. Decrease the mean PSQI score
14. Decrease total mood disturbance (POMS)

11.2. Analysis Populations

The study analysis populations will consist of the following:

Modified Intention-to-Treat (mITT) Analysis Set: The mITT set is defined as subjects randomized to participate in the study that took at least one dose of investigational product and had a non-missing VAS craving primary endpoint.

Evaluable Analysis Set: The evaluable analysis set for the secondary endpoints is defined as those subjects randomized to the study who took 2 tablets per day for at least 80% of days in Weeks 1-5.

Safety Analysis Set: The safety analysis set includes all subjects who took at least one dose of investigational product.

The mITT analysis set will be used for all efficacy endpoints listed in Section 11.1. Safety analyses will be conducted on the safety analysis set. The Evaluable analysis set will only be used for the primary and confirmatory efficacy endpoints and for select secondary efficacy endpoints. Selected secondary efficacy endpoints include for the evaluable subset analysis:

- Weekly percentage of heavy drinking days
- Mean number of drinks per week
- PACS scores

11.3. 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 confirmatory endpoints will use multiplicity adjustment to maintain a family-wise 0.05 significance level. P-values for secondary endpoints of < 0.05 will be considered statistically significant with no adjustment. Endpoint data will also be screened for outliers and skewness.

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.

11.4. Analysis Addressing the Primary Efficacy Endpoint

Each subject will have an initial alcohol cue for "strength" of alcohol craving score from the VAS that is the primary endpoint. Analysis of covariance (ANCOVA) will be used to compare groups with the "strength" of alcohol craving value as the dependent variable and the pretreatment "strength' of alcohol craving score from the first alcohol cue as an independent fixed effect. Clinical site will also be included as an independent factor. There are 3 comparisons (ANS-6637 600 mg vs placebo; ANS-6637 200 mg vs. placebo; ANS-6637 600 mg vs. ANS-6637 200 mg) and Tukey's method will be used to maintain a family-wise 0.05 significance level.

No imputation for missing endpoint data will be performed.

11.5. Secondary Efficacy Endpoints Analysis

There are 3 additional questions asked during the cue sessions for each beverage cue. Each of these questions will be analyzed in the same manner as the primary endpoint. Tukey's method will be used within question and not across questions. An average of the 4 questions will also be analyzed in the same manner. The difference between the first alcohol cue and water cue for each VAS item will be computed at both the pre and post treatment time points. The difference values for each VAS item and the average difference will be analyzed similarly to the primary endpoint. Continuous secondary endpoints (percent heavy drinking days, percent very heavy drinking days, percent days abstinent, drinks per week, drinks per drinking day, number of cigarettes smoked per week, PACS, POMS, and PSQI score) during the last 4 weeks of treatment period will be analyzed using a mixed-effects model with site, assessment time, and baseline drinking as fixed factors. Models will also include time by treatment group interaction term. Additional covariates may be included that are significantly correlated with outcome and/or if there are differences across the treatment groups.

Analysis of the dichotomous secondary endpoints (percentage subjects with no heavy drinking days, percentage subjects abstinent from alcohol, percentage of subjects achieving at least a one and two-level shift in WHO alcohol consumption, and no nicotine use among subjects with any use in the week prior to randomization) during the last 4-weeks of the treatment period will be conducted via logistic regression. Covariates may be included provided there are a sufficient number of events.

No imputation for missing secondary endpoint data will be performed.

11.6. Safety Outcomes

AEs will be coded using the most recent version of the Medical Dictionary of Regulatory Activities (MedDRA) preferred terms and will be grouped by system, organ, and class (SOC) designation. The severity, frequency, and relationship of AEs to investigational product will be presented by preferred term by SOC grouping. Listings of each individual AE including start date, stop date, severity, relationship, outcome, and duration will be provided. Each AE (based on preferred terminology) will be counted once only for a given study participant. If the same AE occurred on multiple occasions, the highest severity will be assumed. Thus, study participants are not counted multiple times in a given numerator in the calculation of frequencies for a specific AE. The elicited AEs of flushing, heat sensation, heart rate increases or palpitations will be presented as counts and frequencies over all subjects and separately over the subjects who reported any of these as counts and frequencies of reported events by treatment groups and severity score for ALDH2 deficient and ALDH2 non-deficient subjects. A separate listing of AEs and summary table will be presented for subjects who took a drug that was a CYP3A-sensitive substrate at any time during the study. Laboratory data, vital signs, ECG results, alcohol breathalyzer results, urine drug test results, and CIWA scores will be reported as summary statistics. The numbers and proportion of subjects who reported CIWA scores ≥ 10 at any time after the start of dosing will be presented. Changes in clinical laboratory tests and vital signs will also be presented as summary statistics of change from baseline. The number and percentage of subjects with a higher ACQ-SF-R score post cue session will be provided by treatment group.

11.7. Baseline Descriptive Statistics

Summaries of the characteristics of the subjects in each of the study groups at baseline will be prepared for the mITT, evaluable, and safety analysis sets. Baseline characteristics will be compared between the ANS-6637 groups and placebo groups using appropriate statistical methods.

11.8. Compliance and Participation Outcomes

Medication compliance is defined as the amount of investigational products taken as a proportion of the total amount prescribed. Compliance will also be evaluated by determining the proportion of subjects who were prescribed ANS-6637, reported taking ANS-6637 (by AiCure assessment), and had a plasma sample with detectable GS-548351, the active moiety of ANS-6637. The participation rate is the percentage of subjects with complete drinking data. Compliance and participation rates will be reported on a weekly basis and across the entire trial duration. Compliance by GS-548351 plasma levels will be reported as number and percentage of subjects with a level above the limit of detection at each time point. Descriptive statistics will also be provided.

11.9. Exploratory Endpoints

The VAS responses to the second alcohol cue will be explored to determine if there are differences and if these differences might have any influence on the endpoints to guide for the design of future studies.

11.10. Sample Size Justification

Analysis of covariance with clinical site and baseline cue as the covariates will be used to model the primary outcome – the VAS craving score for the first alcohol cue. Statistical power was estimated using [PASS 13] with the following parameters. The treatment effect parameter—a maximal 3-point medication-placebo difference on the strength of VAS craving in response to the alcohol cue—was obtained from Roberts (2017), which used a similar cue reactivity paradigm. The mean values on this outcome at Week 2 are assumed to be 11 for 600 mg ANS-667, 12.5 for 200 mg ANS-667, and 14 for placebo and a standard deviation between subjects of 4. Clinical site and the baseline VAS craving for the alcohol cue were assumed to have correlations of 0.13 and 0.39, respectively, with the primary outcome. These correlations, coupled with assumed standard deviations for site and baseline VAS craving for the alcohol cue of 0.82 and 4, respectively, yield explained variation of 26%. These assumptions, with a sample size of 27 subjects per arm (81 total subjects), provides 82% statistical power with a 0.05 two-sided significance level. Using the Tukey test to adjust for the pairwise treatment arm comparisons reduces the power to 81% with a family-wise Type 1 error of 0.05 in 1000 simulations given the above assumptions.

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). Clinical monitors will review source documents and CRFs 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 NIAAA. 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 CRF 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 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 NIAAA if contacted by a regulatory agency about an inspection.

13. Ethics

13.1. Central Institutional Review Board

The study will be conducted under a protocol reviewed by a 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) Parts 50 and 56 and the Belmont Principles.

13.2. 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.

13.2.1. 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.

13.2.2. 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 central 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, CRFs, 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 NIAAA 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 CRF 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. Payments will be in the amounts equivalent to $30 for the screening visit, $50 for the randomization visit, $100 for the Week 2 cue reactivity session, $75 for Visits 4, 5, and 6, $90 for the final clinic visit (Visit 7), and $100 for the final telephone follow-up, for a total of $595.

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 NIAAA or designated clinical monitors.

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 provided by Fast-Track. 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.

Data from this study will be submitted to the National Institute on Alcohol Abuse and Alcoholism Database (NIAAADA) at the National Institutes of Health (NIH). NIAAADA is a data repository that allows researchers studying alcohol use disorder to collect and share de-identified information with each other.

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

NIAAA has registered 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.

17. References

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