The Influence of ANS-6637 on Midazolam Pharmacokinetics in Healthy Volunteers (SEARCH PK)

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Table of Abbreviations

λZ	Apparent elimination rate constant
AE	Adverse event
ALDH-2	Aldehyde dehyrdrogenase
AhR	Human aryl hydrocarbon
ANS	Amygdala Neurosciences
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the curve
AUC(0-tau)	Area under the concentration vs. time curve 0- end of the dosing interval
AUC(0-24)	Area under the concentration vs. time curve from 0-24hrs
$AUC_{0\text{-}inf}$	Area under the concentration vs. time curve extrapolated to infinity
AUC (0-_{last})	Area under the concentration vs. time curve 0 to last quantifiable point
CBC	Complete blood count
CNS	Central Nervous System
CPRS	Comprehensive Psychopathology Scale
CPRU	Clinical Pharmacokinetics Research Unit
C_{max}	Maximum observed plasma concentration
C_{min}	Minimum observed plasma concentration
Ctau	Concentration observed at the end of the dosing interval
Cl/F	Apparent oral clearance
CSO	Clinical Safety Office
CYP	Cytochrome P450
DDI	Drug drug interaction
DOPAC	3,4-dihydroxyphenylacetic acid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders-5
ECG	Electrocardiogram
EtOH	Ethanol
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IDU	Injection drug use
IRB	Institutional Review Board
IV	Intravenous
NAc	Nucleus accumbens
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NIAID	National Institute of Allergy and Infectious Diseases
NIDA	National Institute on Drug Abuse
NIH	National Institutes of Health
NIMH	National Institute of Mental Health
NINDS	National Institute of Neurological Disorders and Stroke
MAD	Multiple ascending doses
MAO	Monoamine oxidase

MDZ	Midazolam
OUD	Ppioid use disorder
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
PANAS	Positive Affect Negative Affect Scale
PK	Pharmacokinetic(s)
PXR	Human pregnane X receptor
SAD	Single ascending doses
SMC	Safety Monitoring Committee
SRCP	Safety Review and Communications Plan
TEAE	Treatment emergent adverse events
$t_{½}$	Terminal half-life
THP	Tetrahydropapaveroline
Tmax	Time to maximum plasma concentration
Vd/F	Apparent volume of distribution
VTA	Ventral tegmental area

Protocol Summary

Full Title:	The Influence of ANS-6637 on Midazolam Pharmacokinetics in Healthy Volunteers
Short Title:	SEARCH PK
Clinical Phase:	I
IND Sponsor:	Office of Clinical Research Policy and Regulatory Operations (OCRPRO)
Conducted by:	National Institute of Allergy and Infectious Diseases (NIAID)
Principal Investigator:	Henry Masur, MD
Sample Size:	N = 12
Accrual Ceiling:	50
Study Population:	Healthy volunteers.
Accrual Period:	6-9 months
Study Design:	Open label, fixed sequence intra-subject drug-drug interaction study designed to evaluate the effect of CYP3A4 inhibition by ANS-6637 and GS-548351 (active metabolite) on single dose midazolam and 1-hydroxymidazolam pharmacokinetics. The study will include 12 healthy volunteers. Subjects will receive (1) midazolam 5 mg po single dose on Day 1 followed by (2) Drug free period on Day 2 followed by (3) ANS-6637 600 mg po daily (Days 3-7) to reach steady state followed by (4) ANS-6637 600 mg po single dose + midazolam 5mg po single dose on Day 8
Study Duration:	Start Date: February 2019 End Date: July-August 2020
Study Agent:	ANS-6637 600 mg

Primary Objective: To evaluate the impact of steady state ANS-6637/GS-548351 on the single dose pharmacokinetics of midazolam (MDZ) and 1-hydroxymidazolam in healthy volunteers

Secondary Objective: The secondary objectives of this study are to

(1) Describe the steady state pharmacokinetics of ANS-6637 and GS-548351

(2) Evaluate the safety and tolerability of administration of ANS-6637 alone or in combination with MDZ

Exploratory Objective: Exploratory objectives of this study are to evaluate:

1. the metabolism of dopamine before and after steady state ANS-6637 in healthy volunteers
2. pharmacogenomics of cytochrome P450 (CYP) enzymes and drug transporters involved in midazolam and ANS-6637 metabolism and transport
3. psychological well being as measured by a change in scale scores at baseline and when ANS-6637 has reached steady state

Primary Endpoints: Plasma area under the concentration time curve 0-infinity ($AUC_{0\text{-}inf}$), maximum total plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}),apparent elimination rate constant (λZ), terminal half-life ($t_{½}$), apparent oral clearance (CL/F), apparent volume of distribution (Vd/F) and minimum total plasma concentration (C_{min}) of midazolam (MDZ), 1'-hydroxymidazolam

Secondary Endpoints: (1) Plasma area under the concentration time curve 0-24 hours ($AUC_{0\text{-}24}$), Plasma area under the concentration time curve 0-last quantifiable point ($AUC_{0\text{-}last}$), time to maximum plasma concentration (t_{max}),apparent elimination rate constant (λZ), terminal half-life ($t_{½}$), apparent oral clearance (CL/F), Vd/F and minimum total plasma concentration (C_{min}) of ANS-6637 and GS-548351.

(2) number of Grade 1-4 adverse events, as defined by the DAIDS Toxicity Table Version 2.1, July, 2017.[20]

Exploratory Endpoints: The following will be measured:

- Dopamine metabolites including urine and plasma dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) levels at baseline and steady state of ANS-6637
- Pharmacogenomics of CYP and drug transporters involved in midazolam and ANS-6637 metabolism and transport
- Psychological assessments include the Comprehensive Psychopathology Scale (CPRS), Snaith Hamilton pleasure scale (SHAPS), and the Positive Affect Negative Affect Scale (PANAS) at baseline and when ANS-6637 has reached steady state

Précis

Opioid use causes a myriad of effects which contribute to significant morbidity and early mortality, and is associated with risky sexual behavior and injection drug use (IDU), two major forms of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) transmission in urban and suburban United States[1-2]. Through these high-risk behaviors, persons with opioid use disorder (OUD) develop both direct comorbidities (e.g. blood stream infections and infectious endocarditis), as well as risk-associated illnesses (e.g. sexually transmitted infections, HCV and hepatitis B virus [HBV]) which have considerable downstream health care effects[3-4]. As such, there is a need for pharmacologic agents in the treatment of OUD that go beyond avoidance of withdrawal and facilitate decreased frequency or complete cessation of opioid use.

The biologic mechanism of OUD, common to all forms of addiction, is a conditioned drug cue-related response in the CNS, causing a dopamine surge[5]. If effective, a central pharmacologic strategy targeting the aberrant reward circuitry seen in OUD could potentially reduce drug craving and result in opioid abstinence.

In the SEARCH Pharmacokinetic (PK) investigation, we aim to understand the pharmacokinetic signal of the novel, oral agent ANS-6637, an aldehyde dehydrogenase 2 (ALDH-2) inhibitor that has the potential to reduce dopamine surge in the CNS and inhibit opioid craving. In preclinical studies, the active metabolite of ANS-6637, GS-548351, showed substrate dependent inhibition of CYP3A in vitro, with little or no inhibitory effect on the activities of other cytochrome P450 (CYP) enzymes. As such, the current investigation seeks to explore the potential inhibition of CYP3A by ANS-6637 with the FDA-recommended CYP3A probe substrate, midazolam[6].

1 Background Information and Scientific Rationale

1.1 Background Information

Substance use and addiction are multi-dimensional, involving psychologic factors in addition to the physiologic mechanisms of withdrawal. One of these factors is the experience of drug craving, which has been conceptualized in various ways including: wanting to re-experience effects of a drug, a strong subjective drive, irresistible urge promoting drug use, obsessive thoughts, relief from unpleasant withdrawal symptoms, the incentive to self-administer a drug, the expectation of positive outcomes, a cognitive appraisal process, and non-automatic cognitive processing[7]. Craving predicts future drug use and is a core feature of substance use disorder diagnosis in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5)[8].

The physiologic experience of craving is common among all drugs of abuse, and lies in the central reward system, which involves communication between the ventral tegmental area (VTA), the nucleus accumbens (NAc), and the pre-frontal cortex. Dopamine released from the VTA into the NAc appears to be a critical pathway in substance use disorder, with surges of dopamine associated with euphoric effects[5,9]. With addiction, a conditioned response to stimulus related cues (drug, alcohol, nicotine) is a surge in dopamine levels within the NAc, triggering craving, drug seeking behavior and/or relapse, physiologically underpinning the cycle of addiction.[5]

Daidzin, a component of Kudzu extracts, is an ancient Chinese remedy for alcoholism, and a selective and reversible competitive inhibitor of aldehyde dehydrogenase 2 (ALDH-2). In animal studies, ALDH-2 inhibition has been shown to suppress alcohol intake and cocaine self-administration and cue-induced reinstatement of relapse-like behavior in rats[10-14]. The proposed molecular mechanism by which ALDH-2 inhibition induces this response is through potentiation of a specific substrate- tetrahydropapaveroline (THP)- which exerts negative feedback to suppress ongoing dopamine production. Dopamine, when metabolized by monoamine oxidase (MAO), produces the metabolite DOPAL (3,4-dihydroxyphenylacetaldehyde). DOPAL must be further metabolized by ALDH-2, but with its inhibition, DOPAL levels increase, condensing with dopamine to form THP in VTA neurons. THP selectively inhibits phosphorylated (activated) tyrosine hydroxylase to reduce dopamine production via negative-feedback signaling, preventing high dopamine levels in the CNS.[15]

1.1.1 Study Team Background

This pharmacokinetic investigation is the first in a series of studies involving ANS-6637 to be performed under the 6-year SEARCH Program. SEARCH represents a multidisciplinary, cross-institution, public-private-academic collaboration between the National Institute of Allergy and Infectious Diseases

(NIAID), National Institutes of Health (NIH) Clinical Center, National Institute of Neurological Disorders and Stroke (NINDS), National Institute on Drug Abuse (NIDA), National Institute of Mental Health (NIMH), and Amygdala Neurosciences (ANS). The overall approach and operations will be led by Henry Masur (PI), Chief of Critical Care Medicine at NIH Clinical Center, and Director of DC PFAP. Given the unique biologic and behavioral aspects of opiod use disorder (OUD), the project requires special expertise in neurobiology, behavioral evaluation, and psychological assessment to fully characterize the effectiveness of ALDH-2 inhibition and bring credibility to the research. The neurobiologic component of our investigation will be guided by David Goldstein MD, PhD, NINDS Senior Investigator, Neurocardiology Section, and Avi Nath, MD, NINDS Clinical Director. The behavioral research component of our investigation will be guided by Kenzie Preston, PhD, NIDA Chief of Clinical Pharmacology and Therapeutics Research Branch, and Karran Phillips, MD, Clinical Director of the NIDA Archway Center in Baltimore. The psychological assessments within the SEARCH investigation will be guided by Maryland Pao, MD, Clinical Director of the NIMH Intramural Research Program, as well as David Goldman, MD, Clinical Director of National Institute on Alcohol Abuse and Alcoholism (NIAAA), and Nancy Diazgranados, MD, Deputy Scientific Director of NIAAA, and Vijay Ramchandani, NIAAA.

1.1.2 Description of the Study Agent/Intervention(s)

A. ANS-6637

The compound ANS-6637 is a prodrug of GS-548351, created by Amygdala Neurosciences, is a highly selective ALDH-2 inhibitor, which has been shown to limit dopaminergic surges in animal models. Administration of ANS-6637 in rats resulted in suppressed tobacco, alcohol, or cocaine self-administration and prevented cue-induced drug-seeking behavior[10, 9]. Importantly, baseline levels of dopamine in the rat model were not affected (since THP does not inhibit activities of other enzymes involved in dopamine metabolism), but rather a targeted suppression of dopaminergic surges. An initial ANS-6637 Phase 1 trial demonstrated that the drug was well-tolerated with only mild AEs, and a Phase 1b study completed in 2017 (NCT03203499) showed that ANS-6637 was safe and well-tolerated even during excessive alcohol drinking, demonstrating that ALDH-2 inhibition is suitable even for patients with comorbid alcohol use.

B. Midazolam

Midazolam is a short-acting, water-soluble benzodiazepine indicated for use as a sedative and amnestic agent. In pharmacokinetic studies, particularly drug-drug interaction (DDI) studies examining CYP3A activity, the use of midazolam (MDZ) (intravenous [IV] and oral) as a CYP3A enzyme selective probe is well established. The October 2017 Draft FDA Guidance for Industry titled “Clinical Drug Interaction Studies – Study Design, Data Analysis and Clinical Implications Guidance for Industry” [18] recommends the use of MDZ as a sensitive CYP3A4 phenotyping substrate drug. Midazolam exhibits kinetic features ideal for

determining CYP3A activity as it undergoes extensive metabolism (> 97%) that is predominantly, if not exclusively, mediated by CYP3A subfamily. The half-life of MDZ in the blood is relatively short ($T_{1/2}$: approximately 60-90 minutes) and the elimination of the primary and secondary metabolites is formation rate limited. Neither MDZ, nor its major 1´-hydroxy metabolite, are substrates of the P glycoprotein transporter present in tissues, such as the intestinal tract and liver. Orally administered MDZ is rapidly and nearly completely absorbed (T_{max} 15-30 minutes). These properties of MDZ make it the probe drug of choice for conducting short duration pharmacokinetic studies designed to determine hepatic and intestinal CYP3A dependent metabolism.[16]

Midazolam acts as a CNS depressant. In the current study, 5 mg of MDZ syrup will be administered as a single dose (0.07 to 0.08 mg/kg for a 60-70 kg person) for probing CYP3A activity. This dose is much lower than that indicated for use in pediatric patients as a single dose (0.25 to 1 mg/kg with a maximum dose of 20 mg) for pre-procedural sedation and anxiolysis. Time to onset of effect is most frequently reported as 10 to 20 minutes, with recovery from effects within 20 minutes in most subjects. Sedation and respiratory depression are most common pharmacologic and possible adverse events associated with MDZ.

Further information regarding MDZ is available in the midazolam hydrochloride syrup package insert.

1.1.3 Summary of Previous Pre-Clinical Studies ANS-6637

Preclinical Pharmacology and Toxicology
Further information is available in the IB.

Preclinical Drug Metabolism and Pharmacokinetics
ANS-6637 is a methylene phosphate prodrug designed to be cleaved in the intestinal lumen by phosphatases located on the apical membrane of enterocytes allowing the active metabolite, GS-548351, to be absorbed. ANS-6637 is rapidly hydrolyzed to GS-548351 in intestinal S9 fractions and by Caco 2 cell monolayers. Additionally, no intact prodrug is detected in portal vein or jugular vein blood samples drawn following dosing of ANS-6637 to rats. Consistent with the high bioavailability of GS-548351 following dosing of ANS-6637 seen in nonclinical species, GS-548351 shows high forward permeability across Caco-2 monolayers, with low efflux, and has been shown to be a moderate substrate for P-gp and BCRP in cell-based assays. GS-548351 is not considered a substrate of the hepatic transporters OATP1B1 and OATP1B3.

GS-548351 has a moderately high volume of distribution, greater than that of total body water in nonclinical species. Consistent with the low systemic clearance observed in nonclinical species, GS-548351 has shown moderate metabolic stability in hepatic microsomal fractions and primary hepatocytes of preclinical species (rat, dog, cynomolgus and rhesus monkey) and high stability

in human hepatic microsomal fractions and primary hepatocytes. This is consistent with the finding that there was no detectable metabolism of GS-548351 by 7 recombinant human cytochrome P450 (CYP) enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2B6, CYP2C8, and CYP3A4).

GS-548351 had little or no inhibitory effect on the activities of human hepatic microsomal enzymes including CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, and CYP2E1 (IC_{50} >100 μM) and is thus unlikely to affect the pharmacokinetics of drugs metabolized by these enzymes. The IC_{50} of GS-548351 against CYP2C8 and CYP2C9 was determined to be 73.2 μM and 69.7 μM respectively, whereas inhibition of CYP3A showed substrate-dependence with midazolam 1'-hydroxylase being the most sensitive to inhibition than testosterone (IC_{50} values of 10.9 and 21.5 μM using midazolam and testosterone as substrates, respectively). The Ki for inhibition of CYP3A4 metabolism of midazolam was determined to be 1.27 μM. No evidence of time-dependent inhibition of CYP1A or CYP3A4 was observed. GS-548351 showed no significant inhibition of the conjugating uridine glucuronosyltransferase enzyme, UGT1A1, in vitro.

Based on the observed low levels of GS-548351-induced activation of human aryl hydrocarbon (AhR) or human pregnane X receptor (PXR), GS-548351 is unlikely to cause drug interactions through activation of either of these human xenobiotic receptors. Efficacious exposure of GS-548351 is unknown as yet; however, if clinically efficacious levels of GS-548351 exceed 15 μM, possible induction of activation of PXR may be observed. It should be noted however, stimulation with GS-548351 at 50 μM resulted in a 5 fold activation of PXR which is a third (~30%) of the maximum reported with rifampicin which is a known clinically relevant inducer of PXR. Concentrations of GS-548351 up to 50 μM led to < 1% of the AhR activation, and therefore clinical interaction through this pathyway are unlikely. GS-548351 shows no inhibition of efflux transporters P-gp and BCRP in cell based assays, but was a weak inhibitor of OATP1B1 and OATP1B3 with IC_{50} values of 22.9 μM and 22.4 μM, respectively.

Elimination of GS-548351 and its metabolites is likely to occur through a mixture of urinary, biliary and fecal excretion.

1.1.4 Summary of Relevant Clinical Studies

Clinical Trials of ANS-6637

A. A Phase 1, Double-Blind, Randomized, Placebo-Controlled, First in Human Study of ANS-6637 to Assess the Safety, Tolerability, and Pharmacokinetics of Single and Multiple Ascending Oral Doses in Healthy Smoker and Non-Smoker Subjects (Study GS-US-272-0101)

Study Design

Study GS-US-272-0101 is a Phase 1, double-blind, randomized, placebo-controlled, multicenter, first in human (FIH) study that targeted healthy non-smoker and smoker males and non-childbearing potential females aged 18 to 65 years of age, inclusive. Single ascending doses (SAD), multiple ascending doses (MAD), and a food effect cohort were conducted. The primary endpoints were to evaluate the safety, tolerability, and pharmacokinetic (PK) profile of single and multiple doses of ANS-6637 in healthy non-smokers and smokers. The secondary endpoint was to evaluate the effect of food on the PK of ANS-6637 and its metabolite(s) in healthy non-smokers using a cross-over study design. Up to 130 subjects were planned for enrollment.

Cohorts 1 through 4 included sequential dose escalation of a single oral dose of ANS-6637 in healthy non-smokers. Four sequential cohorts of 8 subjects each (6 receiving ANS-6637 and 2 receiving matching placebo) were enrolled for single dose treatment, with each cohort receiving a progressively higher dose of ANS-6637 (100, 300, 600, and 900 mg). Based on review of safety, tolerability, and PK data from the SAD non-smoker cohorts, a single SAD smoker cohort of 12 subjects (9 receiving ANS-6637 and 3 receiving matching placebo) was enrolled. SAD smoker Cohort 6 was administered a single oral dose of 100 mg ANS-6637.

Cohort 8 investigated the effect of food (high-fat meal) on the PK of ANS-6637 (300 mg single oral dose) in a parallel 2-sequence, 2-period cross-over cohort with 10 healthy non-smoker subjects (8 receiving GS-6337 and 2 receiving matching placebo).

Cohorts 9 through 12 included sequential dose escalation of multiple oral doses of ANS-6637 in healthy non-smokers. Four sequential cohorts of 8 subjects each (6 receiving ANS-6637 and 2 receiving matching placebo) were enrolled for multiple dose treatment, with each cohort receiving a progressively higher dose of ANS-6637 (100, 300, 600, and 900 mg). With the exception of the 100 mg dose level, all doses were administered once daily for 10 days. At 100 mg, ANS-6637 was administered twice daily for 9 days followed by a single AM dose on Day 10. Based on review of safety, tolerability, and PK data from the MAD non-smoker cohorts at the corresponding dose level, two MAD smoker cohorts of 12 subjects each (9 receiving ANS-6637 and 3 receiving matching placebo) were enrolled. MAD Smoker Cohorts 13 and 14 were administered multiple oral doses of ANS-6637 (300 and 600 mg, respectively) once daily for 10 days.

SAD non-smoker Cohort 5 (up to 1600 mg) and SAD smoker Cohort 7 (up to 1600 mg) were not enrolled.

Subject Disposition and Demographics

A total of 111 subjects were enrolled in the study, 108 subjects completed the study and 3 subjects were prematurely discontinued. No etiology for early termination occurred more than once. One subject was withdrawn after experiencing a pruritic rash. The other subject was withdrawn by the study Investigator after an electrocardiogram (ECG) change that demonstrated inverted T waves consistent with mild ischemia. The final subject was discontinued due to a protocol violation and replaced accordingly.

Safety Results

No deaths, severe AEs, or pregnancies were reported during the study. One subject, a nonsmoker in the ANS-6637 900 mg MAD cohort, had 4 serious adverse events (SAEs; hyperbilirubinemia and abnormal alanine aminotransferase [ALT], aspartate aminotransferase [AST], and alkaline phosphatase [ALP]) on Day 21, 11 days after completing study drug. These events were assessed by the investigator as mild in severity and related to study drug. All of the SAEs resolved by Day 68. Two subjects, both of whom received ANS-6637, had AEs leading to discontinuation of study drug. These included rash, burning sensation, and pruritus (all mild in severity) in 1 subject and electrocardiogram T-wave inversion (mild in severity) in the other subject. A total of 23 of 84 subjects in the ANS-6637 groups and 9 of 27 subjects in the placebo groups had treatment-emergent AEs, most of which were mild in severity. Adverse events were not dose-dependent, and no consistent pattern of AEs was observed between smokers and nonsmokers. For subjects in the SAD cohorts who received ANS-6637, the only AE reported for >1 subject was rhinitis. For subjects in the MAD cohorts who received ANS-6637, AEs reported in >1 subject included dizziness, pruritus, rash, abdominal pain, dry mouth, fatigue, and nausea. Fourteen subjects who received ANS-6637 and 2 subjects who received placebo had AEs that were reported as related to study drug. One subject who received ANS-6637 had an AE (erythema) reported as related to treatment procedures.

Most subjects had ≥1 treatment-emergent laboratory abnormality. Transient decreases from baseline in TSH and increases in free T4 were observed in some subjects. Despite the changes in TSH and free T_4 levels, subjects remained asymptomatic with no changes in their vital signs or overt signs or symptoms of thyroid dysfunction. No other patterns of laboratory abnormalities indicating a relationship to study drug or dose were observed across cohorts.

No subject had notable changes in vital sign parameters during the study, and no vital sign result was recorded as an AE. One nonserious AE of ECG T-wave inversion was reported for 1 nonsmoker receiving ANS-6637; no other subject had a clinically significant safety ECG result during the study.

Pharmacokinetic Results

ANS-6637 was rapidly converted to GS-548351 upon oral administration. Plasma and urine levels of ANS-6637 were generally below quantifiable limit (BQL) (<1 ng/mL and <50 ng/mL, respectively) at most time points at all doses tested (100 to 900 mg) in both the single- and multiple-dose cohorts.

The PK parameters of GS-548351 when ANS-6637 was administered as a single dose in smokers and nonsmokers are shown in Table 1. 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% CV). The median terminal half-life ($t_{1/2}$) 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 and nonsmokers were comparable.

Table 1. GS-548351 Mean (CV) Plasma PK Parameters Following Single Doses of ANS-6637 in Healthy Smokers and Nonsmokers

	Smokers	Nonsmokers			
GS-548351 Mean (CV) PK Parameter	**Cohort 6 100 mg (N=9)**	**Cohort 1 100 mg (N=6)**	**Cohort 2 300 mg (N = 6)**	**Cohort 3 600 mg (N = 6)**	**Cohort 4 900 mg (N=6)**
Cmax (ng/mL)	1718.9 (13.9)	1771.1 (27.4)	5756.7 (28.6)	13,433.3 (13.9)	14,300.0 (18.5)
Tmax (h)a	2.00 (2.00, 4.00)	3.00 (2.00, 3.00)	2.00 (2.00, 3.05)	2.00 (2.00, 3.00)	3.01 (3.00, 6.00)
t1/2 (h)a	16.49 (15.31, 19.22)	19.48 (17.77, 20.27)	17.43 (16.04, 19.07)	18.12 (17.32, 18.57)	18.96 (15.82, 19.43)
AUC0-last (h•ng/mL)	24,809.6 (17.8)	26,012.4 (26.6)	90,688.0 (24.8)	184,251.0 (21.1)	233,279.0 (16.7)
$AUC_{0\text{-inf}}$ (h•ng/mL)	28,658.8 (22.0)	30,845.7 (25.7)	106,699.8 (27.6)	211,984.2 (24.2)	278,039.6 (16.5)

a Median Q1, Q3

Mean steady-state PK parameters of GS-548351 following once- or twice-daily dosing of ANS-6637 under fasted conditions for 10 days are presented in Table 2. Median plasma $t_{1/2}$ values ranged from 16.41 to 19.24 hours, which were comparable with median values observed in the single-dose cohorts. Upon multiple dosing, GS-548351 exhibited slightly less than dose-proportional PK (C_{max} and AUC(0-tau)). GS-548351 plasma concentrations accumulated upon multiple dosing in accordance with its half-life and dosing interval at lower doses of ANS-6637 (100 mg twice daily and 300 mg once daily). However, at the 600- and 900-mg once daily dose levels, there was less accumulation of GS-548351 from Days 1 to 10 of dosing compared with the lower dose levels. The PK parameters (C_{max}, AUC(0-tau), and C_{tau}) in nonsmokers and smokers in the 300- and 600-mg once-daily cohorts were comparable.

Table 2. Mean (CV) PK Plasma Parameters Following Multiple Doses of ANS-6637 in Healthy Smokers and Nonsmokers on Day 10

	Smokers		Non-Smokers			
GS-548351 Mean (CV) PK Parameter	**Cohort 13 300 mg QD (N = 9)**	**Cohort 14 600 mg QD (N = 9)**	**Cohort 9 100 mg BID (N = 6)**	**Cohort 10 300 mg QD (N = 5)**	**Cohort 11 600 mg QD (N = 5)**	**Cohort 12 900 mg QD (N = 6)**
Cmax (ng/mL)	7175.6 (15.4)	11,687.8 (31.3)	4480.0 (23.8)	7534.0 (20.4)	13,080.0 (12.4)	18,200.0 (9.4)
Ctau (ng/mL)	1926.7 (39.2)	2720.0 (35.1)	2478.3 (36.4)	1810.0 (13.4)	2784.0 (28.7)	3571.7 (17.2)
Tmax (h)a	2.00 (1.50, 3.00)	1.50 (1.50, 3.00)	4.00 (4.00, 4.03)	1.50 (1.50, 3.00)	2.00 (2.00, 4.00)	3.00 (3.00, 4.00)
t1/2 (h)a	17.21 (14.37, 17.96)	16.41 (13.17, 20.23)	18.85 (17.63, 21.78)	17.65 (16.70, 18.25)	16.87 (16.77, 17.46)	19.24 (14.07, 22.55)
AUC(0-tau) (h•ng/mL)	87,329.3 (22.7)	129,314.8 (30.6)	40,183.6 (29.4)	85,038.0 (16.2)	141,653.2 (17.9)	187,870.5 (8.7)

BID = twice daily; QD = once daily; a Median (Q1, Q3)

GS-548351 PK parameters 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 are summarized in Table 3. 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 ($AUC_{0\text{-}inf}$) 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 3. Comparison of GS-548351 Plasma PK Parameters Following a Single Dose of ANS-6637 (300 mg) Administered Under Fed (High-Fat Meal) or Fasted Conditions in Healthy Nonsmokers

GS-548351 PK Parameter	Mean (%CV)		% Geometric Least Squares Mean Ratio (Fed/Fasted) (90% CI)
	ANS-6637 Fed (N = 8)	ANS-6637 Fasted (N = 8)	
C_{max} (ng/mL)	4,313.8 (28.0)	5,382.5 (16.7)	77.25 (59.83, 99.73)
$AUC_{0\text{-last}}$ (h•ng/mL)	83,102.9 (28.5)	89,901.4 (15.3)	89.37 (71.38, 111.88)
$AUC_{0\text{-inf}}$ (h•ng/mL)	98,970.1 (27.9)	103,914.1 (17.2)	93.01 (78.06, 110.83)

a Median (Q1, Q3)

Urine concentrations of GS-548351 were determined from predose up to 48 hours postdose in all subjects who received ANS-6637. The mean $\%Dose_{excreted}$ as GS-548351 following a single dose of ANS-6637 at doses from 100 to 900 mg ranged from 3.63% to 5.66%. The mean $\%Dose_{excreted}$ as GS-548351 in the multiple dose cohorts of ANS-6637 at doses from 100 to 900 mg ranged from 1.93% to 4.91%. The renal elimination of GS-548351 was comparable in nonsmokers and smokers in the single and multiple dose cohorts.

B. Studies GS-1180 and GS-1610

Results from ANS-6637/Alcohol DDI studies (GS-US-272-1180 and Study GS-US-272-1610) conducted by the previous IND owner should not be considered predictive because the study designs were flawed and the study results are not considered definitive: (i) subjects' pre-study drinking experience was not assessed or recorded and alcohol naïve subjects might have been enrolled; (ii) subjects were awoken at 5 am, fed a medium breakfast at 6 am, denied hydration starting at 8 am and dosed with ethanol (EtOH) at 9 am - this irregular schedule may have had a negative effect on EtOH tolerability; (iii) EtOH was consumed over a 3- to10 minute period, which is considerably faster than binge drinking, as defined by National Institute on Alcohol Abuse and Alcoholism (NIAAA); (iv) the number of subjects studied was too small to inform interaction predictions - only one subject received ANS-6637 at 200 mg and only one subject received ANS-6637 at 50 mg (this subject was later determined to be a

ALDH-2 heterozygote) before the study was stopped; and (v) HR stopping rules were below generally acceptable limits of HR increases based on age-predicated maximum HRs, which in turn may have forced premature discontinuation of the studies by the sponsor.

C. A Phase 1b, Proof of Concept, Dose-Ranging Study to Evaluate the Safety of the Co-administration of Ascending Doses of ANS-6637 and Ethanol in Healthy Male Alcohol Drinkers (ANS-A-C1-001)[19]

Study Design

This was 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 EtOH in healthy male alcohol drinkers. The study included a screening visit, a qualification visit, a Treatment Phase with up to 6 ascending-dose treatment cohorts, and follow- up. A total of up to 48 subjects were to be enrolled to evaluate up to 6 dose levels of ANS-6637 (8 subjects per cohort). Planned dose levels included doses of 25 mg, 50 mg, 100 mg, 200 mg, 400 mg and 600 mg. Dose escalation was determined based upon the safety results of preceding dose levels. Following completion of each cohort, blinded safety data were reviewed by the investigator and medical monitor in order to determine if it was safe to escalate to the next dose level.

Within 27 days of a standard medical screening visit, subjects attended a 1-day Qualification Phase visit. Eligible subjects consumed a standardized moderate-fat meal (550–650 calories; approximately 25–30% fat), and approximately 3 hours later, began a session of repeat EtOH administration, during which they received up to 5 standard drinks (14 grams of EtOH each, approximately 1 standard drink) over a 2-hour period to evaluate their tolerance to repeat administration of EtOH in a time frame approximating a “real-world” drinking episode and likely to cause intoxication. Subjects who were able to tolerate the repeat EtOH administration were eligible for the Treatment Phase. There was a minimum washout interval of approximately 44 hours between initiation of the repeat EtOH administration in the Qualification Phase and study drug administration in the Treatment Phase.

During the Treatment Phase, subjects were confined to the clinical site beginning on the day prior to dose administration (Day 0) until Day 3 (approximately 48 hours post-dose). Within each cohort, 8 subjects were randomized to receive one dose of ANS-6637 (25 mg, 50 mg, 100 mg, 200 mg, 400 mg or 600 mg) or placebo in a 3:1 fashion, such that a total of 6 subjects received ANS-6637 and 2 subjects received placebo at each dose level. As a safety precaution, no more than 4 subjects were administered study drug on the same day (a dosing subcohort), and individual subject dosing was staggered by approximately 30 to 45 minutes.

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 EtOH administration, during which they received up to 5 doses of EtOH (14 grams each) every 30 minutes, or until a stopping criterion applied . Safety assessments, pharmacodynamic assessments and pharmacokinetic blood sample collections were obtained up to 48 hours post-dose. Subjects were discharged after 48-hour post-dose procedures were completed and the investigator deemed it safe to do so. Subjects with clinically significant adverse events (AEs) were not to be discharged until deemed stable in the opinion of the investigator.

A follow-up visit was conducted approximately 7 (±2) days following discharge from the Treatment Phase or after early discontinuation from the study. A follow-up telephone call was made to subjects with ongoing AEs. Each subject participated in the study for approximately 6 weeks, from screening to follow-up.

Subject Disposition and Demographics

A total of 147 subjects were screened for enrollment into the study. Of those subjects, 48 subjects were eligible for the Qualification Phase, and 99 subjects did not meet study-specific screening requirements. The main reasons for failing screening criteria were based on the following: vital signs (Inclusion #5), current alcohol use (Inclusion #6), inability to comply with study requirements and restrictions (Inclusion #10), and subject was considered unsuitable or unlikely to comply (Exclusion #18). Of those who entered the Qualification Phase, 48 met Qualification Phase criteria and 8 did not meet these criteria and were not eligible for the Treatment Phase.

A total of 8 eligible subjects were randomized to each of the 6 dosing cohorts (6 x ANS-6637–treated and 2 x placebo-treated subjects per cohort) and received study drug in the Treatment Phase; all 48 randomized subjects were included in the Safety population. Two (5.6%) ANS-6637–treated subjects were discontinued early from the study: 1 subject in the 100 mg dosing cohort and 1 subject in the 200 mg dosing cohort were discontinued as they were lost to follow-up.

Safety Results

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

A total of 32 (88.9%) ANS-6637–treated subjects and 9 (75.0%) placebo-treated subjects reported a TEAE. The highest incidence of TEAEs was observed with ANS-6637 100 mg, 200 mg and 400 mg, with all subjects (6 [100%]) reporting at least 1 TEAE, followed by ANS-6637 600 mg and 50 mg (5 [83.3%] subjects each) and placebo (9 [75.0%] subjects); the lowest incidence was observed with ANS-6637 25 mg (4 [66.7%] subjects). 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 EtOH) 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-NCS 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 timepoints tested. Administration of ANS-6637 doses >25 mg resulted in increases in mean heart rate on Day 1 beginning 15 minutes after first EtOH 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 EtOH administration until the last measured timepoint (135 minutes after first EtOH 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.

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

A number of CS findings related to general appearance on physical examinations were reported, the majority of which were related to flushing observed on Day 1. On the ER 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–ER 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 (Grade 4) observed between 45 minutes and 135 minutes after the first EtOH administration, corresponding to between 2 and 5 drinks consumed. Flushing was observed in far fewer subjects following placebo than with ANS-6637.

1.2 Rationale for Current Study

Based on preliminary results from previous studies (GS-US-272-0101), there is minimal systemic exposure of ANS-6637 with plasma levels below the limit of quantitation (1 ng/mL) at most timepoints and no accumulation upon multiple dosing. As such, the prodrug ANS-6637 is unlikely to cause drug-drug interactions.

GS-548351, active metabolite of ANS-6637, showed substrate dependent inhibition of CYP3A in vitro (IC50 values of 10.9 and 21.5 μM using midazolam and testosterone as substrates, respectively). GS-548351 showed little or no inhibitory effect (IC_{50} >100 μM) on the activities of other CYP enzymes including CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2D6, and CYP2E1. GS-548351 was tested for potential time-dependent inhibition against CYP1A and CYP3A. No evidence of time dependent inhibition was observed. It is notable that GS-548351 showed no detectable inhibition of the nicotine-metabolizing enzyme CYP2A6 (IC50 >200 μM) and the EtOH-metabolizing enzyme CYP2E1 (IC50 >200 μM).

Based on the above results, the current investigation aims to evaluate the potential inhibition of CYP3A by ANS-6637 with the recommended probe substrate, MDZ. Healthy, non-smoker subjects will be selected for this study to remove any potential confounding effects of smoking, co-morbidities, and/or concomitant medications in the target population.

1.3 Rationale for Dose Selection

Preliminary PK data from study GS-US-272-0101 indicate that ANS-6637 is rapidly converted to the active compound, GS-548351, prior to reaching systemic circulation, and is below the limit of quantitation (<1 ng/mL) in plasma at most time points. GS-548351 is well-absorbed, with low clearance (CL/F 2 to 3 L/hr) and estimated half-life of 17 to 20 hours, and has low inter-subject PK variability

(%CV <30%). When ANS-6637 was administered as single ascending doses in the fasted state, GS-548351 exhibits approximately dose proportional PK (C_{max} and $AUC_{0\text{-}inf}$) following single doses of 100 mg to 600 mg ANS-6637 and less than dose proportional PK from 600 mg to 900 mg. Upon multiple dosing of ANS-6637, less accumulation was observed at the 600 and 900mg dose levels compared to lower dose levels.

Currently, once daily dosing of 100 mg, 300 mg, and 600 mg ANS-6637 are being considered for proof-of-concept/Phase 2 evaluation. As such, ANS-6637 600 mg daily was chosen to evaluate ANS-6637 as a potential perpetrator of CYP3A4 mediated drug-interactions. Additionally, single and multiple ANS-6637 doses (10 days) of up to 900 mg, which provides approximately 3-fold higher exposures, were well tolerated in Study GS-US-272-0101. Treatment-emergent AEs were usually mild and were not dose-dependent, and no consistent pattern of AEs was observed between smokers and nonsmokers in study GS-US-272-0101. One subject, a nonsmoker in the ANS-6637 900 mg MAD cohort, had 4 SAEs of hyperbilirubinaemia and abnormal ALT, AST, and ALP. The selection of 600 mg dose of ANS-6637 in this study is supported by the blinded safety and PK data of up to 900 mg ANS-6637 single dose and up to 900 mg ANS-6637 once daily dosing for 10 days in the FIH study (GS-US-272-0101).

This study will use MDZ as a selective CYP3A probe. The oral dose (5 mg) selected for use in this study is substantially lower than the maximum recommended MDZ dose of 20 mg, and has been frequently used in PK studies as a CYP3A probe drug. Data in literature indicate that this dose is associated with minimal clinical effects in healthy subjects.

2 Study Objectives

2.1 Primary Objective

To evaluate the impact of steady state ANS-6637/GS-548351 on the single dose pharmacokinetics of midazolam (MDZ) and 1-hydroxymidazolam in healthy volunteers

2.2 Secondary Objectives

The secondary objectives of this study are to (1) describe the steady state pharmacokinetics of ANS-6637 and GS-548351 and to (2) evaluate the safety and tolerability of administration of ANS-6637 alone or in combination with MDZ.

2.3 Exploratory Objectives

Exploratory Objective: Exploratory objectives of this study are to evaluate:

- the metabolism of dopamine before and after steady state ANS-6637 in healthy volunteers
- pharmacogenomics of cytochrome P450 enzymes and drug transporters associated with midazolam and ANS-6637 metabolism and transport
- psychological well being as measured by a change in scale scores at baseline and when ANS-6637 has reached steady state

3 Study Design

Following screening and Day 0 assessments, 12 eligible subjects will be enrolled and admitted as an inpatient to the NIH beginning Day 0 and will be discharged after the completion of assessments on Day 9. Subjects will return to the clinic on Day 15 for a follow up visit 7 (±1) days after the last dose of study drug on Day 8. See Figure 1 for details.On Day 1, subjects will receive Treatment A:

- 0 hrs or pre-dose blood draw will be collected from the subject
- Subjects will be provided with a standardized breakfast that consists of a well-balanced, moderate fat meal containing 500 calories, which will be provided by the NIH metabolic unit kitchen.
- During breakfast (within the first 5 minutes), single dose of MDZ 5 mg [2.5 mL of the 2 mg/mL oral syrup] will be administered via directly observed

therapy (DOT). Subjects will be administered this dose with 240mL of water by the nursing staff.
- Intensive PK blood sampling will occur at hours 0.5, 1, 2, 3, 4, 6, 8, 12, 22, and 24 hours postdose. A window of +/- 10 minutes will be given around each draw time point.
- All subjects will be restricted from unsupervised ambulation until 2 hours post MDZ administration.
- Subjects will be provided with lunch no sooner than 4 hours after the standardized breakfast.

On Day 2, there will be a drug-free period where no drug will be administered.

On Days 3 through 7, Treatment B, ANS-6637 600 mg [6 x 100 mg tablets], will be administered once a day via DOT in the morning with food. On Day 3 only, safety laboratory measures will be completed prior to dosing, and vital signs will be collected predose and approximately 2 hours postdose to monitor the effects of post ANS-6637 administration. All safety labs will be assessed prior to ANS-6637 dosing by the study team. Pausing and halting criteria would apply if there were safety lab results which met the defined criteria. On Day 7, psychological assessments will be completed.

On Day 8, Treatment C:

- Subjects will be provided with a standardized breakfast that consists of a well-balanced, moderate fat meal containing 500 calories, which will be provided by the NIH metabolic unit kitchen.
- 0 hrs or pre-dose blood draw will be collected from the subject
- During breakfast (within the first 5 minutes), single dose of MDZ 5 mg [2.5 mL of the 2 mg/mL oral syrup] will be administered via directly observed therapy (DOT) in combination with ANS-6637 600 mg [6 x 100 mg tablets]. Subjects will be administered the two medications with 240mL of water by the nursing staff.
- Intensive PK blood sampling will occur at hours 0.5, 1, 2, 3, 4, 6, 8, 12, 22, and 24 hours postdose. A window of +/- 10 minutes will be given around each draw time point.
- Subjects will be provided with lunch no sooner than 4 hours after the standardized breakfast. All subjects will be restricted from unsupervised ambulation until 2 hours post MDZ administration.

On Day 9, safety labs will be drawn, patients will be evaluated for adverse events, and dischaged as medically appropriate.

While inpatient at the study center, grapefruit juice, grapefruits, and Seville orange juice, as well as tea, coffee, chocolate, and other foods and beverages containing caffeine and other methyl xanthines (eg theophylline, theobromine, tea leaves, yerba mate, kola nuts and guarana berries)[21] will be prohibited.

On Day 15 (with a one day window), subjects will return to outpatient NIAID clinic for clinical and safety laboratory assessment, psychological assessments, review of AEs and contraindicated medications, and review of study restrictions.

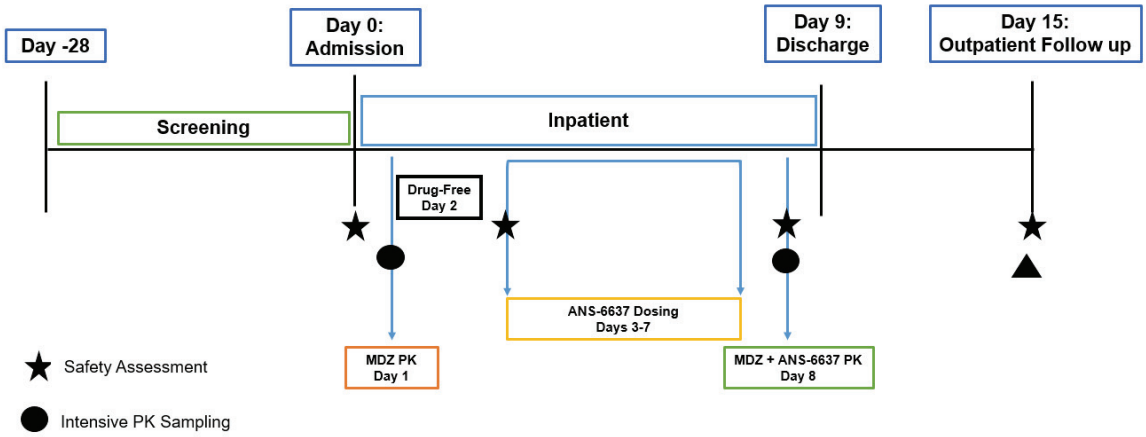

Figure 1. Study Schema.

Day 1: MDZ 5 mg [2.5 mL of the 2 mg/mL oral syrup], single dose, administered AM, fasted condition; Day 2 will be a drug-free period. Day 3-7: ANS-6637 600 mg [6 x 100 mg tablets], once daily, administered AM, fasted condition; Day 8: ANS-6637 600 mg [6 x 100 mg tablets] and MDZ 5 mg [2.5 mL of the 2 mg/mL oral syrup], co-administered in the morning, single dose, fasted condition

3.1 Description of the Study Design

This is an open label, fixed sequence intra-subject drug-drug interaction study designed to evaluate the effect of CYP3A4 inhibition by ANS-6637 and GS-548351 (active metabolite) on single dose midazolam and 1-hydroxymidazolam pharmacokinetics. The study will include 12 healthy volunteers. Subjects will receive (1) midazolam 5 mg po single dose on Day 1 followed by (2) Drug free period on Day 2 followed by (3) ANS-6637 600 mg po daily (Days 3-7) to reach steady state followed by (4) ANS-6637 600mg po single dose + midazolam 5 mg po single dose on Day 8. The study will be performed on a both outpatient and inpatient basis. Up to 50 subjects will be screened to enroll a total of 12 healthy volunteers until the study is completed or terminated, which we anticipate will take approximately 6 months. Subjects who are not able or choose not to complete the entire study will be replaced.

3.2 Study Endpoints

3.2.1 Primary Endpoint

The primary endpoints of this study will be assessed by characterization of plasma area under the concentration time curve 0-infinity ($AUC_{0\text{-}inf}$),

maximum total plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}),apparent elimination rate constant (λZ), terminal half-life ($t_{½}$), apparent oral clearance (CL/F), apparent volume of distribution (Vd/F) and minimum total plasma concentration (C_{min}) of midazolam (MDZ), 1'-hydroxymidazolam, $AUC_{0\text{-}inf,}$ C_{max}, t_{max}, λZ, $t_{½}$, CL/F, Vd/F and C_{min} of MDZ and 1-hydroxymidazolam.

3.2.2 Secondary Endpoints

The secondary endpoints of this study will be:

(1) Plasma area under the concentration time curve 0-24 hours ($AUC_{0\text{-}24}$), Plasma area under the concentration time curve 0-last quantifiable point ($AUC_{0\text{-}last}$), time to maximum plasma concentration (t_{max}),apparent elimination rate constant (λZ), terminal half-life ($t_{½}$), CL/F, Vd/F and minimum total plasma concentration (C_{min}) of ANS-6637 and GS-548351.

(2) number of Grade 1-4 adverse events, as defined by the DAIDS Toxicity Table Version 2.1, July, 2017.[20]

3.2.3 Exploratory Endpoints

Exploratory endpoint of this study will be the (1) levels of dopamine metabolites including urine and plasma dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) at baseline and steady state of ANS-6637, (2) pharmacogenomics of CYP and drug transporters, and (3) Assessments include the Comprehensive Psychopathology Scale (CPRS), Snaith Hamilton pleasure scale (SHAPS), and the Positive Affect Negative Affect Scale (PANAS) at baseline and at steady state of ANS-6637

4 Study Population

4.1 Rationale for Subject Selection

Up to 50 subjects will be screened to enroll a total of 12 healthy volunteers in this open–label, fixed sequence study. Participation of all ethnic groups and genders will be actively encouraged. This will be done through the study team and through the Patient Recruitment and Public Liaison Office at NIH, which recruits subjects for volunteer studies conducted at the NIH Clinical Center. There is an active effort to recruit minorities and women through outreach programs in the Washington, DC metropolitan area.

Healthy volunteers will be studied in order to eliminate the influence of confounding variables, such as medications, on study results. This study is not designed to assess the influence of gender, age, and/or ethnicity on the drug–drug interaction (if observed) between ANS-6637 and MDZ.

This study is being conducted at a single site, NIH. Individuals over the age of 65 years of age will not be included in the study. Pregnant women, children, cognitively impaired individuals, prisoners or other institutionalized individuals, or other individuals who are likely to be vulnerable will not participate in this study. A discussion of the exclusion of children, individuals over the age of 65 years of age, and individuals who are pregnant or breastfeeding is included in section 4.4 of this protocol.

4.2 Subject Inclusion Criteria

A subject will be considered eligible for this study only if all of the following criteria are met:

1. Must have the ability to understand and must personally sign a written informed consent form, which must be obtained prior to initiation of study procedures.

2. Must be between 18 and 65 years of age, inclusive.

3. Must have discontinued use of nicotine and nicotine containing products including vaping or juuling from 90 days prior to study drug dosing and throughout the study duration.

4. Must be willing to abstain from any food or beverages containing alcohol 72 hours prior to first dose and through follow-up visit.

5. Must be willing to abstain from cannabis 72 hours prior to first dose and through follow-up visit.

6. Must be willing to abstain from caffeine (including tea, coffee, chocolate) or grapefruit, Seville orange juice or other methyl xanthine containing foods (e.g. theophylline, theobromine, tea leaves, yerba mate, kola nuts and guarana berries)[21] 72 hours prior to the first dose and through follow-up visit.

7. Must have a body mass index (BMI) from 19 to 30 kg/m^2 (inclusive) at screening.

8. Must be human immunodeficiency virus type 1 (HIV-1) antibody negative at screening.

9. Must be hepatitis B (HBV) surface antigen negative at screening.

10. Must be hepatitis C (HCV) antibody or RNA negative at screening.

11. Male subjects must refrain from sperm donation from clinic admission, throughout the study period, and continuing for at least 90 days following the last dose of study drug.

12. Subjects must refrain from blood donation from clinic admission, throughout the study period, and continuing for at least 30 days following the last dose of study drug.

13. Must be willing to comply with contraception guidelines below
    Contraception:
    The fetal risks associated with ANS-6637 are not known, but pre-clinical animal data demonstrate some risk. Subjects must agree not to become pregnant or impregnate a female. Females of childbearing potential must have a reproductive risk assessment done to determine the risk of undetectable pregnancy at study start [i.e. sexual and contraceptive history for 30 days preceeding screening] pregnancy test at screening and baseline (Day 0). For the duration of the study, subject and their partners must practice <u>two</u> non-hormonal methods of birth control, having begun no less than 30 days, without interruption, prior to screening. They must continue to use both methods until 3 months after stopping the study drug. Two of the three methods of birth control listed below MUST be used, or an alternative combination offering very high efficacy, per the PI, in consultation with the Sponsor Medical Monitor may be considered:

    1. Male or female condoms [but not both] with a spermicide
    2. Diaphragm with a spermicide
    3. Intrauterine device (IUD)

    If pregnancy is suspected or should occur, subjects must notify the study staff immediately.

14. Must, in the opinion of the Investigator, be in good health based upon medical history and physical examination, and screening laboratory evaluations. SEE BELOW

15. Judged to be healthy based on medical history, physical examination, vital signs, and clinical laboratory tests at screening and Day 0: liver

function tests (AST, ALT, Tbili) ≤ upper limit of normal [ULN], platelets (PLT) >150,000/µL, hemoglobin (Hgb) >13 g/dL (males); >12 g/dL (females), CK ≤ 2x ULN, Amylase/lipase < ULN, thyroid function tests [TSH and T4] within normal range, fasting total cholesterol <240 mg/dL, or fasting triglycerides <240 mg/dL, per DAIDS AE table and Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Trials AE table for total bilirubin [Tbili] only.

16. Must be willing and able to comply with all study requirements.

4.3 Subject Exclusion Criteria

A subject will be ineligible for this study if 1 or more of the following criteria are met:

1. Therapy with any prescription, over-the-counter (OTC), herbal, or holistic medications, including hormonal contraceptives by any route, within 5 half-lives of the agent prior to receipt of any study medications will not be permitted with the following exception: Intermittent or short-course therapy (<14 days) with prescription or OTC medications, herbals, or holistic medications within the screening period prior to starting study drugs may be permitted after review by the investigators on a case-by-case basis for potential drug interactions. Receipt of influenza vaccination will be allowed prior to, during, and/or after the study.

2. Have any serious or active medical, surgical, or psychiatric conditions which, in the opinion of the Investigator, would interfere with subject treatment, assessment, or compliance with the protocol.

3. Have previously participated in an investigational trial involving administration of any investigational compound within 30 days prior to screening.

4. Have current, or a history of mild to severe alcohol use, cannabis or other substance use disorder including any use of illicit drugs as defined by DSM-5 criteria, within 12 months of first study dose

5. Renal impairment (chronic renal insufficiency of any chronic kidney disease stage, or acute renal failure not induced by drug therapy defined as eGFR <90 mL/min)

6. Have a positive urine drug test (ethanol, cannabis, barbiturates, cocaine, opiates, or amphetamines) at Screening or Day 0.

7. History of flushing or intolerance related to alcohol consumption using the NIAAA screening assessment questionnaire tool for alcohol flushing

8. Inability to obtain venous access for sample collection.

9. Have a history of significant drug sensitivity or drug allergy to any benzodiazepines.

10. Known hypersensitivity to formulation excipients: microcrystalline cellulose, croscarmellose sodium, magnesium stearate, polyvinyl alcohol, titanium dioxide, polyethylene glycol, and talc.

11. Have been treated with systemic steroids, immunosuppressant therapies or chemotherapeutic agents within 3 months of study screening or expected to receive these agents during the study (e.g., corticosteroids, immunoglobulins, and other immune- or cytokine-based therapies).

12. Presence or history of clinically significant cardiovascular disease, cardiomyopathy, and/or cardiac conduction abnormalities.

13. Have clinically significant ECG abnormalities or any of the following ECG abnormalities at Screening: PR >220 msec; QRS >120 msec; QTcF >450 msec; HR <40 beats per minute; second or third degree heart block.

14. Have a history or family history of Long QT Syndrome, Brugada syndrome, Wolfe-Parkinson-White Syndrome, or have a family history of sudden cardiac death or unexplained death in an otherwise healthy individual between the ages of 1 and 30 years.

15. Have history of syncope, palpitations, unexplained dizziness or chronic nausea or headaches.

16. Have an implanted defibrillator or pacemaker.

17. Have a history of liver disease, including Gilbert's Disease.

18. Any clinically significant electrolyte abnormality (outside of NIH normal reference ranges) at screening (e.g., hypokalemia, hypocalcemia, hypomagnesemia) or any condition that could lead to abnormal electrolyte disturbances (eg, eating disorder).

19. Have a history of taking dopamine antagonists/anti-psychotics.

20. Are unable to comply with study requirements.

21. Positive urine toxicology screen

Co-enrollment Guidelines: Co-enrollment in other trials will not be allowed during this study, other than enrollment on observational studies

4.4 Justification for Exclusion of Women, Minorities, and Children (Special Populations)

Children: Because this is not a therapeutic trial, there is no potential benefit for children to enroll, only a potential risk for those participants. In addition, age–related metabolic and transport processes in children differ from those of adults. As such, our research hypothesis is not applicable to this population.

Individuals Older than 65 Years of Age: As in children, changes in metabolic processes due to aging may confound findings from individuals greater than 65 years of age. As there is no potential benefit for these individuals to enroll, individuals greater than 65 years old will be excluded.

Individuals who are pregnant or breastfeeding:
Pregnancy: Pregnant women are excluded from this study because the effects of ANS-6637 on the embryogenesis and fetal development in humans are unknown.

Breast-feeding: Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with MDZ and ANS-6637, women who are breastfeeding are also excluded from study participation.

5 Study Agent/Interventions

ANS-6637:

How Supplied:

The study drug, ANS-6637, is supplied by Amygdala Neurosciences as white, round 100 mg tablets. Each bottle contains 30 tablets.

Storage:

ANS-6637 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).

Hazards and Precautions:

Measures that minimize drug contact with the body should always be considered during handling, preparation, and disposal procedures. Any unused study drug will be disposed of in accordance with the NIH clinical center pharmacy standard operating procedures.

Dose:

Dosing and Administration: 600 mg (6 x 100 mg tablets)

Route of Administration: Oral

Dose Adjustments/Modifications/Delays

Duration of Therapy: 6 days (Days 3-8)

Use of Ancillary Medications/OTC Products/Foods: As per Inclusion criteria

Subject Access to Study Agent at Study Closure: None

Midazolam:[22]

How Supplied:

This is a formulary drug and will be provided by the NIH clinical center central pharmacy.

Storage:

Store at 20° to 25°C (68° to 77°F)

Hazards and Precautions:

Measures that minimize drug contact with the body should always be considered during handling, preparation, and disposal procedures. Any unused study drug will be disposed of in accordance with the NIH clinical center pharmacy standard operating procedures.

Dose:

Dosing and Administration: 2.5mL

Route of Administration: oral

Duration of Therapy: Day 1 x 1 single dose and Day 8 x 1 single dose

Use of Ancillary Medications/OTC Products/Foods: As per Inclusion criteria

Subject Access to Study Agent at Study Closure: None

Study Drugs Disposition and Dispensation:

Study agents will be distributed via the NIH Central Pharmacy according to standard pharmacy procedures.

Prohibited Medications and Procedures

To minimize potential drug interactions or AEs in healthy volunteers, therapy with any prescription, OTC, herbal, or holistic medications, excluding occasional use of ibuprofen, naproxen, loperamide, or antihistamines (on non-PK days), will not be permitted throughout the study period on or after Study Day 0 unless discussed with and approved by the primary investigator.

6 Study Schedule

Table 4. Study Schedule Overview

	Screening[a] (Day -28 to 0)	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 15 (+/- 1)	ET[i]
Written Informed Consent	X												
Review Study Restrictions	X	X									X	X	X
Medical History	X												
Physical Exam	X	X			X					X		X	X
Psychological Assessments[l]		X							X			X	
Height	X												
Weight	X	X										X	X
Vital Signs	X	X	X	X	X[c]	X	X	X	X	X[c]	X	X	X
HIV-1, HBV, HCV	X												
CBC w/differential	X	X			X[j]					X[j]	X	X	X
Chemistry[d]	X[q]	X[q]			X[j]					X[j]	X	X	X
TSH and T4	X										X		
Urinalysis	X	X			X[j]					X[j]	X	X	X
Serum or Urine Pregnancy Test[e]	X	X										X	
Urine Drug, Alcohol Screen	X	X											
12-Lead ECG	X	X								X[j]		X	X
Review AEs and Con Meds		X[p]	X	X	X	X	X	X	X	X	X	X	X
Inpatient		X	X	X	X	X	X	X	X	X	X[h]		
Study Drug Administration			A[m]		B[m]	B[m]	B[m]	B[m]	B[m]	C[m]			
Intensive PK			X[b]							X[b]			
Pharmacogenomics			X[k]										
Dopamine Metabolites		X[n]								X[n]			
Research Storage Samples: Paxgene DNA, serum, plasma	X[o]												
Research Storage Samples: Paxgene RNA, PBMCs (CPT)		X[o]									X[o]		

Pulse Oximetry[f]			**X**							**X**			
Restricted Unsupervised Ambulation			**X**							**X**			

[a] Prospective subjects should be screened no more than 28 days prior to administration of the first dose of study drug.

[b] Intensive PK sampling will occur relative to the morning dosing on Days 1 and 8 at the following time points: 0 (predose), 0.5, 1, 2, 3, 4, 6, 8, 12, 22, and 24 hours postdose. Subjects will be required to fast (no food or drink, except water), starting from midnight (00:00) or earlier prior to Day 1 and 8, as appropriate, to ensure an approximate 8-hour fast. All PK samples will be collected, processed and stored by the Clinical Pharmacokinetics Research Unit. Jomy George PharmD 301-496-2997.

[c] Vital signs (supine blood pressure, heart rate, respiration rate, and oral temperature): On Days 3 and 8 only, vital signs will be collected predose and approximately 2 hours postdose to monitor the effects of post ANS-6637 administration.

[d] Fasting safety laboratory assessments: basic chemistry [acute care] panel: sodium potassium, calcium, chloride, bicarbonate BUN, creatinine, glucose, albumin; hepatic panel [alkaline phosphatase, AST, ALT, total bilirubin, direct and indirect bilirubin]; mineral panel [calcium, magnesium, phosphorus], lipid panel [total cholesterol, HDL, LDL, triglycerides] total protein, uric acid, amylase and lipase.

[e] Females only. Result must be negative prior to drug administration.

[f] Pulse oximetry: Days 1 and 8 (predose), 0.5, 1, 1.5, 2, and 3 hours post MDZ dosing.

[g] Following MDZ administration on Days 1 and 8, subjects will be restricted from unsupervised ambulation until 2 hours postdose.

[h] Subjects will be discharged from the clinic on Day 9 following all morning assessments.

[i] The following assessments will be performed within 72 hours of early termination from the study

[j] Assessment to occur prior to morning dose.

[k] Sample collection for pharmacogenomics will be drawn at PK time point 0. Send to Frederick as whole blood to be frozen and stored. (NIH Contact: Cathy Rehm 301-594-0012 or Frederick Contact: Ven Natarajan .301-846-1248

[l] Assessments include the Comprehensive Psychopathology Scale (CPRS), Snaith Hamilton pleasure scale (SHAPS), and the Positive Affect Negative Affect Scale (PANAS). All assessments will be administered verbally by a study team physician.

[m] A= Midazolam 5 mg po single dose; B= ANS 6637 600 mg po daily; C= Midazolam 5 mg po x 1 + ANS 6637 600 mg po x 1 will be administered under directly observed therapy (DOT)

[n] Plasma and urine will be collected, processed and stored for analysis of dopamine metabolites by Dr. Goldstein's Lab Building 10, Room 8N252. Attention Patti Sullivan: 301-402-2052 (675-1110)

[o] Blood for research will be collected and stored at NCI Frederick Central Repository. (NIH Contact: Cathy Rehm 301-594-0012

[p] Only reviewing concomitant medications

[q] Includes CK

6.1 Screening (Day -28 to 0)

Subjects who meet general eligibility requirements will be identified using either a face–to–face interview or a telephone call. The study will be briefly described to the subject, and he or she will be scheduled for a screening visit. Subjects will be instructed to fast (no food or drink, except water), starting from midnight (00:00) or earlier, as appropriate, on the evening prior to the Screening visit to ensure an approximate 8-hour fast prior to the fasted blood sample collection the next morning.

On the day of the screening visit, the study coordinator or principal/associate investigator will thoroughly go over the study and standard informed consent form with the subject. The subject will be encouraged to ask questions concerning any issues relating to the study. The consent process will take place in a private room or office at the NIH.

After signing the standard informed consent form, a copy of the consent form will be given to the subject and will be assigned a study identification number. The subject will undergo initial screening, vital signs (supine blood pressure, heart rate, respiration rate, and oral temperature), height, weight, history, physical exam, and labwork. The complete physical examination conducted at Screening will include a review of medical history, allergy history, sexual activity and reproductive and contraceptive history, history of alcohol, illicit drug, and nicotine-containing products, and medication history including medications taken within the last 30 days. Subjects will also be educated as to rationale and risks, and instructed on protocol requirements, including:

- Subjects will not be allowed to leave the NIH campus, but will be permitted to leave the 5th Floor Inpatient Unit and Clinical Center.

- Subjects cannot have consumed food or beverages containing alcohol, caffeine, or xanthine 72 hours prior to the first dose of study drug and during the course of the study through the follow-up visit.

- Subjects will be required to have refrained from the consumption of food and beverages containing alcohol products 72 hours prior to the first dose of ANS-6637 and during the course of the study through the follow-up visit.

- Subjects will be required to have refrained from the use of nicotine or nicotine-containing products 90 days prior to first dose of study drug, and during the course of the study through the follow-up visit.

- Subjects will be required to have refrained from consumption of grapefruit juice, grapefruits, and Seville orange juice 72 hours prior to the first dose of study drug and during the course of the study through the follow-up visit.

- Subjects will be required to have refrained from potentially reproductive sexual activity [females] and to refrain from unprotected potentially reproductive sexual activity [males and females] beginning 30 days prior to screening and continuing for 3 months after dosing

- Subjects will be encouraged to avoid strenuous or prolonged exercise, as well as saunas, steambaths, and sunbathing or other prolonged UV exposure, e.g., in a tanning salon, from the Screening evaluation until completion of the follow-up visit, as these activities are known to affect certain clinical laboratory test parameters, (e.g., creatine kinase (CK)) and will provide false indicators of a potentially treatment-related toxicity.

If a subject is unable to comply with any of the restrictions described above, the subject's continued participation in the study will be reevaluated by the study team.

Following the screening visit and review of the laboratory results, if the subject meets the inclusion criteria and does not exhibit any of the exclusion criteria, the subject will be instructed to return to the clinic for admission assessments on Day 0. Subjects should be scheduled for Day 0 in under 28 days from screening assessment. If the subject does not begin the treatment phase within this 28-day window, all screening evaluation procedures must be repeated. Screening labs may be repeated once within 28 days prior to administration of study drug to rule out laboratory error.

If a subject exhibits any of the exclusion criteria, then the subject's enrollment in the study will be discontinued.

6.2 Day 0

Subjects will be asked to arrive at NIH Clinical Center on Day 0 for admission assessments. Vital signs, complete physical exam, psychopathology assessments, weight, ECG, and labwork will be completed as outlined in Table 4. Adverse events, contraindicated medications, and restrictions listed above will be reviewed.

Prior to dosing on Day 1, the results of the clinical and laboratory evaluations will be reviewed by the Investigator to confirm it is safe to proceed with participation in the study.

6.3 Study Phase

6.3.1 Day 1-15 Assessments

6.3.1.1 Vital Signs

Vital signs will be collected using the standard equipment and operating procedure of the NIH Clinical Center. Vital signs will be collected daily while the participant is inpatient and include supine blood pressure, heart rate, respiration rate, and oral temperature. On Days 3 and 8 only, vital signs will be collected pre-dose and approximately 2 hours post-dose to monitor the effects post ANS-6637 administration.

6.3.1.2 Safety Assessments

6.3.1.2.1 Electrocardiogram

Electrocardiograms will be recorded using the standard equipment and operating procedure of the NIH Clinical Center.

The Investigator or other qualified individuals will review ECGs to assess for changes in ECG intervals and morphology as compared to pretreatment ECGs. Collection of additional ECGs for routine safety monitoring at additional time points or days is at the discretion of the Investigator based on clinical necessity.

6.3.1.2.2 Blood Sampling

The following labs will be collected during the study, as outlined per Table 4:

- Complete blood count (CBC) with differential.
- Serum safety laboratory assessments (fasting): basic chemistry [acute care] panel: sodium potassium, calcium, chloride, bicarbonate BUN, creatinine, glucose, albumin; hepatic panel [alkaline phosphatase, AST, ALT, total bilirubin, direct and indirect bilirubin]; mineral panel [calcium, magnesium, phosphorus], lipid panel [total cholesterol, HDL, LDL, triglycerides] total protein, uric acid, amylase and lipase, T4, thyroid stimulating hormone (TSH)
- Female subjects of childbearing potential will have a point of care (POC) urine pregnancy test conducted prior to drug administration. In the event the subject is unable to provide urine, they will have the option of a serum pregnancy test.
- HIV, HBV, and HCV testing (Screening only)

6.3.1.2.3 Psychological Assessments

On Days 0, 7, and 15, the following psychological assessments will be administered by a study team physician:

- Comprehensive Psychopathology Scale (CPRS)
- Snaith Hamilton pleasure scale (SHAPS)
- Positive Affect Negative Affect Scale (PANAS)

6.3.1.2.4 Urine Sampling

Urine samples will be collected for urinalysis, pregnancy test (females of childbearing potential only) and urine alcohol and drug screening asessments.

6.3.1.2.5 Pulse Oximetry

Subjects will have pulse oximetry readings collected to monitor respiratory function following MDZ dosing according to the following schedule:

Days 1 and 8 (pre-dose), 0.5, 1, 1.5, 2, and 3 hours post MDZ dosing

6.3.1.2.6 Adverse Event Assessment/Con Meds/Protocol Restrictions

Evaluation for AEs, review of concomitant medications, and review of protocol restrictions will occur at each study visit as per Table 4.

6.3.1.3 Pharmacokinetic Assessments

Days 1 and 8: 0 (pre-dose), 0.5, 1, 2, 3, 4, 6, 8, 12, 22, and 24 hours postdose. A window of +/- 10 minutes will be given around each draw time.

Subjects will be required to fast (no food or drink, except water), starting from midnight (00:00) or earlier prior to Day 1 and 8, as appropriate, to ensure an approximate 8-hour fast.

Plasma concentrations of MDZ and 1'-hydroxymidazolam (Days 1 and 8) and ANS-6637 and GS-548351 (Day 8) will be determined at the time intervals as above, and PK evaluated.

6.3.1.4 Pharmacogenomics Sample

A single sample will be collected at time 0 (pre-dose) on Day 1

6.3.1.5 Dopamine Metabolites

Plasma and urine levels of dopamine and DOPAC will be collected on Day 0 and on Day 8 prior to ANS-6637/MDZ dosing

6.4 Final Study Visit

The subject will be seen at Day 15 (+/- 1 day) for a final study visit. At this visit, the subject will undergo study procedures including weight, vital signs, symptom-based physical examination, and a review of study restrictions, adverse events, and contraindicated medications. The subject will also complete an ECG, and laboratory work including CBC, chemistry, and urinalysis.

6.5 Early Termination Visit

If a subject discontinues study treatment dosing, every attempt should be made to keep the subject in the study and continue to perform the required study-related follow-up procedures.

In this case, the subject would undergo the early termination (ET) evaluations and procedures outlined in Table 4 within 72 hours of permanently discontinuing the study drug. Evaluations indicating abnormal results believed to be possibly or probably related to study treatment at the early termination (ET) visit will be repeated weekly or as often as deemed appropriate by the study team until the abnormality resolves, returns to baseline visit levels, or is otherwise explained.

If this is not possible or acceptable to the subject or study team, the subject may be withdrawn from the study.

6.6 Recontact of Subjects After Early Trial Termination or After Day 15 Visit

Subjects will be recontacted after early trial termination or after the day 15 visit on a monthly basis to reinforce pregnancy and contraceptive precautions for appropriate subjects and/or if clinically indicated for 90 days.

7 Study Procedures/Evaluations

7.1 Pharmacokinetic Evaluations

MDZ and ANS-6637 concentrations will be determined by PPD Bioanalytical Laboratory (Middleton, WI) using liquid chromatography/mass spectrometry.

The total amount of blood that will be drawn from subjects for this investigation will be approximately 259 mL (including screening, safety monitoring, and PK sampling). This amount is below the Clinical Center Policy Guideline M95-9, which is 550 mL over 8 weeks. (See Appendix F)

7.1.1 Specimen Collection Procedure

On the day prior to intensive PK monitoring, polypropylene sample storage tubes will be labeled with a code that identifies the subject, the date, the name of the study, the scheduled sample time, and the study day.

All blood samples will be collected into EDTA*K2 (purple top) tubes. After the samples are drawn, they are placed in a refrigerator at the location where the sample(s) was/were procured for a given study subject. Individuals collecting the

samples for processing are aware of the sampling schedule and where each of the samples will be temporarily held in the refrigerator until pickup (typically a matter of minutes up to 12 hours).

7.1.2 Specimen Preparation, Handling and Shipping

7.1.2.1. Midazolam, 1-Hydroxymidazolam Preparation, ANS-6637, and GS-548351

7.1.2.1.1 Sample Preparation:

- One (1) 6 mL Vacutainer tube containing K_2EDTA (lavender-top blood collection tubes (one for MDZ and one for ANS-6637), 13 x 100 mm, BD Vacutainer catalog #367863 or equivalent) will be collected for PK analysis at each of the time points specified in the clinical protocol. Fill tube as completely as possible to ensure sufficient sample volume for the required tests.

- Immediately after the sample is drawn, gently invert the tube 5 to 10 times to thoroughly mix the anticoagulant and place the tube in the refrigerator (Samples can be stored at room temperature for up to 60 minutes prior to completing the processing procedure).

  Samples will then transported to the Clinical Pharmacokinetics Research Unit (CPRU) located within the NIH Clinical Center where they will be processed and stored for batched shipment to PPD for analysis.

- The samples will be centrufuged at room temperature or under refrigeration 2ºC to 8 ºC at 2500 to 3000 rpm (approximately 650 to 1450 x g) for 10 to 15 minutes to achieve a clear plasma layer over the red cells (the speed and time may be varied according to the make and model of centrifuge used). Immediately transfer (approximately) equal portions of the plasma to two (2) properly labeled polypropylene sample storage tubes (Simport 5 mL tubes, P/N T309-5A or equivalent), cap and freeze samples at -80 °C (excursions are permitted between -92°C to 72°C) until shipment.

- The exact time that each blood sample is collected will be written by a team member collecting the sample on a PK sampling sheet (which includes the scheduled sampling times, times of the drug dose(s), time of last meal, and places for vital signs to be recorded). These times will be recorded in a spreadsheet file that will be password protected and used during data analysis. The PK sampling sheets will be retained by the study coordinator, and at the CPRU. A computer database will be used to store the following information: sample code, the study day, date, time of sample collection,

scheduled time of sample collection, time that the serum/plasma was harvested and time that the samples were stored in freezer.

Note: Use of gel separation blood collection tubes is not recommended for PK sample analysis, as drug may be absorbed by the barrier gel.

7.1.2.1.2 Sample Shipment:

- Sample shipment preparation is detailed in Appendix D as per PPD Laboratories
- PPD will be notified by e-mail, facsimile, or telephone prior to sending the shipment
- Detailed sample inventory information must accompany the samples.
- Frozen samples should be shipped via overnight courier with an adequate amount of dry ice Monday through Wednesday to the following:

  PPD
  Attn: Jay Schaefgen, Sr. Group Leader Specimen Management
  3230 Deming Way
  Middleton, WI 53562
  Phone: (608) 662-7706
  Fax: (608) 662-9025
  Email: DemingWaySampleMgmnt@ppdi.com

- Upon arrival, the shipment will be unpacked and the contents verified and documented. If requested, the individual responsible at the shipment point of origin will be notified of sample disposition.

7.2 Pharmacogenomic Sample

7.2.1 Sample Preparation:

- One (1) 10 mL of whole blood will be collected for pharmacogenomics analysis.

7.2.2 Sample Shipment:

- Samples collected for pharmacogenomics will be stored at the NCI at Frederick Central Repository. Samples and data will be stored using codes assigned by the investigators or their designees. All stored computer data will be password protected. Only investigators will have access to the samples and data.
- NIH Contact: Cathy Rehm. Frederick Contact: Ven Natarajan.

7.3 Dopamine Metabolites Sample

7.3.1 Blood Sample Collection

- The patient should be in the supine position for at least 15 minutes before and during collection of the blood sample. To avoid the acute effects of the stress of venipuncture on plasma catechols it is essential that an indwelling i.v. be placed in a vein at least 15 minutes before the blood sample is drawn. The sample may be drawn through an indwelling butterfly i.v. set or through an i.v. cannula (normal saline to keep the line patent), via a 3-way stopcock and Vacutainer adapter into a waste tube (saline void) and then into a chilled evacuated glass collection tube containing heparin in any form as an anticoagulant (e.g., a green top Vacutainer tube). It is preferable, but not essential, to draw the sample without a tourniquet.

- The blood sample (10 mL) should be stored on ice until centrifuged (preferably at 4 ^{o}C) to separate the plasma within 2 hours of blood collection. The plasma should be separated into plastic tubes clearly marked with the date and patient ID and frozen immediately (e.g., on dry ice). The sample should be stored at -70 ^{o}C or colder. Catechols in plasma stored at -20 ^{o}C are not stable.

7.3.2 Urine Sample Collection:

- The patient will empty their bladder, and the time is recorded. All the urine for the next 3.5 hours will be collected in a pre-weighed container. At the end of the urine collection the container is re-weighed, and the difference will be recorded as the volume excreted in the 3.5 hours.

- The urine should then be acidified with 6N HCl, with amount specifications as per Dr. Goldstein's lab.

- The urine will then be transferred to a plastic cryotube with a label stating protocol number, date and time of the end of the sampling, and volume of urine.

7.3.3 Sample Shipment:

Attention: Patti Sullivan

Building 10, Room 8N252
10 Center Drive, MSC 1620
National Institutes of Health
Bethesda, MD 20892-1620
Phone: (301) 402-2052 (backup: 675-1110)
Fax: (301) 402-0180
E-mail: psullivan1@ninds.nih.gov

7.4 Research Storage Samples

7.4.1 Sample Prep

- The following research storage samples will be collected
    - Paxgene DNA at Screening: 8.5 mL
    - Serum at Screening: 5 mL
    - Plasma (PST) at Screening: 4.5 mL
    - Paxgene RNA at Day 0 and Day 9: 2.5 mL
    - PBMCs (CPT) at Day 0 and Day 9: 8 mL

7.4.2 Sample Shipment

- Samples collected for research will be stored at the NCI at Frederick Central Repository. Samples and data will be stored using codes assigned by the investigators or their designees. All stored computer data will be password protected. Only investigators will have access to the samples and data.
- NIH Contact: Cathy Rehm

8 Potential Risks and Benefits

8.1 Potential Risks

Potential research–related risks to study participants include adverse effects of MDZ, ANS-6637, and adverse events associated with blood collection by venous catheter or venipuncture.

Blood Drawing

The risks associated with drawing blood include pain, inflammation, and hematoma formation secondary to needle stick and catheter insertion, and an extremely minor risk for infection. The major risk of anemia is minimized by limiting the amount of blood drawn to under 550 mL over an 8 week period. The

maximum amount of blood to be drawn from any subject for the entirety of this investigation (approximately 2 weeks) is approximately 259 mL. (See Appendix F) Pain associated with blood draws will be partially mitigated by the use of a heplock catheter.

Midazolam

Midazolam is a short-acting, water-soluble benzodiazepine indicated for use as a sedative and amnestic agent.[17] Midazolam acts as a CNS depressant. In the current study, 5 mg of MDZ syrup will be administered as a single dose (0.07 to 0.08 mg/kg for a 60-70 kg person) for probing CYP3A activity. This dose is much lower than that indicated for use in pediatric patients as a single dose (0.25 to 1 mg/kg with a maximum dose of 20 mg) for pre-procedural sedation and anxiolysis. The recommended dose for adults is administered IV or IM and varies on the clinical indication (maximum single dose of 10mg). Time to onset of effect is most frequently reported as 10 to 20 minutes, with recovery from effects within 20 minutes in most subjects. The most common adverse event reported with clinically indicated doses of midazolam include respiratory depression (10%). Other adverse events which have been reported with midazolam include hypotension (3%), drowsiness (1%), headache (1%), nausea (3%), vomiting (3%), and hiccups (4%). The following are more rare adverse events and reported in less than 1% of patients: acidic taste, bronchospasms, dyspnea, euphoria, delirium, amnesia, and skin rash.

ANS-6637

ANS-6637 is an investigational agent. In animal studies, developmental toxicity and fetal malformations were observed in the presence of maternal toxicity at high doses of ANS-6637. The clinical significance of these findings and the specific effects of ANS-6637 on human embryogenesis and fetal development are unknown. Given these findings, females of childbearing potential who are unwilling to be abstinent or use an acceptable method of birth control listed above are excluded from current study. Male subjects with female partners of childbearing potential must use effective contraception while taking ANS-6637 and must refrain from sperm donation while on study and for at least 90 days after taking the last dose of the study drug.

Single and multiple oral doses of ANS-6637 up to 900 mg once daily were generally well tolerated in healthy nonsmoking and smoking subjects. No deaths, severe AEs, or pregnancies were reported. Treatment-emergent AEs were usually mild and were not dose-dependent, and no consistent pattern of AEs was observed. One subject, a nonsmoker who received ANS-6637, had 4 SAEs (hyperbilirubinemia and abnormal ALT, AST, and 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. As such, levels of ALT, AST, ALP, and total bilirubin should be monitored in subjects as reflected in Table 4. Transient abnormal laboratory

test results for TSH and free T4 were observed in some subjects, the clinical importance of which is currently unknown. No other patterns of laboratory abnormalities indicating a relationship to study drug or dose were observed across cohorts. No consistent clinically significant treatment-related effects of ANS-6637 were observed in chemistry and hematology laboratory evaluations, vital sign measurements, or ECG parameters.

An initial ANS-6637 Phase 1 trial demonstrated that the drug was well-tolerated with only mild AEs, and a Phase 1b study completed in 2017 (NCT03203499) showed that ANS-6637 was safe and well-tolerated even during excessive alcohol drinking, demonstrating that ALDH-2 inhibition is suitable even for patients with comorbid alcohol use.

Pharmacogenomic testing: There are no foreseeable risks with pharmacogenomic testing. Results of pharmacogenomic testing will not become a part of the subject's medical record at the NIH. Records containing this information are maintained in a secure manner. Genetic information about the subject will not be revealed to others, including the subject's relatives, without the subject's permission. We will not release any information about the subject to any insurance company or employer unless they sign a document allowing release of information.

8.2 Potential Benefits

This is not a therapeutic trial; therefore, study subjects will not experience direct benefits from their participation. However, the results of this study may help in the care of patients who may receive ANS-6637 in the future.

9 Research Use of Stored Human Samples, Specimens, and Data

Intended Use: Samples and data collected under this protocol will be used to study the presence of MDZ and ANS-6637 and metabolites, dopamine metabolites, and pharmacogenomics markers associated with CYP enzymes and drug transporters. Samples will be stored for future use beyond this study for the purposes of pharmacokinetic evaluations that are not currently being investigated in this study.

Storage: Access to research samples will be limited, as they will be continuously stored in a locked room. Samples and data will be stored using codes assigned by the investigators or their designee(s). All stored computer data will be password protected. As in aforementioned text, subjects will be assigned a study identification number.

Tracking: Detailed tracking information as per Section 7.4.2

Disposition at the Completion of the Protocol: After completion of the protocol (termination), stored data and any remaining samples will be transferred to an active repository protocol for future management. Subjects will be notified at the time of consenting regarding the storage of samples for future use.

In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. Before any sharing of samples, data, or clinical information, either IRB approval must be obtained, or the NIH Office of Human Subjects Research Protections must determine that the research is exempt from IRB oversight. OHSRP can make this determination for some research where the samples or data have no personal identifying information about the study subject, and the researcher is not able to ascertain it.

Loss or Destruction:
Any substantial loss or unanticipated destruction of samples (i.e. due to freezer malfunction) or data (i.e. misplacing a printout of data with identifiers) that affects the investigator's ability to adequately analyze the study data will be reported to the IRB.

10 Data Sharing Plan

What data will be shared?
The investigator will share human data generated in this study for future research as follows:
- De-identified or identified data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?
Data will be shared through
- BTRIS (automatic for activities in the Clinical Center).
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared?
- At the time of publication or shortly thereafter.

11 Remuneration Plan for Subjects

Volunteers will be compensated for their time and inconvenience.Study subjects who complete the entire protocol will receive a total of $3000. Subjects who do

not complete all study procedures will receive compensation based on the extent of their participation.

The following schedule will be utilized for this study:

Compensation schedule	
Screening	$100
Day 0	$250
Day 1-2	$500
Day 3	$200
Day 2-7	$200 x 6 = 1200
Day 8-9	$500
Day 15	$250
Total	**$3000**

If the subject requires additional clinical follow-up outside of scheduled study visits based on medically advisory investigator discretion (e.g. development of an AE), the subject may be compensated an amount consistent with NIH CC policies and guidelines for additional follow-up visits/procedures. Payment will be issued following the end-of study visit. Travel and/or lodging expenses may be provided as per PI discretion.

12 Assessment of Safety

12.1 Toxicity Scale

The Investigator will grade the severity of each AE according to the “Division of Aids Table for Grading the Severity of Adult and Pediatric Adverse Events” Version 2.1, July 2017[20]

Specification of Safety Parameters

Policy Link: https://federation.ih.gov/ohsr/nih/pnp.php

OHRP Link: http://www.hhs.gov/ohp/policy/advevntguid.html

FDA Link- IND: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.32

12.2 Recording/Documentation

At each contact with the subject, information regarding adverse events will be elicited by appropriate questioning and examinations. All events, both expected/unexpected and related/unrelated will be recorded on a source document. Source documents will include: progress notes, laboratory reports, consult notes, phone call summaries, survey tools and data collection tools. Source documents will be reviewed in a timely manner by the research team. All reportable adverse events that are identified will be recorded [in CRIMSON, or the appropriate case report form (CRF), or source document]. The start date, the stop date, the severity of each reportable event, and the PI's judgment of the AEs relationship and expectedness to the study agent/intervention will also be recorded [in CRIMSON, or on the appropriate CRF, or source document].

12.3 Definitions

Adverse Event (AE)
An adverse event is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g. abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the research.

Adverse Reaction (AR)
An adverse event that is caused by an investigational agent (drug or biologic).

Suspected Adverse Reaction (SAR)
An adverse event for which there is a reasonable possibility that the investigational agent caused the adverse event. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction which implies a high degree of certainty.

Serious Adverse Event (SAE)
A serious adverse event is an AE that results in one or more of the following outcomes:

- death
- life-threatening event (places the subject at immediate risk of death from the event as it occurred)
- an inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly/birth defect; or
- a medically important event*

* Medical and scientific judgment should be exercised in deciding events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Unexpected Adverse Event
An AE is unexpected if it is not listed in the Investigator's Brochure or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. It is the responsibility of the IND sponsor to make this determination.

Serious and Unexpected Suspected Adverse Reaction (SUSAR)
A SUSAR is a Suspected Adverse Reaction that is both Serious and Unexpected.

Unanticipated Problem (UP):
An Unanticipated Problem is any event, incident, experience, or outcome that is

1. unexpected in terms of nature, severity, or frequency in relation to
   a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
   b. the characteristics of the subject population being studied; and
2. possibly, probably, or definitely related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. (Per the IND Sponsor, an AE with a serious outcome will be considered increased risk.)

Unanticipated problem that is not an Adverse Event (UPnonAE):
An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered a non-serious UP. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

Protocol Deviation: Any change, divergence, or departure from the IRB approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as:

1. Those that occur because a member of the research team deviates from the protocol.
2. Those that are identified before they occur, but cannot be prevented.
3. Those that are discovered after they occur.

Serious Protocol Deviation: A deviation that meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of subjects or others.

Non-compliance: The failure to comply with applicable NIH Human Research Protection Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human subjects. Non-compliance is further characterized as

1. Serious: Non-compliance that
   a. Increases risks, or causes harm, to participants
   b. Decreases potential benefits to participants
   c. Compromises the integrity of the NIH-HRPP
   d. Invalidates the study data
2. Continuing: Non-compliance that is recurring
3. Minor: Non-compliance that is neither serious nor continuing

12.4 Documenting, Recording, and Reporting Adverse Events

All AEs occurring from the time the informed consent is signed through the specified 15 day follow-up period will be documented, recorded, and reported.

At each contact with the subject, information regarding AEs will be elicited by appropriate questioning and examinations and will be:

- immediately documented in the subject's medical record/source document,
- recorded in CRIMSON, and
- reported as outlined below (e.g., IND Sponsor, Institutional Review Board [IRB], and Food and Drug Administration [FDA]).

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.

All abnormal laboratory findings will be reviewed on a routine basis by the PI to identify potential safety signals. An abnormal lab not included on the toxicity table should be assessed in a similar fashion to the criteria above.

12.4.1 Investigator Assessment Of Adverse Events

The Investigator (or designee) will assess all AEs with respect to **Seriousness** (criteria listed above), **Severity** (intensity or grade), and **Causality** (relationship to study agent and relationship to research) according to the following guidelines listed.

12.4.2 Severity

The Investigator (or designee) will grade the severity of each AE according to the ""Division of Aids Table for Grading the Severity of Adult and Pediatric Adverse Events" Version 2.1, July 2017[20]

Some Grade 1 lab parameters on the DAIDS Toxicity Table (Fibrinogen, Potassium (low), Uric Acid (males only, elevated) fall within the NIH lab reference range for normal values. These normal values will not be reported as Grade 1 AEs. The Grade 1 values for these tests will be reported as follows:

- Fibrinogen: 100-176 mg/dL
- Potassium (low): 3.0-3.3 mmol/L
- Uric Acid (males): 8.7-10.0 mg/dL

12.4.3 Causality

Causality (likelihood that the event is caused by the study agent(s)) will be assessed considering the factors listed under the following categories.

Definitely Related

- reasonable temporal relationship
- follows a known response pattern
- evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related

- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship OR
- good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationship OR
- definitely due to an alternative etiology

Note: Other factors (e.g., dechallenge, rechallenge) should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator

(or designee) may revise the causality assessment as additional information becomes available.

12.5 Investigator Reporting Responsibilities to the Sponsor

12.5.1 Adverse Events

Line listings and /or cumulative listings of AEs will be generated from the centralized database and will be submitted to the IND Sponsor when needed for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

12.5.2 Specific Adverse Event Reporting Requirements

All SAEs (regardless of relationship and whether or not they are also UPs) must be reported on the Safety Expedited Report Form (SERF) and sent to the Clinical Safety Office (CSO) by fax or e-mail attachment. Deaths and immediately life threatening SAEs must be reported to the CSO within 1 business day after the site becomes aware of the event. All other SAEs must be reported within 3 business days of site awareness.

CSO CONTACT INFORMATION:

Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704
Phone: 301-846-5301
Fax: 301-846-6224
Email: rchspsafety@mail.nih.gov

SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE CRF and the SERF.

SAEs that occur after the study 15 day follow-up period that are reported to and are assessed by the investigator to be possibly, probably, or definitely related to study drug must be reported to the CSO.

12.5.3 Unanticipated Problems

Unanticipated Problems that are also AEs must be reported to the CSO and sent by fax or e-mail attachment no later than 7 calendar days of site awareness of the event. UPs that are not AEs are not reported to the CSO.

Report all UPs that are also AEs to the CSO on the NIH Problem Report Form.

12.5.4 Reporting of Pregnancy

All pregnancies will be reported on the Pregnancy Notification/Outcome Form to the CSO within 1 business day from site awareness.

Pregnancy outcome data (e.g., delivery outcome, spontaneous or elective termination of the pregnancy) will be reported to the CSO within 3 business days of the site's awareness.

Although pregnancy itself is not an AE, events that meet SAE criteria during pregnancy, delivery, or in the neonate (e.g., congenital anomaly/birth defect) are reportable on the SERF.

In the event of pregnancy:

- Discontinue the study agent and procedures but continue to follow-up for safety.
- Report to SMC and the IRB.
- Advise research subject to notify the obstetrician of study participation and study agent.
- Due to the known potential reproductive toxicity of the study agent, any pregnancy that overlaps study participation or the 3 months thereafter, AND that the study team becomes aware of, will, within the limits of subject voluntary agreement and consent, be followed to completion. The outcome will be reported to the sponsor within 7 days of study team awareness. This procedure will be subject to IRB stipulation as to approaching and consenting of the subject or partner.

12.6 Investigator's Reporting Responsibilities

12.6.1 Expedited Reporting to the NIAID IRB

Serious and non-serious Unanticipated Problems, deaths, serious deviations, and serious or continuing non-compliance will be reported within 7 calendar days of investigator awareness. Serious Adverse Events that are possibly, probably, or definitely related to the research will be reported to the NIAID IRB within 7 calendar days of investigator's awareness, regardless of expectedness.

12.6.2 Waiver of Reporting Anticipated Protocol Deviations, Expected non-UP AEs and Deaths

Anticipated deviations in the conduct of the protocol will not be reported to the IRB unless they occur at a rate greater than anticipated by the study team. Expected adverse events will not be reported to the IRB unless they occur at a rate greater than that known to occur in normal healthy populations. If the rate of these events exceeds the rate expected by the study team, the events will be classified and reported as though they are unanticipated problems. Deaths unrelated to any research procedures will be reported at the time of continuing review.

12.6.3 Annual Reporting to the NIAID IRB

The following items will be reported to the NIAID IRB in summary at the time of Continuing Review:

- Serious and non-serious unanticipated problems
- Expedited serious adverse events that are possibly, probably, or definitely related to the research
- Serious adverse events that are not related to the research
- All adverse events, except expected AEs granted a waiver of reporting.
- Serious and non-serious protocol deviations
- Serious, continuing, and minor non-compliance
- Any trends or events which in the opinion of the investigator should be reported
- Type and duration of the follow-up of subjects after adverse events

12.6.4 Reporting to FDA

Serious and unexpected suspected adverse reactions (SUSARs) as defined in 21 CFR 312.32 and determined by the IND Sponsor will be reported to FDA, all participating investigators, and to the Pharmaceutical Sponsor, Amygdala Neurosciences, as IND Safety Reports.

The IND Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33

12.7 Pausing Rules Individual Subject

Pausing is the suspension of administration of study agent to a single subject until a decision is made whether or not to resume administration of the study agent.

The pausing criteria for a single subject in this study include any of the following:

- A subject experiences an SAE that is possibly, probably, or definitely related to a study agent;

- A subject experiences one Grade 3 or greater AE that is/are possibly, probably, or definitely related to a study agent;
- Any safety issue that the Site Investigator determines should pause administration of a study agent to a single subject.

12.7.1 Reporting a Pause

If a pausing criteria is met, a description of the AE(s) or safety issue must be reported by the PI, within 1 business day, to the CSO, the IRB and SMC by fax or email.

12.7.2 Resumption of Paused Study

The PI in collaboration with the CSO, and SMC will determine whether or not it is safe to resume administration of the study agent to the subject. The PI will notify the IRB of the decision on resumption of the study agent.

12.7.3 Discontinuation of Study Agent

Subjects who do not resume study agent will continue to be followed for safety.

If a subject discontinues study treatment dosing, every attempt should be made to keep the subject in the study and continue to perform the required study-related follow-up procedures.

In this case, the subject would undergo the early termination (ET) evaluations and procedures outlined in Table 4 within 72 hours of permanently discontinuing the study drug. Evaluations indicating abnormal results believed to be possibly or probably related to study treatment at the early termination (ET) visit should be repeated weekly or as often as deemed appropriate by the Principal Investigator until the abnormality resolves, returns to baseline visit levels, or is otherwise explained.

If this is not possible or acceptable to the subject or study team, the subject may be withdrawn from the study.

12.8 Halting Rules for the Protocol

Halting the study requires immediate discontinuation of study agent administered for all subjects and suspension of enrollment until a decision is made whether or not to continue enrollment and study agent administration.

The halting rules are:

1. One or more subjects experience the same or similar SAEs that are possibly, probably, or definitely related to the study agent.

OR

2. Two or more of the same or similar AE in different subjects that are grade 3 or above and are possibly, probably, or definitely related to the study agent.

OR

3. Two or more subjects experience Grade 2 events in transaminase elevations that are possibly, probably, or definitely related to the study agent.

OR

4. Any safety issue that the PI and/or the CSO determines should halt the study.

The PI, IRB, the CSO, SMC, the pharmaceutical supporter(s), the FDA, or other government agencies, as part of their duties to ensure that research subjects are protected; may discontinue the study at any time. Subsequent review of serious, unexpected and related adverse events by the Medical Monitor, DSMB, ethics review committee or IRB, the sponsor(s), the FDA, and other regulatory authorities may also result in suspension of further trial interventions/administration of study agent at a site. The FDA, other regulatory authorities, and the study sponsor(s) retain the authority to suspend additional enrollment and Study Agent(s)/Intervention(s) administration for the entire study as applicable.

12.9 Reporting a Study Halt

If a halting rule is met, a description of the adverse event(s) or safety issue must be reported by the PI, within one business day, to the CSO, the IRB and SMC by fax or email.

12.10 Resumption of a Halted Study

The IND Sponsor, in collaboration with the PI, and SMC will determine if it is safe to resume the study.

The PI will notify the IRB of the decision on resumption of the study.

12.11 Premature Withdrawal of a Subject

An individual subject will be withdrawn for any of the following:

--An individual subject's decision. (The investigator should attempt to determine the reason for the subject's decision.)

--Non-compliance with study procedures to the extent that it is potentially harmful to the subject or to the integrity of the study data.

--A change in the subject's baseline condition after enrollment so that the subject no longer meets one or more of the following inclusion/exclusion criteria:

1. Have taken any prescription medications or over-the-counter medications including herbal products, nutritional supplements, antacids, and vitamins within 2 weeks of commencing study drug dosing.

2. Have any serious or active medical, surgical, or psychiatric conditions which, in the opinion of the Investigator, would interfere with subject treatment, assessment, or compliance with the protocol.

3. A positive urine drug and/or alcohol screen

--The investigator determines that continued participation in the study would not be in the best interest of the subject.

12.12 Replacement of a Subject Who Discontinues Study Treatment

Subjects who do not complete the protocol will be replaced. If a subject is replaced, all the data will still be included for the safety assessment.

12.13 Safety Monitoring Committee (SMC)

An independent SMC consisting of 3 individuals will review the study prior to initiation and at specific time points as agreed upon by the SMC. The SMC will focus on participant safety and will include subject matter experts. The independent experts do not have direct involvement in the conduct of the study and have no significant conflicts of interest as defined by NIAID policy.

Prior to each SMC review, the principal investigator will submit data as requested by the SMC. After each SMC review, a recommendation as to whether the study is to continue, be modified, or be terminated will be provided in a summary report. All SAEs and all UPs will be reported by the principal investigator to the SMC at the same time they are submitted to the IRB. The SMC will be notified within 1 business day if pausing or halting rules are met, and the SMC will provide a recommendation for continuation, modification, or termination of the

study. The principal investigator will submit the written SMC summary reports with recommendations to the IRB.

13 Clinical Monitoring Structure

13.1 Site Monitoring Plan

As per ICH-GCP 5.18 and *FDA 21 CFR 312.50* clinical protocols are required to be adequately monitored by the study sponsor. *This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines.* Monitors under contract to the NIAID/Office of Clinical Research Policy and Regulatory Operations (OCRPRO) will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points in CRIMSON, and prompt reporting of all SAEs; 3) to compare abstracted information entered into CRIMSON with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators' are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms), and pertinent hospital, including CRIMSON or clinical records readily available for inspection by the local IRB, FDA, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the Principal Investigator and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status and regulatory obligations.

13.2 Safety Monitoring Plan

13.2.1 Safety Review and Communications Plan (SRCP)

A Safety Review and Communication Plan (SRCP) has been developed for the protocol. The SRCP is an internal communications document between the PI and the CSO, which delineates the safety oversight responsibilities of the PI, the CSO, and other stakeholders. The SRCP also includes the overall plan for conducting periodic safety surveillance assessments.

Following written standard operating procedures, the study investigators will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements.

A Medical Monitor, representing the IND Sponsor (OCRPRO), has been appointed for oversight of safety in this clinical study. The SMM will be responsible for performing safety assessments as outlined in a Safety Review and Communications Plan (SRCP).

14 Statistical Considerations

Study Endpoints:
Primary: The primary endpoint of this study will be assessed by characterization of plasma PK parameters $AUC_{0\text{-}inf}$ and C_{max} of analytes MDZ and 1'-hydroxymidazolam

Secondary: The secondary endpoint of this study will be the number of Grade 1-4 adverse events, as defined by the DAIDS Toxicity Table.

Exploratory: Exploratory endpoint of this study will be the (1) levels of dopamine metabolites including urine and plasma dopamine and DOPAC at baseline and steady state of ANS-6637, (2) pharmacogenomics of CYP and drug transporters, and (3) Assessments include the Comprehensive Psychopathology Scale (CPRS), Snaith Hamilton pleasure scale (SHAPS), and the Positive Affect Negative Affect Scale (PANAS) at baseline and at steady state of ANS-6637.

14.1 Sample Size Justification

Sample size/power calculations require estimation of the standard deviation of the difference, $D=Y_2-Y_1$, where Y_1 and Y_2 are the Midazolam log AUCs before and after ANS-6637 is given. This standard deviation was back-calculated to be approximately 0.35 from the confidence interval given in Malati et al (2012). Power for different numbers of participants was calculated using the noncentral t-distribution.

Table 5 below shows that a sample size of 12 yields approximately 90% power to demonstrate that the GMR is between 0.70 and 1/0.70=1.43, if the true GMR is 1.

Table 5. Probability that a 90% confidence interval demonstrates that the GMR is between 0.70 and 1/0.70=1.43, if the true GMR is 1.

The first row is the sample size.

n=10	n=11	n=12	n=13	n=14	n=15
.814	.864	.902	.930	.950	.964

14.2 Pharmacokinetic Analysis

Midazolam, 1-hydroxy midazolam, ANS-6637, and GS-548351 plasma PK parameters will be calculated for each subject when midazolam was dosed alone and in combination with ANS-6637 by applying a noncompartmental approach using WinNonlin® Professional Version 7.0 (Pharsight Corp., Mountain View, California). The key PK parameters that will be calculated for midazolam, 1`-hydroxy midazolam, ANS-6637 and GS-548351 include but not limited to AUC_{0-inf}, AUC (0-24), C_{max}, T_{max}, Kel, Vd, and $t_{1/2}$. The apparent elimination rate constant (λZ) will be determined by calculating the absolute value of the slope of the log-linear regression of at least 3 points of the plasma concentration-time plot. T1/2 will be calculated from λZ, using the formula ln(2)/ λZ. The area under the concentration vs. time curve from time zero to 24 hours post-dose ($AUC_{0–24}$) will be calculated using the linear trapezoidal rule. The area under the concentration vs. time curve from time zero to infinity hours post-dose (AUC_{0-inf}) will be calculated as $AUC_{0-inf} = AUC_{0-24} + C'/\lambda_Z$ (where C' is the concentration predicted by regression line for the last time point with a plasma concentration above the quantification limit). The CL/F will be determined as Dose/AUC, and apparent VdλZ/F calculated as Dose/(λZ*AUC).

For this study, a potentially clinically important effect of ANS-6637/GS-548351 on midazolam pharmacokinetics would be concluded if the 90% confidence interval (CI) for the GMR AUC_{0-inf} will not be contained within the prespecified "no-effect" 70–143% CI limit. If the GMRs fell below 70% or above 143%, then a clinically important effect would be concluded. A clinically important effect will be considered a drug interaction of sufficient magnitude to require dose adjustment of drugs predominantly metabolized by CYP3A when co-administered with ANS-6637.

14.3 Statistical Analysis:

The goal is to show that the effect of ANS-6637 on Midazolam $AUC_{0\text{-}inf}$ (hereafter referred to as simply AUC) is a geometric mean ratio between 0.70 and 1/0.70=1.43. This is accomplished with two 1-sided tests, each at significance level 5%. The first tests GMR=0.70 versus the alternative hypothesis that GMR>0.70, and the second tests GMR=1.43 versus the alternative hypothesis that GMR<1.43. This is equivalent to constructing a 90% confidence interval for the GMR and determining whether the interval lies entirely between 0.70 and 1.43. The procedure begins by applying a logarithmic transformation to the AUC. It is assumed that log AUC follows an approximate normal distribution. A 90% confidence interval for the mean difference in Midazolam log AUC before and after ANS-6637 is constructed using paired t-statistic methodology. The estimated mean difference and confidence limits are then exponentiated to produce an estimate and 90% confidence interval for the GMR.

14.4 Safety Analysis:

AEs will be assessed through physical examination, interview, psychological assessments, clinical laboratory test findings, 12-lead ECG traces, and vital signs measurements. All safety data collected on or after the date that study drug was first administered up to the seven (7) days after the last dose of study drug will be summarized by treatment group (according to the study drug received) using safety analysis set.

15 Ethics/Protection of Human Subjects

15.1 Informed Consent Process

Informed consent is a process where information is presented to enable individuals to voluntarily decide whether or not to participate as a research subject. It is an on-going conversation between the human research subject and the researchers which begins before consent is given and continues until the end of the subject's involvement in the research. Discussions about the research will provide essential information about the study and include: purpose, duration, experimental procedures, alternatives, risks and benefits. Subjects will be given the opportunity to ask questions and have them answered.

The subjects will sign the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The researcher will document the signing of the

consent form in the subject's medical record. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

15.1.1 Non-English–Speaking Participants

If a non-English speaking participant is unexpectedly eligible for enrollment, the participant will be provided with the CC Short Written Consent Form for Non-English Speaking Research Participants in the participant's native language and a verbal explanation of the purpose, procedures and risks of the study as described in MAS Policy M77-2, NIH HRPP SOP 12 and 45 CFR 46.117(b)(2). The IRB-approved English consent form will serve as basis for the verbal explanation of the study. The investigator will obtain an interpreter unless the investigator is fluent in the prospective participant's language. Preferably, the interpreter will be someone who is independent of the participant (i.e., not a family member). Interpreters provided by the CC will be used whenever possible. The interpreter will interpret all oral communications (English to target language and conversely) between the Investigator and a Limited English Proficient (LEP) participant, facilitate discussions, and clarify information as necessary.

The IRB-approved English consent form will be signed by the investigator obtaining consent and a witness to the oral presentation. The CC Short Written Consent Form will be signed by the participant and a witness who observed the presentation of information. The interpreter may sign the consent document as the witness and, in this case, will note "Interpreter" under the signature line. A copy of both signed forms will be provided to the participant to take home.

The investigator obtaining consent will document the consent process in the participant's medical record (CRIMSON), including the name of the interpreter. Further, all instances of use of the CC Short Written Consent Form will be reported to the IRB at the time of annual review. If the CC Short Written Consent Form is used three times or more for the same language, this will be reported to the IRB immediately.

15.1.2 Participation of NIH Employees

NIH employees and members of their immediate families may participate in this protocol. We will follow the Guidelines for the Inclusion of Employees in NIH Research Studies and will give each employee a copy of the "NIH information sheet on Employee Research Participation".

For NIH employees:

- Neither participation nor refusal to participate will have an effect, either beneficial or adverse, on the participant's employment or work situation.

- The NIH information sheet regarding NIH employee research participation will be distributed to all potential subjects who are NIH employees.

- The employee subject's privacy and confidentiality will be preserved in accordance with NIH Clinical Center and NIAID policies, which define the scope and limitations of the protections.

- For NIH employee subjects, consent will be obtained by an individual independent of the employee's team. Those in a supervisory position to any employee and co-workers of the employee will not obtain consent.

- The importance of maintaining confidentiality when obtaining potentially sensitive and private information from co-workers or subordinates will be reviewed with the study staff at least annually and more often if warranted.

15.2 Subject Confidentiality

All records will be kept confidential to the extent provided by federal, state and local law. The study monitors and other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records. Records will be kept locked and data will be coded. Any personally identifiable information maintained for this study will be kept on restricted-access computers and networks. Personally identifiable information will only be shared with individuals authorized to receive it under this protocol. Individuals not authorized to receive personally identifiable information will be provided with coded information only, as needed. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, [the FDA], the NIAID, the OHRP, [the pharmaceutical supporter(s)], or the sponsor's designee.

16 Data Handling and Record Keeping

16.1 Data Capture and Management

Study data will be maintained in CRIMSON and collected directly from subjects during study visits and telephone calls, or will be abstracted from subjects' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the

data abstracted for this study. Data entry into CRIMSON will be performed by authorized individuals. The Investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

16.2 Record Retention

The principal investigator is responsible for retaining all essential documents listed in the ICH Good Clinical Practice Guideline. All essential documentation for all study subjects are to be maintained by the investigators in a secure storage facility for a minimum of 5-7 years, and in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever is longest. All stored records are to be kept confidential to the extent provided by federal, state, and local law.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. NIAID must be notified in writing and written OCRPRO/NIAID permission shall be obtained by the site prior to destruction or relocation of research records.

Appendix A: Scientific References

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Appendix B: Toxicity Table

https://rsc.tech-res.com/docs/default-source/safety/division-of-aids-(daids)-table-for-grading-the-severity-of-adult-and-pediatric-adverse-events-corrected-v-2-1.pdf

Appendix C: ANS-6637 Investigator's Brochure

Appendix D: PPD Laboratory Specimen Shipping and Handling Instructions

PK Sample Collection and Processing

Analyte(s): ANS-6637, GS-548351, Midazolam and 1-Hydroxymidazolam

Matrix: Human Plasma (K_2EDTA)

- One (1) 6 mL Vacutainer tube containing K_2EDTA (lavender-top blood collection tubes, 13 x 100 mm, BD Vacutainer catalog #367863 or equivalent) will be collected for PK analysis at each of the time points specified in the clinical protocol. Fill tube as completely as possible to ensure sufficient sample volume for the required tests.

- Immediately after the sample is drawn, gently invert the tube 5 to 10 times to thoroughly mix the anticoagulant and place the tube at room temperature (Samples can be stored at room temperature for up to 60 minutes prior to completing the processing procedure.)

- Centrifuge the sample at room temperature or under refrigeration 2 ºC to 8 ºC at 2500 to 3000 rpm (approximately 650 to 1450 x g) for 10 to 15 minutes to achieve a clear plasma layer over the red cells (the speed and time may be varied according to the make and model of centrifuge used). Immediately transfer (approximately) equal portions of the plasma to two (2) properly labeled polypropylene sample storage tubes (Simport 5 mL tubes, P/N T309-5A or equivalent), cap and freeze samples at -70 °C until shipment.

  Note: Use of gel separation blood collection tubes is not recommended for PK sample analysis, as drug may be absorbed by the barrier gel.

Sample Shipment

- All sample shipments should be preceded by a phone call, e-mail, or fax prior to their receipt. All HIV positive or other known infectious sample shipments must be preceded by a phone call, e-mail, or facsimile prior to their receipt.
- Detailed sample inventory information must accompany the samples. Lack of paperwork or illegible information will delay sample login and project initiation. Samples that are unclearly or incompletely labeled may be subject to additional handling fees. Submission of sample inventory information in electronic form is encouraged.
- Frozen samples should be shipped via overnight courier with an adequate amount of dry ice Monday through Wednesday to the following:

PPD

Attn: Jay Schaefgen, Sr. Group Leader Specimen Management

3230 Deming Way

Middleton, WI 53562

Phone: (608) 662-7706

Fax: (608) 662-9025

Email: DemingWaySampleMgmnt@ppdi.com

- Notify PPD by e-mail, facsimile, or telephone that a shipment is leaving your location. Upon arrival, the shipment will be unpacked and the contents verified and documented. If requested, the individual responsible at the shipment point of origin will be notified of sample disposition.

Note: For international shipments, PPD recommends the use of World Courier.

Appendix E: Suggested Study Initiation Calendar

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	Day 0*	**Day 1 ***	**Day 2***	**Day 3***	**Day 4***	**Day 5***
Day 6*	**Day 7***	**Day 8***	**Day 9***	Day 10	Day 11	Day 12
Day 13	Day 14	**Day 15**				
*Days study patients will be inpatient at NIH (Admission: Day 0, Discharge: Day 9) Bolded are study visit days at NIH						

OR

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
		Day 0 *	**Day 1***	**Day 2***	**Day 3***	**Day 4***
Day 5*	**Day 6***	**Day 7***	**Day 8***	**Day 9***	Day 10	Day 11
Day 12	Day 13	Day 14	**Day 15**			
*Days study patients will be inpatient at NIH (Admission: Day 0, Discharge: Day 9) Bolded are study visit days at NIH						

Appendix F: Blood Volumes for Specimen Collection

Evaluation	Study day							Early termination visit
	Screening (days −28 to 0)	Baseline[a] (day 0)	1-2 (PK)	3	8-9 (PK)	9	15	
HIV-1 antigen/antibody testing	8							
Viral markers hepatitis screen (HBV, HCV)	8							
Chemistry[a]	4	4		4	4	4	4	4
Mineral Panel	(4)	(4)		(4)	(4)	(4)	(4)	(4)
Lipid Panel	(4)	(4)		(4)	(4)	(4)	(4)	(4)
Amylase/lipase	(4)	(4)		(4)	(4)	(4)	(4)	(4)
TSH/T4	(4)					(4)		
Uric Acid	(4)	(4)		(4)	(4)	(4)	(4)	(4)
Serum pregnancy test[b]	(4)	(4)		(4)	(4)	(4)	(4)	(4)
CBC/diff	3	3		3	3	3	3	3
PK blood[c]			66		66			
Pharmacogenomics[d]			10					
Dopamine Metabolites		10			10			
Storage Sample	18	10.5				10.5		
Daily volume (mL)	41	27.5	76	7	83	17.5	7	
Cumulative volume (mL)	41	68.5	144.5	151.5	234.5	252	**259**	

CBC/diff = complete blood count with differential; PK = pharmacokinetics.

[a] Fasting serum chemistry: alkaline phosphatase, AST, ALT, total bilirubin, , direct and indirect bilirubin, total protein, albumin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, phosphorus, magnesium, potassium, sodium, uric acid, total cholesterol, HDL, LDL, triglycerides, TSH/T4, and amylase, lipase

[b] Females only. This can be performed using blood or urine sample.

[c] Intensive PK sampling will occur relative to the morning dosing on Days 1 and 8 at the following time points: 0 (predose), 0.5, 1, 2, 3, 4, 6, 8, 12, 22, and 24 hours postdose

[d] Sample collection for pharmacogenomics. Send to Frederick as whole blood to be frozen and stored.
