Phase 1 Evaluation of (2R,6R)-Hydroxynorketamine

Protocol

A Double-Blind, Placebo-controlled, Single Ascending Dose (SAD), Multiple Ascending Dose (MAD) and CSF Capture Study of the Safety, Pharmacokinetics and Pharmacodynamics of (2R,6R)-Hydroxynorketamine in Healthy Volunteers

Protocol Status: Final
Protocol Date: 11-15-2023
Protocol Version: 7.0

Investigational Product: (2R,6R)-Hydroxynorketamine

Protocol Reference Number: HNK-378227-04
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National Institute of Mental Health

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History of revisions to the Phase 1 protocol.

The following table presents the noteworthy changes for each version of the Phase 1 protocol for (2R,6R)-Hydroxynorketamine.

Protocol Version	Date	Section	Description of Change
2.0	10/23/2020	Study Contacts	Updated the study contact information for the pharmacokinetics lead to Robert Tweig.
2.0	10/23/2020	9.3	Removal of relatedness aspects of the stopping rules.
2.0	10/23/2020	11.7	Removal of unnecessary details describing the IDP, Diluent and administration equipment. This information is contained within the MOP.
2.0	10/23/2020	11.9	Addition of reporting language for SAEs.
2.0	11/11/2020	11.9	Addition of capture of abuse-related AE's
3.0	3/3/2021	Study Contacts	Change in PK lead to Satya Avula
4.0	3/21/2022	Study Contacts	Change in study PI to Shruti Raja
4.0	3/21/2022	Synopsis	Edits and additional details (cohort #, dose) around the MAD study protocols
4.0	3/21/2022	Synopsis	Additional of the CSF Capture study protocols
4.0	3/21/2022	Tables	Revised MAD study schedule of assessments and procedures
4.0	3/21/2022	Tables	Added CSF Capture study schedule of assessments and procedures
4.0	3/21/2022	Figures	Added Visual representation of MAD and CSF Capture study timelines
4.0	3/21/2022	TOC	Table of contents changes
4.0	3/21/2022	7.0	Addition of CSF Capture study design section
4.0	3/21/2022	Multiple	References to the CSF Capture study design added throughout multiple sections
4.0	3/21/2022	12.9	Addition of AE response procedures
4.0	3/21/2022	Tables 11& 12	Addition of CSF Capture study blood draw schedule and CSF draw schedule
5.0	5/24/2022		Corrections and Updates for consistency
6.0			Remove CSF Capture Cohort #10, revise Cohort #9
7.0	11/15/2023	Synopsis	Updated objectives to reflect study design

SPONSOR AND MEDICAL MONITOR APPROVAL

I have read the following and approve it:

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SYNOPSIS

Title: A Double-Blind, Placebo-controlled, Single Ascending Dose (SAD), Multiple Ascending Dose (MAD) and CSF Capture Study of the Safety, Pharmacokinetics and Pharmacodynamics of (2R,6R)-Hydroxynorketamine in Healthy Volunteers
Sponsor: National Institute of Mental Health
Name of drug substance: (2R,6R)-Hydroxynorketamine hydrochloride
Name of investigational drug product: (2R,6R)-Hydroxynorketamine hydrochloride solution for injection
Clinical Phase: Phase 1
Clinical Site: Duke Clinical Research Institute, Duke University School of Medicine
Objectives: Primary: ● Determine the safe dose range of (2R,6R)-Hydroxynorketamine ● Determine the pharmacokinetics of (2R,6R)-Hydroxynorketamine administered in single ascending doses ● Determine the pharmacokinetics of (2R,6R)-Hydroxynorketamine administered in multiple ascending doses Exploratory: ● Collect quantitative electroencephalography (qEEG) data following administration of (2R,6R)-Hydroxynorketamine in healthy Subjects as a pharmacodynamic biomarker ● Assess the concentration of (2R,6R)-Hydroxynorketamine in cerebral spinal fluid (CSF) following a single IV administration
Methodology/study design: A 6-cohort single ascending dose (SAD) study will be conducted in healthy volunteers utilizing a slow-infusion intravenous (IV) route of administration. Standard safety, pharmacokinetics (PK) and qEEG monitoring will be evaluated at all dose levels. Subsequently, a 2-cohort multiple ascending dose (MAD) study will be conducted. Doses will be administered on days 1,4, 8, and 11. Standard safety parameters will be monitored, and PK will be evaluated at all dose levels. Finally, a single cohort of healthy volunteers will receive a single dose by slow-infusion IV and have PK samples collected from both blood and cerebrospinal fluid (CSF).
Study drugs, formulation, dose and route of administration: (2R,6R)-Hydroxynorketamine hydrochloride will be administered intravenously over a 40-minute period as a solution in a 25 mM sodium phosphate 0.9% w/v saline solution. The SAD doses will range from 0.1 mg/kg to 4.0 mg/kg and the investigational drug product will be diluted into a 53 mL total volume of formulant.

Placebo will be made up of a 0.9% w/v saline solution (53 mL total volume) also administered via slow IV infusion over a 40-minute period.

Subjects:

A total of 48 Subjects are planned to be enrolled in a 6-cohort SAD study (36 in the treatment groups and 12 in the control groups). All SAD cohorts will have 6 Subjects in the treatment group and 2 Subjects in placebo group.

All cohorts in the SAD study will incorporate sentinel dosing which will include 1 active and 1 placebo Subject. All remaining Subjects will be dosed at least 24 hours after the sentinel cohort participants.

A total of 16 Subjects are planned to be enrolled in a 2 cohort MAD study (12 in the treatment groups and 4 in the control groups). All MAD cohorts will have 6 Subjects in the treatment group and 2 Subjects in placebo group.

A total of 6 subjects are planned to be enrolled in a 1 cohort CSF capture study. The CSF cohort will have 4 in the treatment group and 2 in the control group.

Additional cohorts (both SAD and MAD) may be enrolled if it is determined that an intermediate or higher dose level should be evaluated upon review of both safety and PK data. The Institutional Review Board will be notified of this revised approach.

Pharmacokinetics:

Serial PK blood samples will be collected during the SAD portion for each Subject receiving drug and placebo at 9 timepoints (preinfusion, end-of-infusion [approximately 40 minutes], 1, 2, 4, 8, 12, 24, and approximately 48 hr after the start of the infusion). Pharmacokinetic urine samples will be collected during the SAD study for each Subject receiving drug and placebo at set intervals following the initiation-of-infusion (0-4, >4-8, >8-12, >12-24 hr). Pharmacokinetic urine samples (up to 3 per subject) will be collected at key time intervals (0-4h, >4-8h, >8-12h, >12-24h) after the start of infusion.

Serial PK blood samples will be collected for the first and fourth (last) dosing in the MAD study for each Subject receiving drug and placebo. Blood PK samples will be obtained at 8 timepoints (preinfusion, end-of-infusion [approximately 40 minutes], 1, 2, 4, 8, 12, and approximately 24hr after the start of the infusion). Two PK blood samples will be collected for the second and third dosing in the MAD study for each subject receiving drug and placebo at approximately 10 minutes predose and at the end of the infusion.

Serial PK blood samples will be collected for the single dose CSF capture study for each Subject receiving drug and placebo. Blood PK samples will be obtained at 8 timepoints (preinfusion, end-of-infusion [approximately 40 minutes], 1, 2, 4, 8, 12, and approximately 24hr after the start of the infusion). CSF samples will be obtained preinfusion, and at 2 timepoints (1h and 8 hours post infusion).

Blood and CSF samples will be analyzed for PK of (2R,6R)-Hydroxynorketamine using a LC/MS/MS method to capture the following parameters: maximum plasma concentration (C_{max}), time to C_{max} (t_{max}), minimum plasma concentration (C_{min}), area under the plasma concentration-time curve from 0 to infinity ($AUC_{0-\infty}$), area under the plasma concentration-time curve during the dosing interval (AUC_{0-Tau}), systemic plasma clearance (CL), volume of distribution during terminal phase (V_z), renal clearance (CL_R), terminal half-life ($t_{1/2}$).

Safety:

Safety will be assessed throughout the study. Baseline and follow-up safety assessments will include height, body mass index (BMI), weight, temperature, medical, visual and ocular history, physical examinations, ocular examinations, visual acuity, color vision tests, electrocardiograms (ECGs), vital signs (VS), clinical laboratory tests (hematology, serum chemistry, and urinalysis), the Profile of Mood States (POMS), the Columbia-Suicide Severity Rating Scale (C-SSRS), the Clinician Administered Dissociative States Scale (CADSS), and adverse events (AEs). Safety assessments will include AEs, ECGs, VS, clinical laboratory results, and physical observations. Assessment of each Subject's level of alertness/sedation will be accomplished using the Modified Observer's Assessment of Alertness/Sedation (MOAA/S).

Evaluation of safety in the MAD study will utilize the same safety assessments used in the SAD study.

Evaluation of safety in the CSF capture study will utilize the same safety assessments used in the SAD and MAD study with the exception of the CADSS and MOAA/S assessments.

Monitoring of AEs will be governed by change from baselines established during prescreening and Day -1 examinations and clinical laboratory tests. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by system organ class and preferred term, by severity, by relationship to study drug and study procedure, and by study drug dose.

Dose escalation in the SAD study or continued dosing in the MAD study may be stopped according to the predefined halting rules or if a Subject's scores demonstrate acute suicidality on the C-SSRS assessment or at the discretion of the study Principal Investigator and/or sponsor.

Determination of whether to escalate to the next dose level in the SAD study or continue dosing in the MAD will be made by the Principal Investigator in consultation with the Medical Monitor and Study Sponsor.

Test products, dose, and mode of administration for SAD study:

Cohort 1: (2R,6R)-Hydroxynorketamine @ 0.1 mg/kg via slow IV infusion (40 minutes)
Cohort 2: (2R,6R)-Hydroxynorketamine @ 0.25 mg/kg via slow IV infusion (40 minutes)
Cohort 3: (2R,6R)-Hydroxynorketamine @ 0.5 mg/kg via slow IV infusion (40 minutes)
Cohort 4: (2R,6R)-Hydroxynorketamine @ 1.0 mg/kg via slow IV infusion (40 minutes)
Cohort 5: (2R,6R)-Hydroxynorketamine @ 2.0 mg/kg via slow IV infusion (40 minutes)
Cohort 6: (2R,6R)-Hydroxynorketamine @ 4.0 mg/kg via slow IV infusion (40 minutes)

Test products, mode of administration and schedule for MAD study:

Cohort 1: (2R,6R)-Hydroxynorketamine @ 1.0 mg/kg via slow IV infusion (40 minutes) on days 1, 4, 8, 11
Cohort 2: (2R,6R)-Hydroxynorketamine @ 2.0 mg/kg via slow IV infusion (40 minutes) on days 1, 4, 8, 11

Test products, mode of administration and schedule for CSF capture study:

Cohort 1: (2R,6R)-Hydroxynorketamine @ 0.25 mg/kg via slow IV infusion (40 minutes)

Control product (placebo) will be sterile saline also administered via slow IV infusion (40 minutes).

The highest dose administered in the MAD will not exceed the highest tolerable SAD dose.

Duration of patient participation in study:

Participants in the SAD study: each Subject will participate in one treatment period. A treatment period consists of residential stay on days -1 to 2 inclusive with 1 follow up visit (Day 3) and phone call (Day 5-8).

Participants in the MAD study: each Subject will participate in one treatment period. A treatment period consists of residential stay on days -1 to 5, with discharge the morning of day 5 and re-admission on day 712 inclusive with 1 follow up phone call (Day 15) and phone call (Day 17-19).

Participants in the CSF capture study: each Subject will participate in one treatment period. A treatment period consists of residential stay on days -1 to 2 inclusive with 1 follow up visit (Day 3) and phone call (Day 5-8).

Study populations:

The SAD, MAD and CSF capture study populations will be made up of healthy male and female adult volunteers (aged 18-65 years) who meet all defined inclusion/exclusion criteria.

Table 1: SAD schedule of assessments and procedures

Procedure	Screening (Day –28 to Day -2)	Day -1	Day 1	Day 2	Day 3	Day 5-8
Informed Consent	X					
Eligibility Criteria	X	X				
Demographics	X					
Height	X					
Weight, BMI	X	X			X	
Medical history	X	X			X	X
Physical Examination*	X	X		X	X	
Vital Signs**	X	X	X	X	X	
ECG**	X	X	X	X	X	
Urine Drug Test	X	X				
Urine Cotinine	X	X				
Alcohol Breath Test	X	X				
Pregnancy Test (Females)^	X	X				
Serology (Hepatitis B, Hepatitis C, HIV)	X					
Clinical Lab. Tests (Hema., Chem., Urinalysis)	X	X	X	X		
Prior and Concom. Meds.	X	X		X	X	X
Ocular Examinations***		X			X	
Modified Observer's Assess. of Alert./Sed. (MOAA/S)****			X			
Suicide Screen (C-SSRS)	X	X	X	X		
Mood and Cognitive Assessment (POMS)		X	X	X		X
Clin. Admin. Diss. States Scale (CADSS)*****		X	X			
IV Drug Administration			X			
Blood Sample Collection (PK)			X	X	X	
Urine Sample Collection (PK)			X	X		
qEEG			X			
Confinement		----------X----------				
Adverse Events			X	X	X	X
Outpatient Visit					X	
Phone Call						X

An early termination visit will be scheduled on an ad hoc basis for any subject who withdraws from the study.
* Physical examinations can be conducted at any additional time at the judgement of the Principal Investigator (eg, to evaluate an adverse event)
^FSH for postmenopausal women at screening visit
** Day 1 Timepoints: 1-hr preinfusion, and 1, 2, 4, 8, and 12 hr after the start of the infusion.
*** Ocular examinations include visual acuity and color vision tests.
**** On Day 1, MOAA/S is performed preinfusion, during the infusion at approximately 20 min after start of infusion, 40 min after start of infusion, and approximately 1 hr after the start of the infusion.
***** On Day 1, CADSS is performed preinfusion and approximately 40 min and 80 min after the start of the infusion.

Table 2: MAD schedule of assessments and procedures

Procedure	Screening (Day –28 to Day -2)	Confinement										Follow-Up	
		Day -1	Day 1	Day 2	Day 4	Day 5	Day 7	Day 8	Day 9	Day 11	Day 12	Day 15	Day 17-19
Informed Consent	X												
Eligibility Criteria	X	X											
Demographics	X												
Height	X												
Weight, BMI	X	X									X		
Medical history	X	X									X	X	X
Physical Examination*	X	X					X				X		
Vital Signs**	X	X	X		X	X	X	X	X	X	X		
ECG**	X	X	X		X	X	X	X	X	X	X		
Urine Drug Test	X	X					X						
Alcohol Breath Test	X	X					X						
Pregnancy Test (Females)^	X	X					X						
Serology (Hepatitis C, Hepatitis B, HIV)	X												
Clinical Lab. Tests (Hema., Chem., Urinalysis)	X		X	X				X	X	X	X		
Prior and Concom. Meds.	X	X	X			X	X				X	X	X
Ocular Examinations***		X		X						X	X		
Modified Observer's Assess. of Alert./Sed. (MOAA/S)****			X		X			X		X			
Suicide Screening (C-SSRS)	X	X	X	X	X	X	X	X	X	X	X	X	X
Mood and Cognitive Assessment (POMS)		X	X	X	X	X	X	X	X	X	X	X	X
Clin. Admin. Diss. States Scale (CADSS)*****			X		X			X		X			
IV Drug Administration			X		X			X		X			
Blood Sample Coll. (PK)			X	X	X			X		X	X		
Check-in & Confinement		X	X	X	X		X	X	X	X			
Check-out******						X					X		
Adverse Events			X	X	X	X		X	X	X	X	X	X
Outpatient Visit													
Phone Call												X	X

An early termination visit will be scheduled on an ad hoc basis for any subject who withdraws from the study.

* Physical examinations can be conducted at any additional time at the judgement of the Principal Investigator (eg, to evaluate an adverse event)

^FSH for postmenopausal women at screening

** Dosing Day timepoints: 1-hr preinfusion, and 1, 2, 4, 8, and 12 hr after the start of the infusion.

*** Ocular examinations include visual acuity and color vision tests.

**** On Dosing days MOAA/S is performed preinfusion, during the infusion at approximately 20min after start of infusion, 40 min after start of infusion, and approximately 1 hr after the start of the infusion.

***** On Dosing days, CADSS is performed preinfusion and approximately 40 min and 80 min after the start of the infusion.

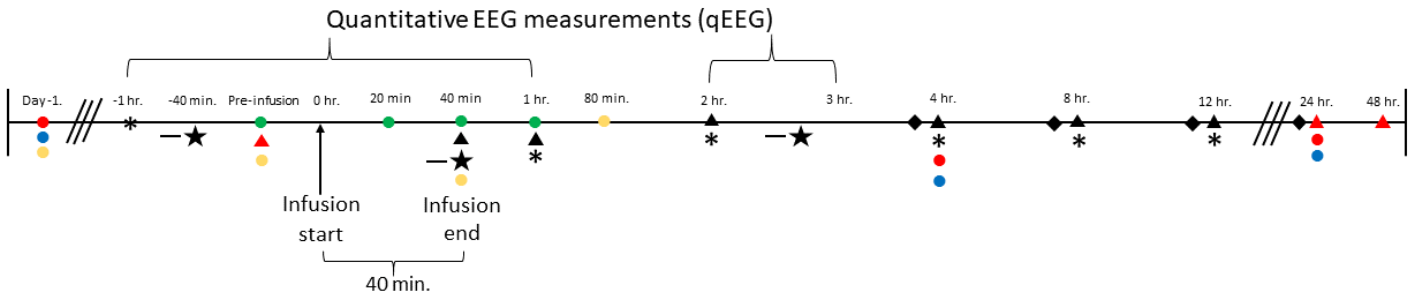
****** See Figure 2 for details regarding check-in/check-out schedule

Table 3: CSF Capture Study schedule of assessments and procedures

Procedure	Screening (Day –28 to Day -2)	Day -1	Day 1	Day 2	Day 3	Day 5-8
Informed Consent	X					
Eligibility Criteria	X	X				
Demographics	X					
Height	X					
Weight, BMI	X	X			X	
Medical history	X	X			X	X
Physical Examination*	X	X		X	X	
Vital Signs**	X	X	X	X	X	
ECG**	X	X	X	X	X	
Urine Drug Test	X	X				
Urine Cotinine	X	X				
Alcohol Breath Test	X	X				
Pregnancy Test (Females)^	X	X				
Serology (Hepatitis B, Hepatitis C, HIV)	X					
Clinical Lab. Tests (Hema., Chem., Urinalysis)	X	X	X	X		
Prior and Concom. Meds.	X	X		X	X	X
Ocular Examinations***		X			X	
Suicide Screen (C-SSRS)	X	X	X	X		
Mood and Cognitive Assessment (POMS)		X	X	X		X
IV Drug Administration			X			
Blood Sample Collection (PK)			X	X		
Cerebrospinal Fluid Collection (PK)			X			
Confinement		----------X----------				
Adverse Events			X	X	X	X
Outpatient Visit					X	
Phone Call						X

An early termination visit will be scheduled on an ad hoc basis for any subject who withdraws from the study.
* Physical examinations can be conducted at any additional time at the judgement of the Principal Investigator (eg, to evaluate an adverse event)
^FSH for postmenopausal women at screening visit
** Day 1 Timepoints: 1-hr preinfusion, and 1, 2, 4, 8, and 12 hr after the start of the infusion.
*** Ocular examinations include visual acuity and color vision tests.

Figure 1: Visual representation of timeline of key events in the SAD dosing period.



▲ Pharmacokinetics and clin. lab blood draw (PK): Pre-infusion, approximately 24 and 48 hrs after start of infusion

▲ Pharmacokinetics blood draw (PK): End-of-infusion (40 min), 1, 2, 4, 8, 12 hrs after start of the infusion

◆ Pharmacokinetics urine capture (PK): Up to 3 per Subject; collected 0-4, >4-8, >8-12, >12-24 hrs after the start of infusion

∗ Vitals/Electrocardiograms (ECG): Approximately 1 hr pre-infusion and 1, 2, 4, 8 and 12 hrs after the start of infusion

● Profile of mood states (POMS): Day -1, within approximately 4 and 24 hrs after the start of infusion

● Columbia-suicide severity rating scale (C-SSRS): Day -1, within approximately 4 and 24 hrs after the start of infusion

● Modified observer's assessment of alertness/sedation (MOAA/S): Pre-infusion, and approximately 20 min, 40 min and 1 hr after start of infusion

● Clinician administered dissociative states scale (CADSS): Day -1, Pre-infusion, approximately 40 min and 80 min after start of infusion

—★ Resting-State EEG and visual evoked potentials (checkerboard): 5 min resting state followed by 5 min visual evoked potential paradigm beginning approximately 40 min pre-infusion, and 40 min and 160 min after the start of infusion

****When multiple procedures occur at the same timepoint, the following guides the approximate order of how procedures should be performed: MOAA/S > PK/lab draw > VS > ECG > EEG > CADSS > C-SSRS > POMS.

Figure 2: Visual representation of timeline of key events in the MAD study.

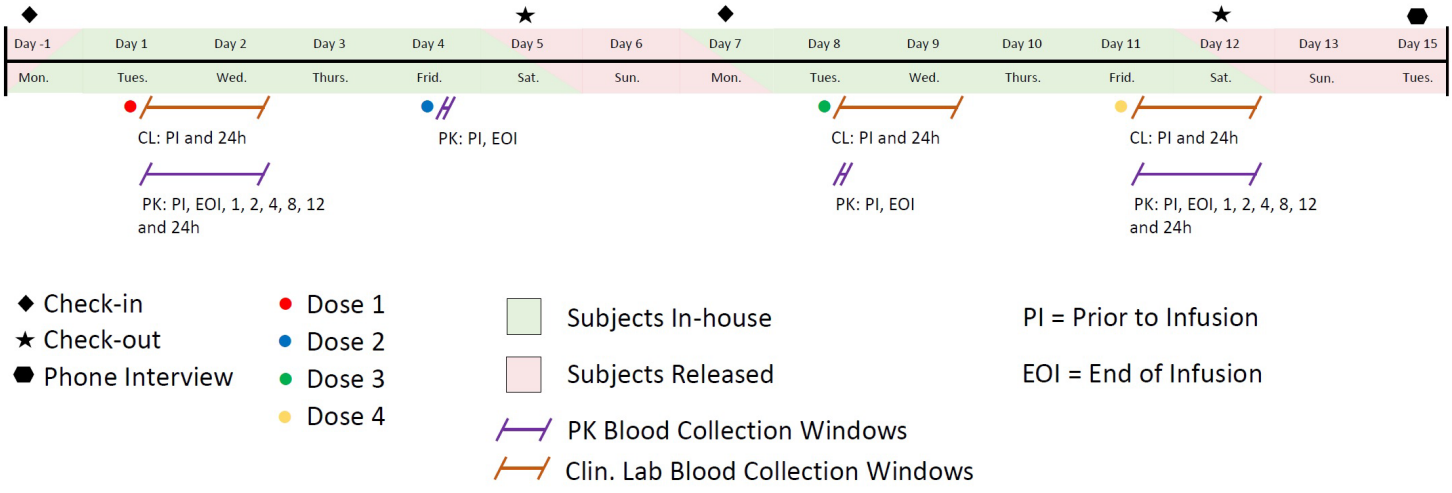


Figure 3: Visual representation of timeline of key events for Dose #1 in the MAD study.

▲ Pharmacokinetics and clin. lab blood draw
▲ Pharmacokinetics blood draw only
* Vitals/Electrocardiograms (ECG): Approximately 1 hr pre-infusion and 1, 2, 4, 8 and 12 hrs after the start of infusion
● Profile of mood states (POMS): Day -1, within approximately 4 and 24 hrs after the start of infusion
● Columbia-suicide severity rating scale (C-SSRS): Day -1, within approximately 4 and 24 hrs after the start of infusion
● Modified observer's assessment of alertness/sedation (MOAA/S): Pre-infusion, and approximately 20 min, 40 min and 1 hr after start of infusion
● Clinician administered dissociative states scale (CADSS): Day -1, Pre-infusion, approximately 40 min and 80 min after start of infusion

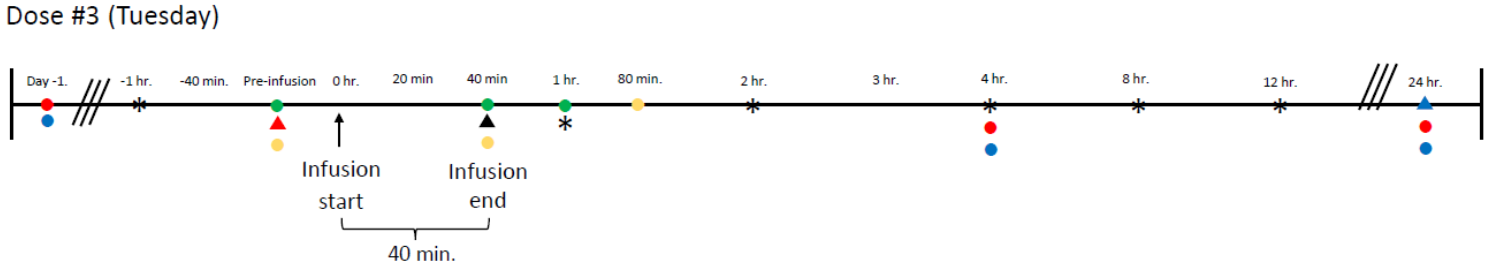
****When multiple procedures occur at the same timepoint, the following guides the approximate order of how procedures should be performed: MOAA/S > PK/lab draw > ECG > CADSS > C-SSRS > POMS.

Figure 4: Visual representation of timeline of key events for Dose #2 in the MAD study.

▲ Pharmacokinetics blood draw only
* Vitals/Electrocardiograms (ECG): Approximately 1 hr pre-infusion and 1, 2, 4, 8 and 12 hrs after the start of infusion
● Profile of mood states (POMS): Day -1, within approximately 4 and 24 hrs after the start of infusion
● Columbia-suicide severity rating scale (C-SSRS): Day -1, within approximately 4 and 24 hrs after the start of infusion
● Modified observer's assessment of alertness/sedation (MOAA/S): Pre-infusion, and approximately 20 min, 40 min and 1 hr after start of infusion
● Clinician administered dissociative states scale (CADSS): Day -1, Pre-infusion, approximately 40 min and 80 min after start of infusion

****When multiple procedures occur at the same timepoint, the following guides the approximate order of how procedures should be performed: MOAA/S > PK/lab draw > ECG > CADSS > C-SSRS > POMS.

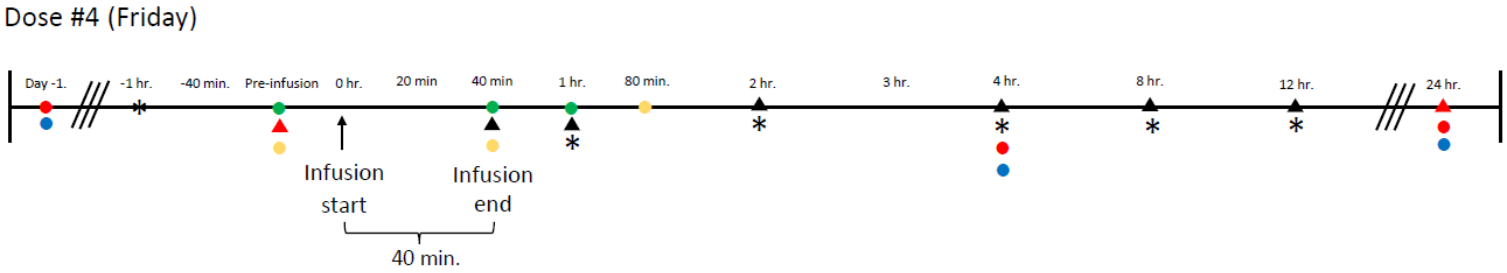
Figure 5: Visual representation of timeline of key events for Dose #3 in the MAD study.



▲ Pharmacokinetics and clin. lab blood draw
▲ Clin. lab blood draw only
▲ Pharmacokinetics blood draw only
* Vitals/Electrocardiograms (ECG): Approximately 1 hr pre-infusion and 1, 2, 4, 8 and 12 hrs after the start of infusion
● Profile of mood states (POMS): Day -1, within approximately 4 and 24 hrs after the start of infusion
● Columbia-suicide severity rating scale (C-SSRS): Day -1, within approximately 4 and 24 hrs after the start of infusion
● Modified observer's assessment of alertness/sedation (MOAA/S): Pre-infusion, and approximately 20 min, 40 min and 1 hr after start of infusion
● Clinician administered dissociative states scale (CADSS): Day -1, Pre-infusion, approximately 40 min and 80 min after start of infusion

****When multiple procedures occur at the same timepoint, the following guides the approximate order of how procedures should be performed: MOAA/S > PK/lab draw > ECG > CADSS > C-SSRS > POMS.

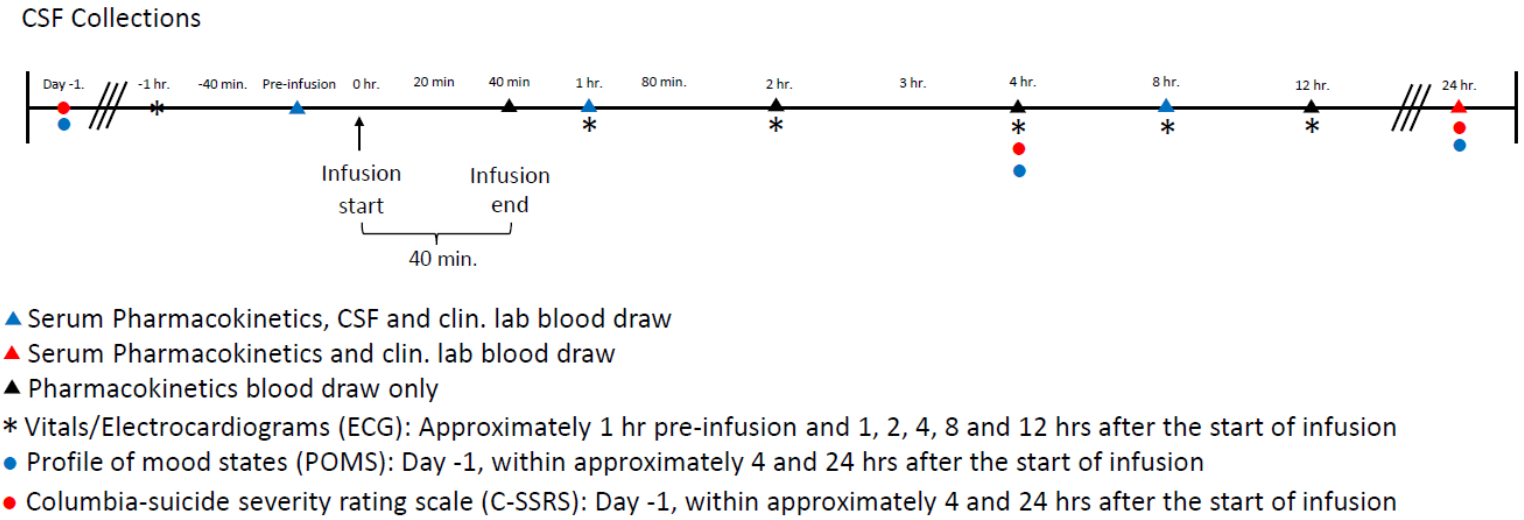
Figure 6: Visual representation of timeline of key events for Dose #4 in the MAD study.



▲ Pharmacokinetics and clin. lab blood draw
▲ Pharmacokinetics blood draw only
* Vitals/Electrocardiograms (ECG): Approximately 1 hr pre-infusion and 1, 2, 4, 8 and 12 hrs after the start of infusion
● Profile of mood states (POMS): Day -1, within approximately 4 and 24 hrs after the start of infusion
● Columbia-suicide severity rating scale (C-SSRS): Day -1, within approximately 4 and 24 hrs after the start of infusion
● Modified observer's assessment of alertness/sedation (MOAA/S): Pre-infusion, and approximately 20 min, 40 min and 1 hr after start of infusion
● Clinician administered dissociative states scale (CADSS): Day -1, Pre-infusion, approximately 40 min and 80 min after start of infusion

****When multiple procedures occur at the same timepoint, the following guides the approximate order of how procedures should be performed: MOAA/S > PK/lab draw > ECG > CADSS > C-SSRS > POMS.

Figure 7: Visual representation of timeline of key events for the CSF Capture study.



****When multiple procedures occur at the same timepoint, the following guides the approximate order of how procedures should be performed: PK/lab draw > ECG > > C-SSRS > POMS.

TABLE OF CONTENTS

Tables and Figures

ABBREVIATIONS

AE	Adverse event
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AUC_{0-72}	Area under the plasma concentration-time curve from 0 to 72 hours
$AUC_{0-\infty}$	Area under the plasma concentration-time curve from 0 to infinity
AUC_{0-last}	Area under the plasma concentration-time curve from 0 to time of last measurable sample
AUC_{0-Tau}	Area under the plasma concentration-time curve during the dosing interval
BMI	Body mass index
C-SSRS	Columbia-Suicide Severity Rating Scale
CADSS	Clinician Administered Dissociative States Scale
CL	Systemic plasma clearance
CL_R	Renal clearance
C_{max}	Maximum plasma concentration
C_{min}	Minimum plasma concentration
CMO	Contract manufacturing organization
CRF	Case report form
CSF	Cerebrospinal Fluid
DCRI	Duke Clinical Research Institute
DEPRU	Duke Early Phase Research Unit
ECG	Electrocardiogram
FDA	Food and Drug Administration
FFT	Fast Fourier Transform
FSH	Follicle-stimulating hormone
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HED	Human equivalent dose
HEK	Human embryonic kidney
hERG	Human ether-á-go-go-related gene
HIV	Human immunodeficiency virus
ICH	International Conference on Harmonisation
IDP	Investigational drug product
IP	Intraperitoneal
IRB	Institutional Review Board
IV	Intravenous
Ln	Log transformation
MAD	Multiple ascending dose
MDD	Major depressive disorder
MedDRA	Medical Dictionary for Regulatory Activities
MOAA/S	Modified Observer's Assessment of Alertness/Sedation

MOP	Manual of Procedures
NMDAR	NMDA-type glutamate receptors
NOAEL	No observed adverse effect levels
PD	Pharmacodynamic
PHI	Protected health information
PI	Principal Investigator
PK	Pharmacokinetic(s)
POMS	Profile of Mood States
PQC	Product quality complaint
qEEG	Quantitative electroencephalography
$RAUC_{(0-\infty)}$	Accumulation ratio for $AUC_{(0-\infty)}$
RC_{max}	Accumulation ratio for C_{max}
SAD	Single ascending dose
SOC	Summarized by system organ class
SQ	Subcutaneous
$t_{1/2}$	Terminal half-life
t_{max}	Time to C_{max}
TRD	Treatment-resistant depression
V_z	Volume of distribution during terminal phase
WHO	World Health Organization

1.0 INTRODUCTION

Currently over 15 million Americans suffer from major depressive disorder (MDD), and up to 40% of these patients are resistant to current first-line pharmacological therapies (1). Furthermore, current first line pharmacological therapies can take over 8 weeks of treatment before full alleviation of the symptoms of depression often occurs. Thus, there is a large unmet medical need for a fast-acting antidepressant that can also effectively treat those patients resistant to current pharmacological therapies.

(2R,6R)-Hydroxynorketamine is a metabolite of the drug ketamine. Ketamine, a racemic mixture of R-(-)ketamine and S-(+)ketamine, is an approved anesthetic and a World Health Organization (WHO) essential medicine (2). Ketamine has been examined as a therapy for treatment-resistant depression (TRD) (3). S-(+)ketamine (Spravato^{TM}) has recently been approved by the United States Food and Drug Administration (FDA) for this indication. However, similar to Ketamine, S-(+)ketamine also has sedative, sensory dissociative and abuse potential. Following administration, ketamine is rapidly metabolized into over twenty characterized metabolites including (2R,6R)-Hydroxynorketamine (4). (2R,6R)-Hydroxynorketamine possesses antidepressant qualities in established rodent models without ketamine's associated sedative, sensory dissociative or addictive actions (5). Based on this background, (2R,6R)-Hydroxynorketamine is being evaluated in this phase 1 study to establish a safe range of exposure prior to being examined in patients with TRD.

2.0 BACKGROUND

2.1 NONCLINICAL STUDIES

At doses ranging from 5 to 125 mg/kg, (2R,6R)-Hydroxynorketamine demonstrated acute and sustained antidepressant-like activity in validated murine models including the forced-swim test, novelty-suppressed feeding test, learned helplessness test, the social defeat/social interaction ratio, and sucrose preference tests (5). At a dose of 10 mg/kg in mice (2R,6R)-Hydroxynorketamine induced an increase in hippocampus gamma-power (30-80 Hz) oscillations like those noted following ketamine administration in human studies (5). In murine models, within this antidepressant-relevant dose range there were no ketamine-related adverse effects observed. These included no change in locomotion activity or rotorod coordination, no prepulse inhibition deficits and no ketamine-like drug discrimination or self-administration (5). The antidepressant-like activity of (2R,6R)-Hydroxynorketamine could be blocked by administering a brain-penetrant AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor antagonist, implicating AMPA receptor involvement (5). (2R,6R)-Hydroxynorketamine possessed no quantifiable binding to the ketamine/MK801 site of the NMDA-type glutamate receptors (NMDAR) up to 100 μM, and modest functional inhibition of the NMDAR was limited to high concentrations (5,6). For example, at 10 μM concentrations, (2R,6R)-Hydroxynorketamine did not modify NMDAR-mediated field excitatory postsynaptic potentials in the CA1 field of mouse hippocampal slices and NMDAR-mediated miniature excitatory postsynaptic currents or NMDA-evoked currents in CA1 pyramidal neurons of rat hippocampal slices (6). (2R,6R)-Hydroxynorketamine protected mice from NMDA-induced lethality with a median effective dose ($ED_{50)}$ of 227.8 mg/kg compared to 6.4 mg/kg for ketamine (6).

2.2 NONCLINICAL SAFETY ASSESSMENTS

As part of the nonclinical development of (2R,6R)-Hydroxynorketamine, key safety pharmacology and toxicology studies were conducted. Safety pharmacology studies included the central nervous system modified Irwin test, respiratory, cardiovascular, and human ether-á-go-go-related gene (hERG) evaluations. Single and repeat dose range finding studies preceded definitive Good Laboratory Practice (GLP) single dose and repeat-dose toxicology studies in two species (rat and dog). Genetic toxicology was evaluated utilizing a micronucleus test in cultured human peripheral blood lymphocytes. An examination of neurotoxicity was conducted by evaluating the induction of neuronal vacuolation and apoptotic cell death in selected regions of the brain including the posterior cingulate and retrosplenial cortex. All safety pharmacology and definitive toxicology studies were performed in accordance with the United States Food and Drug Administration (FDA) GLP guidelines. All in vivo safety pharmacology and toxicology studies were performed utilizing an intravenous (IV) infusion route of administration of (2R,6R)-Hydroxynorketamine.

2.3 SAFETY PHARMACOLOGY

Central Nervous System

Central nervous system safety pharmacology studies of (2R,6R)-Hydroxynorketamine were performed in male RccHan® WIST rats. Single doses of 25, 175, and 300 mg/kg were administered, and each animal was subjected to a modified Irwin assessment at 5 minutes, 1.5, 5, and 24 hours postdose. Transient changes were noted at up to 1.5 hours postdose for animals administered doses ≥175 mg/kg with the majority of findings evident solely at 5-minutes postdose for those rats receiving 300 mg/kg. The changes included decreases in measures associated with motor activity, increases in measures associated with sedation, altered respiration, and decreased body temperature. All test article-related changes at ≥175 mg/kg had resolved by the 5-hour postdose timepoint.

Respiratory

Respiratory safety pharmacology studies of (2R,6R)-Hydroxynorketamine were performed in male RccHan® WIST rats. Single doses of 25, 175, and 300 mg/kg were administered, and each animal was subjected to head-out plethysmography for a duration inclusive of the infusion period through 5.5 hours postdose and 24 hours postdose. Transient changes in respiratory parameters were dose-related in magnitude and had resolved by 1.5 hours after the end of infusion. Respiration rate increases were noted for animals administered ≥25 mg/kg, with compensatory tidal volume decreases observed for animals administered ≥175 mg/kg. Given the compensatory differences in respiration rate and tidal volume, evident changes in minute volume were limited to a brief increase for animals administered 300 mg/kg.

Cardiovascular

Cardiovascular safety pharmacology studies of (2R,6R)-Hydroxynorketamine were performed in beagle dogs. Single doses of 15, 30, and 90 mg/kg were administered to telemetry-instrumented beagles and the cardiovascular function of each animal was assessed by qualitative or quantitative electrocardiography for a duration inclusive of the infusion period (20 minutes) through to 18 hours after the start of the infusion. No qualitative or quantitative electrocardiography effects were noted over this period for all doses. Administration of 15, 30, and 90 mg/kg caused lower systolic pressure (-4 mmHg, -6 mmHg, -16 mmHg, respectively) and arterial pulse pressure (-2 mmHg, -6 mmHg, -12 mmHg, respectively) from 0.25 to 3 hours after the start of infusion. At 90 mg/kg, diastolic pressure and mean arterial pressure were significantly lower (-4 mmHg and -8 mmHg, respectively) from 0.25 to 3 hours after the start of infusion. No (2R, 6R)-Hydroxynorketamine-related effects on heart rate or body temperature were noted through 18 hours after the start of infusion.

hERG

The in vitro effect of (2R,6R)-Hydroxynorketamine on the hERG potassium channels was assessed using cloned hERG expressed in human embryonic kidney (HEK) cells. The HEK cells stably expressing hERG were held at -80 mV and exposed to drug treatments of 100 μM and 300 μM and onset and steady state inhibition of potassium current was measured using a pulse pattern with fixed amplitudes. Each recording ended

with a final application of a supramaximal concentration of the reference substance (E-4031, 500 nM) to assess the contribution of endogenous currents. The remaining uninhibited current was subtracted off-line digitally from the data to determine the potency of hERG inhibition. (2R,6R)-Hydroxynorketamine inhibited hERG current by 4.3% ± 0.1% (Mean ± SEM) at 100 μM (n = 3) and 10.3% ± 0.4% at 300 μM (n = 3) versus 0.2% ± 0.6% (n = 3) in control. The half maximal inhibitory concentration (IC_{50})for the inhibitory effect of (2R,6R)-Hydroxynorketamine on hERG potassium current was not calculated but was estimated to be greater than 300 μM.

2.4 TOXICOLOGY

Range-finding toxicology studies

Dose range-finding studies (single- and repeat-dose) of (2R,6R)-Hydroxynorketamine were performed in rat and dog and were supported by toxicokinetics. In rats, single doses of 150, 300, or 630 mg/kg were administered and repeated doses of 150 or 375 mg/kg/dose were administered on days 1, 4, 8, 11, and 15. The 630 mg/kg single dose was the maximum tolerated dose (MTD) with 1 animal death and 2 animals being sacrificed due to morbidity. A single dose of 300 mg/kg evoked several drug related observations including ataxia and low carriage and dose-dependent hypoactivity and squinting eyes in females administered 150 mg/kg. In the repeated dose study, clinical pathology findings secondary to hypoactivity, hemoconcentration, and a stress response were observed in rats administered ≥150 mg/kg/dose. Additionally, increased liver weights were noted in males administered ≥150 mg/kg/dose. Clinical effects of repeated doses ≤300 mg/kg/dose and 150 mg/kg/dose were considered nonadverse owing to the mild severity or transient nature of the findings and the overall lack of impact on animal health and wellbeing.

In dogs, single doses of 45, 90 or 180 mg/kg and repeated doses of 90 or 180 mg/kg/dose were administered on days 1, 4, 8, and 15. At a single dose of 180 mg/kg, test-article related clinical observations included ataxia and cloudy foamy vomitus. At repeated doses of ≥90 mg/kg/dose excessive salivation and ataxia were noted and emesis, vomitus, and intermittent whole-body tremors were observed in dogs administered repeated doses of 180 mg/kg/dose. Other effects observed in animals administered 180 mg/kg at both single and repeated doses were considered nonadverse owing to the mild severity and/or transient nature of the findings and the overall lack of impact on animal health and wellbeing.

Definitive GLP toxicity studies

Definitive toxicology studies of (2R,6R)-Hydroxynorketamine were performed in two species (rat and dog) and were supported by toxicokinetics. Male and female rats were administered 0, 25, 175, or 350 mg/kg/dose (2R,6R)-Hydroxynorketamine on Days 1, 4, 8, 11, 15, 18, 22, and 25. (2R,6R)-Hydroxynorketamine-related clinical observations for males included dose-dependent squinting eyes and low carriage and red discolored hair coat for males administered ≥175 mg/kg/dose and ataxia, swollen nose, and sternal recumbent body position (one animal) for males administered 350 mg/kg/dose. (2R,6R)-Hydroxynorketamine-related clinical observations for females included

dose-dependent hypoactivity and ataxia for females administered ≥175 mg/kg/dose and squinting eyes for females administered 350 mg/kg/dose. Irregular/labored respiration was also noted for animals (male and female) administered ≤350 mg/kg/dose and it was not clear whether this was related to (2R,6R)-Hydroxynorketamine dosing. All observations were transient, occurred close to t_{max} (1-minute postdose), generally resolved by 60 minutes postdose, and did not correlate with any changes in food consumption or body weight; therefore, they were considered nonadverse for animals administered 175 mg/kg/dose. However, these findings were considered adverse for animals administered 350 mg/kg/dose due to mortality, recumbence, and minimal decrease in male body weight/body weight gain (females experienced no change in body weight). Additionally, at the terminal sacrifice, (2R,6R)-Hydroxynorketamine-related increased liver weight parameters were noted in males administered 350 mg/kg/dose and correlated with the microscopic finding of minimal centrilobular hepatocyte hypertrophy. No (2R,6R)-Hydroxynorketamine-related microscopic findings or effects on organ weight parameters were observed at the recovery sacrifice, which was consistent with reversibility. Thus, the no observed adverse effect level (NOAEL) in rats was determined to be 175 mg/kg/dose. This dose level corresponded to mean maximum plasma concentration (C_{max}) and AUC values of 150,000 ng/mL and 308,000 ng*hr/mL, respectively, in males and 147,000 ng/mL and 315,000 ng*hr/mL, respectively, in females on Day 25 of the dosing phase.

Male and female dogs were administered 0, 8, 90, or 180 mg/kg/dose (2R,6R)-Hydroxynorketamine on Days 1, 4, 8, 11, 15, 18, 22, and 25 of the dosing phase. (2R,6R)-Hydroxynorketamine-related clinical observations for males included vocalization during dosing (dose-dependent) and clear eye discharge for males administered ≥90 mg/kg/dose and ataxia and intermittent whole-body tremors for males administered 180 mg/kg/dose. (2R,6R)-Hydroxynorketamine-related clinical observations for females included dose-dependent ataxia and vocalization during dosing for females administered ≥90 mg/kg/dose and excess salivation, emesis, vomitus, and intermittent hind limb tremors for females administered 180 mg/kg/dose. (2R,6R)-Hydroxynorketamine was associated with higher heart rate in males administered 180 mg/kg/dose; this effect reversed during the recovery phase. Effects for animals administered 180 mg/kg/dose were considered nonadverse owing to the transient nature of these findings and the lack of overall impact on the health and wellbeing of animals. Thus, the NOAEL in dogs was determined to be 180 mg/kg/dose. This dose level corresponded to mean C_{max} and AUC_{0-72} values of 218,000 ng/mL and 316,000 ng*hr/mL, respectively, on Day 25 of the dosing phase.

2.5 GENOTOXICITY

A standard battery of genetic toxicology studies has been conducted on (2R,6R)-Hydroxynorketamine in accordance with the Organization for Economic Co-operation and Development (OECD) Principles on GLP. (2R,6R)-Hydroxynorketamine showed no evidence of mutagenicity in a bacterial reverse mutation test using 5 strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA102) and test concentrations of 5 to 5000 µg/plate in the absence or presence of exogenous metabolic

activation (Aroclor 1254 induced rat liver S9). An in vitro micronucleus assay conducted using human peripheral blood lymphocytes and test concentrations up to 240 µg/mL (approximately 1 mM) showed that (2R,6R)-Hydroxynorketamine did not induce biologically relevant increases in the frequency of micronucleated cells following treatment in the absence and presence of S-9. To complete the test battery, (2R,6R)-Hydroxynorketamine was assessed in an in vivo rat bone marrow micronucleus assay using IV doses of 105, 210, or 420 mg/kg/day for 2 days. Although 1 animal given 210 mg/kg/day was found dead at 2.5 hours post second dose, (2R,6R)-Hydroxynorketamine showed no evidence of induction of micronucleus formation in polychromatic erythrocytes following IV administration at up to 420 mg/kg/day. Hence, (2R,6R)-Hydroxynorketamine showed no evidence of mutagenic, clastogenic, or aneugenic activity in a standard battery of genotoxicity studies.

2.6 NEUROTOXICITY

Although multiple lines of evidence demonstrate that (2R,6R)-Hydroxynorketamine is a weak NMDAR antagonist, it was deemed prudent to assess if (2R,6R)-Hydroxynorketamine induces a specified neurological toxicity involving neuronal vacuolation (referred to as Olney's lesions) that is associated with blockade of the NMDAR. Olney's lesion neurotoxicity studies of (2R,6R)-Hydroxynorketamine were performed using male and female RccHan®:WIST rats. Both single and repeat-dose studies were performed. Single-dose studies involved doses of (2R,6R)-Hydroxynorketamine of 12, 80, 120, and 160 mg/kg. Clinical observations for (2R,6R)-Hydroxynorketamine were consistent with those noted during the GLP toxicology studies. The validated NMDAR antagonist MK-801 was utilized as a positive control and dosed at 0.5 and 0.8 mg/kg via the subcutaneous (SQ) route. MK-801-related Olney-like brain lesions were observed in rats administered both these doses. No microscopic brain lesions were noted in rats administered (2R,6R)-Hydroxynorketamine at any dose. Repeated dose studies employed (2R,6R)-Hydroxynorketamine at 12, 80, or 160 mg/kg/dose administered 4 times over 2 weeks (days 1, 4, 11 and 15). MK-801 was again utilized as a positive control and dosed SQ at 0.5 and 0.8 mg/kg/dose at the same schedule (ie, 4 times over 2 weeks [days 1, 4, 11, and 15]). Data were consistent with that obtained in the single dose study, in that Olney-like brain lesions were observed in rats administered both doses of MK801. In summary, no microscopic brain lesions were noted in rats administered single or repeated doses of (2R,6R)-Hydroxynorketamine at any dose.

2.7 CONCLUSIONS

Based upon multiple nonclinical models of antidepressant effectiveness, there is now strong evidence that (2R,6R)-Hydroxynorketamine possesses acute and sustained antidepressant-like activity akin to ketamine but without the associated ketamine-like adverse effects including sedation, sensory dissociation, and abuse potential. Nonclinical toxicology studies show that there are no predicted safety pharmacology liabilities for (2R,6R)-Hydroxynorketamine including studies exploring the respiratory, central nervous, and cardiovascular systems. There was no evidence of genotoxicity of (2R,6R)-Hydroxynorketamine in a standard battery of genotoxicity studies (Ames test, in

vitro micronucleus test, and rat bone marrow micronucleus test). There was no observed effect of (2R,6R)-Hydroxynorketamine on the hERG potassium channels. Single and repeated-dose range finding and definitive (GLP) toxicology studies in rats and dogs established NOAELs of 175 mg/kg/dose and 180 mg/kg/dose, respectively.

A neurotoxicity assessment of neuronal vacuolation (Olney's lesions) demonstrated that there were no microscopic brain lesions in rats administered (2R,6R)-Hydroxynorketamine at single or repeated doses up to 160 mg/kg. Overall, these data support clinical investigation of (2R,6R)-Hydroxynorketamine at the planned doses and schedule outlined (see section 8.0 Study design rationale).

3.0 STUDY OBJECTIVES AND ENDPOINTS

The primary objective of this study is to determine the safe dose range and tolerability of (2R,6R)-Hydroxynorketamine HCl administered via a slow IV infusion (40 minutes) to healthy volunteers. A second primary objective for this study is to assess the pharmacokinetics (PK) of (2R,6R)-Hydroxynorketamine HCl in serum following single and multiple dose administrations.

The exploratory objective of this study is to collect quantitative electroencephalography (qEEG) data as a PD biomarker (SAD). Another exploratory objective of this study is to assess the concentration of (2R,6R)-Hydroxynorketamine HCl collected from cerebrospinal fluid following administration of a single dose(CSF capture).

Study endpoints include completion of all dosing levels and timepoints with full collection of all safety data, adverse events (AEs) tabulation, clinical laboratory tests, PK endpoints (eg, C_{max}, time to C_{max} [t_{max}], minimum plasma concentration [C_{min}], area under the plasma concentration-time curve from 0 to infinity [$AUC_{0-\infty}$], area under the plasma concentration-time curve during the dosing interval [AUC_{0-Tau}], systemic plasma clearance (CL), volume of distribution during terminal phase [V_Z], renal clearance [CL_R], terminal half-life [$t_{1/2}$]).

4.0 STUDY RISKS AND BENEFITS

As with all clinical studies, there are risks. These risks include multiple venipuncture and blood sample collections. The blood sample collection scheme was designed to collect the minimum number of blood samples to collect the needed samples for thorough clinical laboratory testing and that accurately and completely describe the PK of study drug. This minimizes the number of venipunctures and the total volume of blood collected from each Subject during the study. The amount of blood to be collected from each subject over the course of the study is acceptable based on the American Red Cross standard (http://www.redcrossblood.org/donating-blood/eligibility-requirements).

Subjects in the CSF Capture Study will also undergo lumbar puncture with placement of CSF catheter, a procedure associated with risk of bleeding, infection and post-procedure headache. The volume of CSF to be collected at each time point was calculated to be less than the hourly CSF production to minimize risk of headache and subjects will be kept in a semi-recumbent position while the CSF catheter remains in place. Procedures will be performed utilizing standard aseptic technique to minimize infection risk.

Subjects will be asked to provide protected health information (PHI). All attempts will be made to keep PHI confidential within the limits of the law; however, there is a chance that unauthorized persons will see the subjects' PHI. All study records will be kept in a locked file cabinet or maintained in a locked room at the Duke Early Phase Research Unit (DEPRU). Electronic files will be maintained on secure servers and transferred files with PHI will be password protected. Only people involved in the conduct, oversight, monitoring, or auditing of this study will be allowed access to the PHI that is collected. Any publications from this study will not use information that will identify subjects by name. Organizations that may inspect and/or copy research records maintained at DEPRU for quality assurance and data analysis include the local Institutional Review Board (IRB), National Institute of Mental Health and/or their designees, and the FDA.

A description of this clinical study will be available at http://www.ClinicalTrials.gov, as required by US law. This website will not include information that can identify subjects and at most, it will include a summary of the results.

The potential risks to the subjects enrolled in this study are moderate. To further minimize these risks, experienced personnel will implement the protocol and provide detailed instructions to the subjects. There may be other risks, discomforts, or side effects that are currently unknown. Overall, the risks associated with the research are justified by the potential public health benefits.

The primary benefit to Subjects, aside from the financial compensation, is the contribution to a study that may prove beneficial for the discovery of a new therapy for TRD.

Subjects will be fully informed of the risks and requirements of the study. During the study, Subjects will be given any new information that may affect their decision to continue participation. They will be told that their consent to participate in the study is voluntary and may be withdrawn at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only Subjects who are fully able to understand the risks, benefits, and potential AEs of the study, and provide their consent voluntarily will be enrolled.

5.0 STUDY DESIGN (SAD)

This is a 6-cohort, single site, first-in human, double-blind, placebo-controlled, randomized single ascending dose (SAD) study assessing (2R,6R)-Hydroxynorketamine in healthy male and female volunteers. All Subjects in the SAD study will undergo a screening period of up to 28 days to ensure that all eligibility requirements are met. Key baseline safety metrics will be taken during screening and on the first Day of confinement. Selected safety metrics will be collected during the on-drug portion of the study. A final safety assessment will be conducted via an outpatient visit and follow-up phone call.

All cohorts will include 6 Subjects receiving drug and 2 Subjects receiving placebo. All cohorts will incorporate sentinel dosing of 1 active and 1 placebo Subject. Each Subject will only participate in a single dose level. Table 4 outlines the planned dosing levels and number of Subjects in each cohort.

Each treatment will be administered after Subjects have fasted overnight for at least 8 hours.

Safety monitoring and assessments will be performed as described in sections 8.7 and 12.8. Dosing may be stopped if any of the stopping criteria are met as described in section 10.3. Determination of whether to escalate to the next dose level if stopping criteria are met will be made by the Principal Investigator (PI) in consultation with the Medical Monitor and the Study Sponsor.

Pharmacokinetic blood samples will be collected as described in section 8.6. The qEEG will be performed on all Subjects in the SAD study as described in section 12.10

Table 4: SAD planned dosing levels

Cohort	Dose (mg/kg)	Subjects on Active Drug (n)	Subjects on Placebo (n)
1A (Sentinel 1)	0.1	1	1
1B	0.1	5	1
2A (Sentinel 2)	0.25	1	1
2B	0.25	5	1
3A (Sentinel 3)	0.5	1	1
3B	0.5	5	1
4A (Sentinel 4)	1.0	1	1
4B	1.0	5	1
5A (Sentinel 5)	2.0	1	1
5B	2.0	5	1
6A (Sentinel 6)	4.0	1	1
6B	4.0	5	1

6.0 STUDY DESIGN (MAD)

The multiple ascending dose (MAD) portion of the study is a 2-cohort single site, double-blind, placebo-controlled design assessing (2R,6R)-Hydroxynorketamine in healthy adult male and female volunteers. All Subjects in the MAD study will undergo a screening period of up to 28 days to ensure that all eligibility requirements are met. Key baseline safety metrics will be taken during screening and on the first Day of confinement. Selected safety metrics will be collected during the on-drug portion of the study. A final safety assessment will be conducted via an outpatient visit and follow-up phone call.

All cohorts will include 6 Subjects receiving drug and 2 Subjects receiving placebo. Each Subject will receive a total of 4 doses (drug or placebo) administered on days 1, 4, 8, and 11. Each Subject will only participate in a single dose level. Table 5 outlines the planned dosing levels and number of Subjects in each cohort.

Each dose will be administered after Subjects have fasted overnight for at least 8 hours.

Safety monitoring and assessments will be performed as described in sections 8.7 and 12.8. AE monitoring and reporting will be performed as described in sections 8.7 and 12.8. Dosing may be stopped if any of the stopping criteria are met as described in section 10.3. Determination of whether to escalate to the next dose level if stopping criteria are met will be made by the PI in consultation with the Medical Monitor and the Study Sponsor.

Pharmacokinetic blood samples will be collected as described in section 8.6.

Table 5: MAD planned dosing levels

Cohort	Dose (mg/kg)	Subjects on Active Drug (n)	Subjects on Placebo (n)
1	1.0	6	2
2	2.0	6	2

7.0 STUDY DESIGN (CSF CAPTURE)

The cerebrospinal fluid capture (CSF Capture) portion of the study is a 1-cohort single site, double-blind, placebo-controlled design assessing (2R,6R)-Hydroxynorketamine in healthy adult male and female volunteers. All Subjects in the CSF Capture study will undergo a screening period of up to 28 days to ensure that all eligibility requirements are met. Key baseline safety metrics will be taken during screening and on the first Day of confinement. Selected safety metrics will be collected during the on-drug portion of the study. A final safety assessment will be conducted via an outpatient visit and follow-up phone call.

The cohort will include 5 Subjects receiving drug and 3 Subjects receiving placebo. Each Subject will receive a total of 1 dose (drug or placebo) administered on day 1. Each Subject will only participate in a single dose level. Table 6 outlines the planned dosing level and number of Subjects in each cohort.

Each dose will be administered after Subjects have fasted overnight for at least 8 hours.

Safety monitoring and assessments will be performed as described in sections 8.7 and 12.8. AE monitoring and reporting will be performed as described in sections 8.7 and 12.8. Dosing may be stopped if any of the stopping criteria are met as described in section 10.3. Determination of whether to escalate to the next dose level if stopping criteria are met will be made by the PI in consultation with the Medical Monitor and the Study Sponsor.

Pharmacokinetic blood and CSF samples will be collected as described in section 8.6.

Table 6: CSF Capture study planned dosing levels

Cohort	Dose (mg/kg)	Subjects on Active Drug (n)	Subjects on Placebo (n)
1	0.25	5	3

8.0 STUDY DESIGN RATIONALE

8.1 STUDY DRUG

The antidepressant activity of racemic ketamine and the recent approval of S-(+)ketamine (esketamine, Spravato™) have proven to be major advances for individuals with TRD. However, several issues surround these drugs including unwanted side effects and the potential for abuse. Further, inconsistencies surround the purported mechanism of action (NMDAR antagonism) as other investigational drugs with optimized potency for this target lack consistent antidepressant activity. There are also PD questions given that ketamine is eliminated on a timescale (hours) that is inconsistent with the effect duration (days). (2R,6R)-Hydroxynorketamine is a metabolite following ketamine administration. (2R,6R)-Hydroxynorketamine possesses strong antidepressant activity in multiple models of depression and anxiety. (2R,6R)-Hydroxynorketamine is a weak NMDAR antagonist and, therefore, is devoid of unwanted side-effects including sedation, dissociation and addictive potential as tested in animal models. (2R,6R)-Hydroxynorketamine is manufactured as the HCl salt which yields a single polymorph and crystal form and is stable as both a solid and as a solution in multiple formulants. The investigational drug product (IDP) is a solution-based formulation of (2R,6R)-Hydroxynorketamine in 25 mM phosphate buffer.

8.2 STUDY POPULATION

Healthy male and female adult subjects (18-65 years old) will be enrolled in this study. It is expected that the PK of (2R,6R)-Hydroxynorketamine in these Subjects will be representative of the PK profile in patients with TRD. The cohort size in all SAD and MAD stages is expected to sufficiently evaluate the safety and PK of (2R,6R)-Hydroxynorketamine. Furthermore, these cohort sizes are also expected to provide sufficient qEEG data to evaluate the use of qEEG as a potential PD biomarker.

8.3 DESIGN ASPECTS

The design of the SAD, MAD and CSF Capture studies are intended to mirror the currently accepted best practices for the administration of ketamine in the setting of depression (in MDD and TRD). Each design aspect (route of administration, IDP, PK measurements, qEEG data capture, etc.) should allow for direct comparison to existing clinical data on the use of ketamine as an antidepressant and should inform dose and schedule of (2R,6R)-Hydroxynorketamine in TRD.

8.4 ROUTE OF ADMINISTRATION

Racemic ketamine is being used to treat TRD in specialized clinics utilizing a slow IV infusion. To produce a comparative clinical assessment for (2R,6R)-Hydroxynorketamine an identical route of administration (ie, IV) and time frame (40 min) is adopted.

8.5 PLANNED DOSAGE

The doses of (2R,6R)-Hydroxynorketamine in the SAD study are based upon both the presumed safety ranges as defined by the definitive toxicology studies and the doses which achieve efficacy in various models of depression and anxiety. Efficacious doses of (2R,6R)-Hydroxynorketamine achieved in mouse models of antidepressant efficacy and qEEG gamma changes range from 5 mg/kg to 20 mg/kg. The commonly used dose of 10 mg/kg intraperitoneal (IP) achieved a mouse plasma C_{max} of 2,587 ng/mL and plasma AUC of 772 ng*hr/mL. The human equivalent dose is 0.813 mg/kg utilizing an allometric scaling factor of 12.3. Adjusting for the bioavailability of IP dosing (approximately 50%), the human IV dose estimate can be corrected to 0.406 mg/kg.

The (2R,6R)-Hydroxynorketamine NOAEL in rats is 175 mg/kg/dose. The human equivalent dose is 28.2 mg/kg utilizing an allometric scaling factor of 6.2. This dose level corresponded to mean C_{max} and AUC values of 150,000 ng/mL and 308,000 ng*hr/mL, respectively, in males and 147,000 ng/mL and 315,000 ng*hr/mL, respectively, in females on Day 25 of the dosing phase. The (2R,6R)-Hydroxynorketamine NOAEL in dogs is 180 mg/kg/dose. The human equivalent dose is 100 mg/kg utilizing an allometric scaling factor of 1.8. This dose level corresponded to mean C_{max} and AUC_{0-72} values of 218,000 ng/mL and 316,000 ng*hr/mL, respectively, on Day 25 of the dosing phase. The human equivalent dose is 100 mg/kg utilizing an allometric scaling factor of 1.8.

Table 7: Key dosing levels and associated exposures in mouse, rat, and dog.

	HED (by allometry)	**C_{max}**	**AUC**
Efficacious dose in mouse (10 mg/kg)	0.813 mg/kg	2,587 ng/mL	772 ng*hr/mL
Rat NOAEL (175 mg/kg/dose)	28.2 mg/kg	150,000 ng/mL (m) 147,000 ng/mL (f)	308,000 ng*hr/mL 315,000 ng*hr/mL
Dog NOAEL (180 mg/kg/dose)	100 mg/kg	218,000 ng/mL	316,000 ng*hr/mL

A second approach was applied for human dose estimation based on PK data of (2R,6R)-Hydroxynorketamine postketamine doses in humans using validated methods (7-9). Ketamine and its major metabolites were quantified in plasma after IV infusion at 0.5 mg/kg of racemic ketamine. A parent-metabolite PK model was developed for racemic-ketamine and its major metabolites including rac-norketamine, rac-dehydronorketamine and (2S,6S:2R,6R)-Hydroxynorketamine, and the model parameters were estimated using a stepwise approach. The estimated model parameters for (2S,6S:2R,6R)-Hydroxynorketamine was assumed to be the same as those for (2R,6R)-Hydroxynorketamine to establish these estimations. Concentration of (2R,6R)-Hydroxynorketamine was predicted at different dose levels post a 40 min infusion and the exposure was calculated. Based upon these methods, it was determined that an IV dose of 0.1 mg/kg in human could provide similar exposure as observed in mouse post 10 mg/kg IP dose.

Based upon these collective models, the (2R,6R)-Hydroxynorketamine proposed doses for the SAD study range from 0.1 mg/kg to 4.0 mg/kg. Dose escalations between cohorts are approximately 2-fold increases between cohorts in alignment with common phase 1 clinical trial practice. The highest proposed SAD dose of 4.0 mg/kg is approximately 7X lower than the NOAEL human equivalent dose in the most sensitive species (rat) and 25X lower than the NOAEL human equivalent dose in the less sensitive species (dog) using allometric scaling factors of 6.2 and 1.8, respectively.

The (2R,6R)-Hydroxynorketamine doses selected for the MAD portion are set at 1.0 and 2.0 mg/kg. These doses are deemed acceptable for inclusion in the MAD study based on the PK, PD, safety, and tolerability outcomes obtained from the SAD section study.

The (2R,6R)-Hydroxynorketamine doses selected for the CSF Capture portion are set at 0.25 and 2.0 mg/kg. These doses are deemed acceptable for inclusion in the CSF Capture study based on the PK, PD, safety, and tolerability outcomes obtained from the SAD section study.

8.6 PHARMACOKINETIC EVALUATION

Blood samples will be obtained during the study for measurement of (2R,6R)-Hydroxynorketamine plasma concentrations to provide an unambiguous association between drug dose and systemic drug exposure. Serial PK blood samples will be collected during the SAD and MAD studies for each Subject receiving drug and placebo at 9 timepoints and 20 timepoints, respectively. For PK assessments, there will be an acceptable blood collection window of ± 5 minutes in the first 8 hours after a dose and ± 30 minutes thereafter.

Serial PK blood samples will be collected during the SAD portion for each Subject receiving drug and placebo at 9 timepoints (preinfusion, end-of-infusion [approximately 40 minutes], 1, 2, 4, 8, 12, 24, and approximately 48 hr after the start of the infusion). Pharmacokinetic urine samples (up to 3 per Subject) will be collected during the SAD study for each Subject receiving drug and placebo at set intervals following the initiation-of-infusion (0-4, >4-8, >8-12, >12-24 hr).

Serial PK blood samples will be collected for the first and fourth (last) dosing in the MAD study for each Subject receiving drug and placebo. Blood PK samples will be obtained at 8 timepoints (preinfusion, end-of-infusion [approximately 40 minutes], 1, 2, 4, 8, 12, and approximately 24 hr after the start of the infusion). Two PK blood samples will be collected for the second and third dosing in the MAD study for each Subject receiving drug and placebo at preinfusion and end-of-infusion [approximately 40 minutes] timepoints.

Serial PK blood samples will be collected during the CSF Capture portion for each Subject receiving drug and placebo at 8 timepoints (preinfusion, end-of-infusion [approximately 40 minutes], 1, 2, 4, 8, 12, and approximately 24 hr after the start of the infusion). CSF samples will be collected during the CSF Capture portion for each Subject

receiving drug and placebo at 3 timepoints (preinfusion, 1, and approximately 8 hr after the start of the infusion).

Blood and CSF samples will be stored and shipped to LabCorp (Covance) central laboratories who will perform PK analysis of (2R,6R)-Hydroxynorketamine using a LC/MS/MS method to determine plasma concentration levels in order to calculate the following PK parameters: C_{max}, t_{max}, C_{min}, $AUC_{0-\infty}$, AUC_{0-Tau}, CL, V_z, CL_R, $t_{1/2}$.

8.7 SAFETY AND TOLERABILITY EVALUATIONS

A series of safety assessments will be employed to assess drug-related AEs and assess tolerability in the study described in sections 12.8 and 13.1. Preclinical toxicology studies did not identify any anticipated or toxicology issues and, therefore, standard phase 1 study safety assessments will be employed. Given the nature of the planned indication (TRD) and the relationship of (2R,6R)-Hydroxynorketamine to ketamine, 4 specialty assessments will be included (ie, the Profile of Mood States [POMS], the Columbia-Suicide Severity Rating Scale [C-SSRS], the Clinician Administered Dissociative States Scale [CADSS], and the Modified Observer's Assessment of Alertness/Sedation [MOAA/S]) as described in sections 12.8 and 13.1.

Monitoring of AEs will be governed by change from baseline established during prescreening and Day -1 examinations and clinical laboratory tests as described in sections 12.9 and 13.1. The AEs will be coded using the MedDRA dictionary and summarized by system organ class (SOC) and preferred term, by severity, by relationship to study drug and study procedure, and by study drug dose.

Dose escalation in both the SAD and MAD portions or continued dosing in the MAD part may be stopped according to the predefined halting rules or if a Subject's scores on the C-SSRS assessment demonstrates acute suicidality or at the discretion of the study PI and/or sponsor. Dose escalation in the SAD and MAD studies or continued dosing in the MAD study may also be stopped for any additional reason at the discretion of the PI in consultation with the Medical Monitor and the Study Sponsor.

9.0 SUBJECT SELECTION

9.1 GENERAL CONSIDERATIONS

A full checklist of eligibility criteria must be completed prior to formal enrolment. Eligibility requirements must be re-evaluated and confirmed on Day -1 prior to dosing with the study drug.

9.2 INCLUSION CRITERIA

All volunteers must satisfy the following criteria to be considered for study participation.

1. Be a healthy male or female between 18 and 65 years of age (inclusive).
2. Voluntarily consents to participate in the study and provides written informed consent before the start of any study-specific procedures.
3. Be willing and able to remain in the study unit for the entire duration of the confinement period and return for outpatient visits.
4. Agree to comply with prohibitions and restrictions (section 9.5).
5. Females must have a negative serum β-human chorionic gonadotropin (hCG) pregnancy test at screening and a negative urine pregnancy test on Day -1 prior to study initiation.
6. Females must be of nonchildbearing potential or agree to use appropriate birth control, as defined in section 9.4 Contraception Requirements.
7. Males must be surgically sterile for at least 90 days before screening or agree to use a condom with spermicide when sexually active with a female partner who is not using an acceptable form of birth control during the study and for 90 days after study administration. Males must also agree to not donate sperm starting at enrollment and for 90 days after last study drug administration.
8. BMI (weight [kg]/[m^2]) between 18 and 35 kg/m^2 (inclusive) and weighs between 50 and 120 kg (110 – 264 pounds), inclusive.
9. Blood pressure (after Subject is in a supine position for approximately 5 minutes) between 90 and 145 mmHg systolic (inclusive) and no higher than 90 mmHg diastolic at Screening and Day -1.
10. A 12-lead ECG with no clinically significant abnormality as judged by the Investigator and QTcf interval ≤ 450 milliseconds at Screening and Day -1.
11. Resting pulse rate between 45 and 100 beats per minute at Screening and Day -1.
12. Clinical laboratory findings and VS within normal range, or if outside of the normal ranges, deemed not clinically significant in the opinion of the Investigator.
13. Agree to comply with the rules regarding consumption of alcohol, caffeinated beverages, and tobacco/nicotine products during the study.

9.3 EXCLUSION CRITERIA

All volunteers who do not satisfy the following criteria will be excluded from study participation.

1. History or presence of clinically significant medical illness including (but not limited to) hepatic, cardiovascular, pulmonary, renal, hematologic, endocrine, gastrointestinal, immunologic, dermatologic, neurologic, oncologic, or psychiatric disease that in the opinion of the Investigator would endanger the safety of the Subject or the validity of the study results.
2. Clinically significant acute illness in the 2 weeks prior to dosing.
3. Previous or current participation in any clinical study with an investigational drug, device, or biologic within 30 days or five half-lives of the investigational product to dosing.
4. Preplanned surgery or procedures that would interfere with the conduct of the study.
5. History of severe drug or excipient allergy, or hypersensitivity to be judged at the discretion of the Investigator.
6. Donation or loss of greater than 0.5 L of blood within 90 days before screening or study start. Donation of platelets within 40 days before screening or study start. Donation of plasma within 14 days before screening or study start. Receipt of blood products within 60 days before screening or study start.
7. Recent history (2 years) of alcohol or drug abuse at the discretion of the Investigator or a positive screen for alcohol or drugs of abuse (including marijuana) at screening and upon check-in.
8. Testing positive for hepatitis B, hepatitis C, or HIV, or a history of any of these diseases. Subjects whose results are compatible with prior immunization may be included at the discretion of the Investigator.
9. History of unexplained loss of consciousness, epilepsy, or other seizure disorders, or cerebrovascular disease.
10. Malignancy within 5 years of screening visit (except basal cell or squamous cell skin carcinoma).
11. Inability to adhere to the study unit diet.
12. Use of any prescription or nonprescription medication (including vitamins, herbal preparations, and nutritional supplements) within the 14 days prior to dosing except for common analgesics (acetaminophen, ibuprofen), hormonal contraceptives or hormonal replacement therapy or nonsedating antihistamines. Topical medications may be allowed at the discretion of the Investigator.
13. History or current diagnosis of mental illness including (but not limited to) psychotic disorder, bipolar disorder, schizophrenia, borderline personality disorder, and antisocial personality disorder, generalized anxiety disorder, obsessive compulsive disorder, posttraumatic stress disorder, and eating disorders.

14. History of suicidal or homicidal ideation.
15. Significant primary sleep disorder.
16. Known allergy to ketamine, heparin, or any of the IDP components (see section 11.0 Study Drug Information).
17. Any strenuous exercise in the 2 days prior to study drug administration.
18. Consumption of beverages or food that contain alcohol, grapefruit, poppy seeds, Brussel sprouts, pomegranate, broccoli, char-grilled meat within 2 days prior to drug administration. Allowances for a single isolated incidental consumption may be evaluated and approved pending PI approval for the potential interactions.
19. Use of tobacco or nicotine-containing products within 4 weeks prior to drug administration.
20. Employee of the PI or study center with direct involvement in the proposed study or other studies under the direction of the study PI.
21. Poor peripheral venous access.
22. Close relative (parent, sibling, child) of clinical site employee.
23. Subjects who, in the opinion of the PI or designee, should not participate in this study.

9.4 FERTILITY AND CONTRACEPTION REQUIREMENTS

Females must be of nonchildbearing potential, defined as;

1. Surgically sterile for at least 180 days before study initiation.
2. Postmenopausal for at least 1 year before study initiation. Subjects claiming postmenopausal status will have a Follicle Stimulating Hormone (FSH) test performed at screening to confirm postmenopausal status.

-OR-

Females of childbearing potential must agree to use an allowable form of birth control from screening until 90 days after end-of-study. The following are allowed birth control methods:

1. Implanted or intrauterine hormonal contraceptive use for at least 180 continuous days before drug administration.
2. Oral, path, or injected contraceptives, or vaginal hormonal device in use for at least 90 continuous days before drug administration.
3. Intrauterine device.
4. Vasectomized partner (at least 90 days before drug administration).
5. Nonsurgical permanent sterilization (at least 90 days before drug administration).
6. Double barrier method (for example, diaphragm with spermicide, condoms with spermicide).

7. Abstinence (and agreement to use double barrier method if they become sexually active during the above time).

Male Subjects must agree to one of the following birth control methods from screening to 90 days after end-of-study.

1. Be surgically sterile for at least 90 days before screening.
2. Agree to use a condom with spermicide when sexually active with a female partner who is not using an acceptable method of birth control.
3. Abstinence (with agreement to use a condom with spermicide if they become sexually active during the study).

9.5 PROHIBITIONS AND RESTRICTIONS

Potential Subjects must be willing to adhere to the following prohibitions and restrictions during the study to be eligible for participation.

1. Subjects must remain at the study center until 24 hours after drug administration (SAD) or 24 hours after completion of the final drug administration (MAD).
2. Subjects must continue using an appropriate method of birth control (see section 9.4 Contraception Requirements).
3. Subjects must avoid excess daily consumption of methylxanthine-containing products (eg chocolate, coffee, caffeinated teas or colas) during the confinement period of the study. Excess consumption is defined as >1 serving of chocolate (1.55 ounces), 3 or more cups (0.7 liters) of coffee or caffeinated teas or colas.
4. Subjects must consume standard institutional meals during the confinement period of the study. Excessive food consumption is not permitted.
5. Subjects are not permitted to use tobacco or nicotine containing products including (but not limited to) cigarettes, e-cigarettes, cigars, bidis, kreteks, pipes, chewing tobacco, snuff, and dip during the confinement period of the study.
6. Subjects are not permitted to use drugs of abuse including (but not limited to) alcohol, cannabinoids, opiates, cocaine, amphetamines, benzodiazepines, hallucinogens or barbiturates during the confinement period of the study.
7. Subjects are not permitted to perform strenuous activity or exercise during the confinement period of the study and for 48 hours after completion of the final drug administration.
8. Subjects must be advised not to donate blood for at least 90 days after completion of the study or to participate in any investigational drug study for at least 90 days after completion of the study.

10.0 STUDY WITHDRAWAL, COMPLETION, ESCALATION, AND STOPPING CRITERIA

10.1 WITHDRAWAL

A Subject will be withdrawn from the study for any of the following reasons.

1. Withdrawal of consent.
2. Noncompliance with any of the study requirements including failure to meet eligibility criteria or violation of any of the listed prohibitions and/or restrictions.
3. Discontinuation of study treatment for any reason.
4. Subject is lost to follow-up.

Study drug assigned to the withdrawn Subject may not be assigned to another Subject and must be disposed of according to the instructions (see Drug Product Accountability).

10.2 REPLACEMENT

Subjects who withdraw before receiving the study drug will be replaced. Following dosing, 1 subject per cohort may withdraw before completion and may or may not be replaced, at the discretion of the Investigator. Should more than 1 subject from the same cohort voluntarily withdraw from the study following dosing, those subjects will be replaced unless withdrawal was due to a safety or tolerability issue. Subjects who received any amount of the study drug and who withdraw or are withdrawn from the study will be encouraged to continue follow-up (with subjects' consent) for safety. Subjects withdrawing will be asked to complete an early termination visit if they do not wish to be followed per protocol. Any decision to replace a subject who drops out after receiving the study drug will be documented in the electronic data capture (EDC) system on an electronic case report form (CRF).

10.3 ESCALATION, CONTINUATION, AND STOPPING RULES

Criteria surrounding sentinel halting, study halting and/or dose escalation within the SAD portion of the study and study halting and/or dose continuation within the MAD portion of the study will governed by the following criteria.

<u>Study Halting Rules</u>

If any of the following events occur, the study will be stopped:

- Any subject develops an SAE through the last study visit.
- 2 or more subjects in any cohort experience a Grade 3 (severe) AE, including unsolicited clinical, ECG, VS, and laboratory AEs, that is of the same type (preferred term) and occurring in the same SOC.
- Any subject develops an episode of anaphylaxis within 24 hours after receiving the study drug.

Dose Escalation Halting Rules

The PI must review the following criteria before dose escalation:

- SAD: A review of all available safety data for the cohort to Day 3 demonstrates that the study halting criteria described below have not been met.
- MAD: A review of all available safety data to Day 12, at a minimum, demonstrates that the dose-escalation halting criteria described below have not been met.

If any of the following dose escalation halting rules are met in either the SAD or MAD parts, escalation to the next planned dose cohort will not proceed until all available study data have been reviewed by the PI, the Medical Monitor and the Study Sponsor:

- Any subject develops an SAE.
- Any subject experiences a Grade 3 (severe) AE.
- 2 or more subjects in a cohort experience a Grade 2 (moderate) or greater AE that is of the same type (preferred term) and occurring in the same SOC.

Individual Halting Rules

The following individual halting rules pertain to the MAD study only:

- Any SAE.
- Any other condition that the site PI judges to unduly increase the risk to the subject.

Sentinel Halting Rules

The following are SAD sentinel halting criteria, ie, if any are met, the rest of the cohort will not be dosed until reviewed by the PI, the Medical Monitor and the Study Sponsor:

- Any subject develops a Grade 3 (severe) AE within 24 hours of study drug administration.

If stopping rules are triggered for any reason, the study PI will notify the Medical Monitor and the Study Sponsor and initiate a review that may include unblinding of treatment assignments. A summary of the decision making for all halts will be documented and submitted to the IRB.

In the event that any of the stopping criteria are triggered, the PI in consultation with the Medical Monitor and Study Sponsor may take one of the following courses of action.

1. Temporarily halt dosing while the event is investigated.
2. Declare the prior tested dose level as the MTD.
3. Recommend repeat dosing at the same level.
4. Recommend dosing at an intermediate level.
5. Recommend protocol amendment to increase Subject safety.
6. Discontinue the study.

11.0 STUDY DRUG INFORMATION

11.1 DRUG PRODUCT IDENTITY, MANUFACTURE, SHIPMENT, AND RECEIVING

The Clinical Trial Materials include the IDP, buffered vehicle, and placebo. The IDP is a solution-based formulation of (2R,6R)-Hydroxynorketamine Hydrochloride. The diluting vehicle is a 25 mM sodium phosphate (pH 7) buffer in 0.9% (normal) saline solution. The placebo is 0.9% (normal) saline. The IDP will be manufactured by a Good Manufacturing Practice (GMP) certified contract manufacturing organization (CMO) as a 10 mg/mL (free base equivalent) sterile solution using a 25 mM sodium phosphate (pH 7) buffer in 0.9% (normal) saline. The buffered vehicle is sterile solution using a 25 mM sodium phosphate (pH 7) buffer in 0.9% (normal) saline and will be manufactured by a GMP certified CMO. Placebo will be sourced by the Duke Clinical Research Institute (DCRI).

The IDP will be manufactured, shipped, and stored in individual 100 mL (total volume) Type I clear glass vials containing a total volume of 55 mL of IDP. The IDP will be labeled according to FDA regulatory requirements.

The buffered vehicle will be manufactured, shipped, and stored in individual 100 mL (total volume) Type I sterile glass vials. The buffered vehicle will be labeled according to FDA regulatory requirements.

11.2 INVESTIGATIONAL DRUG PRODUCT, FORMULANT, AND PLACEBO PHYSICAL DESCRIPTION

The IDP, formulant, and placebo are all clear, colorless solutions.

11.3 INVESTIGATIONAL DRUG PRODUCT AND PLACEBO PREPARATION AND QUALITY CONTROL

The stock IDP (ie, the 10 mg/mL solution) and buffered vehicle will be manufactured and shipped by a GMP certified CMO. Dilution of the IDP to the clinically used dose using the buffered vehicle will be performed by the pharmacy staff at the clinical site as described in section 12.7 and the Pharmacy Manual. Placebo will be sourced by the Duke Clinical Research Institute (DCRI).

11.4 INVESTIGATIONAL DRUG PRODUCT, FORMULANT AND PLACEBO ACCOUNTABILITY

The PI is responsible for ensuring that all study drug, vehicle buffer, and placebo at the clinical study facility is accounted for throughout the study (ie, inventoried, stored, used and disposed of according to set procedures). The dispensing of study drug to the Subject must be documented on a designated drug accountability form. All IDP and placebo containers and administration components must be returned to the pharmacy following administration for reconciliation and disposition. Unused study drug must be disposed of according to clinic site procedures.

Study drug, buffer, and placebo must be handled in strict accordance with the protocol, pharmacy manual, and container labels and must be stored in limited access area under the defined environmental conditions. Unused study drug must not be disposed of until the PI or his/her designee has completed drug accounting per a preset schedule.

11.5 INVESTIGATIONAL DRUG PRODUCT QUALITY COMPLAINT PROCEDURE

A product quality complaint (PQC) is defined as any suspicion of a product defect related to manufacturing, labeling, or packaging, ie, any dissatisfaction relative to the identity, quality, durability, or reliability of a product, including its labeling or package integrity. The PQCs may have an impact on the safety and efficacy of the product. Timely, accurate, and complete reporting and analysis of PQC information from clinical studies are crucial for the protection of Subjects, Investigators, and the sponsor, and are mandated by regulatory agencies worldwide. The sponsor has established procedures in conformity with regulatory requirements to ensure appropriate reporting of PQC information.

12.0 STUDY PROCEDURES AND EVALUATIONS

The Time and Events Schedule for the SAD study (Table 1), MAD study (Table 2), and CSF Capture study (Table 3) summarizes all key procedures and evaluations.

12.1 STUDY SPECIFIC MATERIALS

The following study specific materials will be supplied to the clinical site by the Study Sponsor.

Investigator's Brochure
Package Insert for Investigational Drug Product
Manual of Procedures (MOP) including:
- Pharmacy Manual/Site Investigator Product Manual
- Laboratory Manual
- Electronic Data Capture Manual
- qEEG Manual
- Statistical Analysis Plan (safety)
- Statistical Analysis Plan (PK)
- Statistical Analysis Plan (qEEG)

Investigational Drug Product/Placebo Container Labels
Pharmacokinetics Labels
C-SSRS and CADSS Questionnaires
Investigational Drug product: (2R,6R)-Hydroxynorketamine Hydrochloride
Formulant: 25 mM sodium phosphate (pH 7) buffer in 0.9% (normal) saline
Placebo: 0.9% (normal) saline
Diluting Vials: 100 mL (total volume) sterile ALK vial
Diluting Syringes: 1 mL sterile Beckton Dickinson Syringes
Diluting Syringes: 60 mL sterile Beckton Dickinson Syringes
Administration Syringes: 60 mL sterile Beckton Dickinson Syringes
Administration Tubing: Single Use Extension Set, 60-inch (152.4 cm) Tubing (APV = 1.1 mL)
Administration Needles: Protect IV® Plus Safety IV Catheters (Radiopaque), 20G X 1"

12.2 SCREENING

The informed consent documents will be discussed with each potential participant and each Subject will sign an informed consent document for the study before any study-specific procedures are performed. Each potential participant will have the following assessments performed by the Investigator or a designee at screening.

1. Medical history, including psychiatric history.
2. Demographic data, including sex, age, race, ethnicity, body weight, height, body mass index (BMI; kg/m^2), and smoking habits.
3. Physical examination.
4. VS.

5. ECG.
6. Clinical laboratory tests.
7. Serology tests.
8. Urine test for drugs of abuse, cotinine.
9. Alcohol breath test.
10. Serum pregnancy (all female Subjects).
11. FSH test (females claiming postmenopausal status).
12. Verbal confirmation that all remaining inclusion/exclusion criteria are met.
13. C-SSRS.
14. Prior and concomitant medication review.

Subjects not meeting eligibility criteria at Screening may be rescreened once at the discretion of the Investigator. Subjects who meet eligibility criteria at Screening and are not dosed in a cohort (eg, alternates or because a cohort is filled) may be rescreened as needed to participate in future cohorts.

12.3 INFORMED CONSENT

Each Subject must give written consent according to local requirements after the nature of the study has been fully explained. The consent form must be signed before performance of any study-related activity. The consent form that is used must be approved by both the sponsor and by the reviewing IRB and be in a language that the Subject can read and understand. The informed consent should be in accordance with Principals that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and sponsor policy.

Before enrollment in the study, the PI or an authorized member of the investigational staff must explain to potential Subjects the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail. Subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the Subject will receive. Finally, they will be told that the Investigator will maintain a Subject identification register for the purposes of long-term follow up if needed and that their records may be accessed by health authorities and authorized sponsor staff without violating the confidentiality of the Subject, to the extent permitted by the applicable law(s) or regulations. By signing the informed consent form, the Subject is authorizing such access, and agrees to allow his or her study physician to recontact the Subject for the purpose of obtaining consent for additional safety evaluations, if needed.

The Subject will be given sufficient time to read the informed consent form and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by means of the Subject's personally dated signature. After having obtained the consent, a copy of the signed informed consent form must be given to the Subject.

12.4 SUBJECT ASSIGNMENT AND CHECK-IN

For the SAD part, 48 Subjects (6 cohorts of 8 Subjects) will be dosed according to the Time and Events Schedule for the SAD study (Table 1). For the MAD part, 16 Subjects (2 cohorts of 8 Subjects) will be dosed according to the Time and Events Schedule for the MAD study (Table 2). For the CSF Capture part, 8 Subjects (1 cohorts of 8 Subjects) will be dosed according to the Time and Events Schedule for the CSF Capture study (Table 3). Additional cohorts may be enrolled after evaluation if it is determined that intermediate or higher dose levels should be tested. The number of Subjects planned is considered sufficient to characterize the safety and PK in a first-in-human clinical trial. Each Subject will receive an assigned treatment based on the randomization schedule prepared by the clinical site.

Subjects in the SAD study will be randomized to receive either a single dose of active drug or a single dose of matching placebo. Subjects in the MAD study will be randomized to receive either a series of 4 doses of active drug or a series of 4 doses of matching placebo. Subjects in the CSF Capture study will be randomized to receive either a single dose of active drug or a single dose of matching placebo.

For Subjects in the SAD study, the maximum duration of the study from screening to end-of-study will be approximately 33 days. For Subjects in the MAD study, the maximum duration of the study from screening to end-of-study will be approximately 52 days. For Subjects in the CSF Capture study, the maximum duration of the study from screening to end-of-study will be approximately 33 days.

For the SAD, MAD and CSF Capture parts, an appropriate number of alternates will be included as judged by the PI. Alternates not used in a cohort may be dosed in future cohorts if eligibility criteria are re-confirmed.

At check-in (Day -1), Subjects will be admitted to the research center at an appropriate time the Day before study drug administration to ensure a minimum 8-hour fast prior to dosing. At check-in, all Subjects will be evaluated to confirm that they continue to meet all the inclusion criteria and none of the exclusion criteria. An alcohol breath test will be performed. A urine sample will be collected to screen for drugs of abuse and cotinine. If at any time an alcohol, cotinine, or drug test is positive, the Subject will be discontinued from study participation. A positive point-of-care drug test may be sent for confirmation to the clinical laboratory at the discretion of the Investigator (eg, suspicion of a spurious value).

All Subjects will be asked to confirm that they still adhere to the contraceptive criteria, and each Subject’s response will be documented.

A urine sample from each female Subject will be collected for a urine pregnancy test at check in. This test must be negative for the Subject to continue in the study. If an assessment is performed at both screening and Day -1, the Day -1 assessment will be considered baseline. The following additional assessments will be performed on Day -1.

1. Eligibility criteria.
2. Weight.
3. Medical history.
4. Physical examination.
5. VS.
6. ECG.
7. Clinical laboratory tests.
8. Urine tests for drugs of abuse and cotinine.
9. Breath alcohol.
10. Ocular examination.
11. Prior and concomitant medication review.
12. Psychiatric/mental health assessment including the POMS, the CADSS and the C-SSRS assessments.

12.5 FASTING, MEALS, AND BEVERAGES

An optional meal, snack, and/or beverage will be served on the evening of check-in. All Subjects will then be required to fast for at least 8 hours before drug infusion, during the drug infusion and for 1 hour after the start of the infusion. The Subjects will be provided a light meal from 1 hour after the start of the infusion to 2 hours after the start of the infusion. Subjects will be allowed up to 240 mL of water from one hour before until one hour after drug treatment. At all other times, Subjects will be allowed to drink water ad libitum.

All other meals will be provided at appropriate times thereafter. Meal/snack menus will be approximately the same for all cohorts.

12.6 RANDOMIZATION AND BLINDING

All Subjects and clinical staff, (except the unblinded pharmacist staff) will be blinded to treatment. An unblinded pharmacist staff will be required at the Clinical Site to comply with the study's randomization and blinding requirements. At the clinical site, prior to study administration, the PI will be responsible for designating a qualified pharmacy staff to service as the unblinded pharmacy staff in the study. Unblinded pharmacy staff may dose Subjects, but may not participate in Subject assessments.

The designated, unblinded pharmacy staff will be responsible for all drug accountability issues, including preparing, labelling, and dispensing study drug and placebo in accordance with the randomization codes provided, yet remain independent of all Subject assessments. The pharmacy staff will follow the Standard Operating Procedures and Work instructions related to pharmacy services and protocol-specific requirements.

Randomization codes will be provided to the unblinded pharmacy staff. Confirmation of receipt of the randomization code will be required by the Sponsor. The unblinded pharmacy staff will be responsible for maintaining the blind, consistent with protocol

design, throughout the study. All documentation is to be filed in the Pharmacy Manual. Access to this manual will be restricted to the unblinded pharmacy staff.

The Subjects, PI, and all other study personnel involved with the Subject assessments will be blinded to the actual treatment assignment of the Subjects. The PI will be ultimately responsible for ensuring the integrity of the blind, and that it is maintained through the study.

The treatment assignment should only be unblinded at the clinic in the case of dose-limiting toxicity or emergency when the knowledge of the study drug assignment is necessary for the clinical management or welfare of the Subject. Breaking the blind at the clinic under any other circumstance will be considered a protocol violation. The PI is strongly encouraged to contact the sponsor before unblinding the study drug assignment. If the blind is broken for any reason, the Investigator must notify the Sponsor within one day. In addition, the Investigator will record the date and reason for revealing the blinded study drug assignment for that Subject in the source documents and appropriate case report form page(s).

12.7 DRUG PREPARATION, DOSING, AND ADMINISTRATION

The IDP and placebo will be prepared according to the Pharmacy Manual (general description below and provided in the Study Drug Information (section 11).

The dose of IDP will be prepared based upon the specific dose level of the cohort and the Subjects body weight. The exact dose will be prepared by diluting the IDP [a 10 mg/mL solution of (2R,6R)-Hydroxynorketamine Hydrochloride] with buffered vehicle [25 mM sodium phosphate (pH 7) buffer in 0.9% (normal) saline].

12.8 SAFETY MONITORING AND REPORTING

Key safety evaluations will include monitoring of physical examinations, clinical laboratory outcomes, VS, ECG, and Subject self-reporting.

Physical examinations will be conducted according to the Time and Events Schedule for the SAD part (Table 1), MAD part (Table 2), and CSF Capture part (Table 3) and will include evaluations of eyes, ears, nose, throat, heart, peripheral vasculature, lungs, musculoskeletal system, abdomen, neurological function, endocrine system, and skin.

Clinical laboratory tests will be conducted according to the Time and Events Schedule for the SAD study (Table 1), MAD study (Table 2), and CSF Capture study (Table 3) and will be inclusive of tests described in Table 8.

VS and ECGs will be performed according to the Time and Events Schedule for the SAD study (Table 1), MAD study (Table 2), and CSF Capture study (Table 3) ±30 minutes.

Table 8: Clinical laboratory evaluations

Hematology	Chemistry	Urinalysis
• Total white blood cell count • Platelet count • Hemoglobin • Hematocrit • Red blood cell count • Basophil count • Eosinophil count • Lymphocyte count • Monocyte count • Neutrophil count • Reticulocyte count	• Aspartate aminotransferase • Alanine aminotransferase • Bilirubin, total • Creatinine • Sodium • Potassium • Glucose • Blood urea nitrogen • Calcium • Alkaline phosphatase • Bilirubin, direct • Albumin • Protein, total • Magnesium • Pregnancy test (serum) • FSH (confirm postmenopausal status)	• pH • Protein • Blood • Ketones • Glucose • Bilirubin • Nitrites • Specific gravity • Leukocytes • Urobilinogen • Urine pregnancy test If any abnormal value is observed on the urine dipstick test, the sample should be further analyzed with urine microscopy: • White blood cell count • Red blood cell count • Cellular casts • Granular casts • Hyaline casts
Coagulation	**Drugs of Abuse (Urine) & Alcohol Testing (Breath)**	**Serology Screen**
• Activated partial thromboplastin time • Prothrombin time • International normalized ratio	• Cannabinoids • Amphetamines • Barbiturates • Cocaine • Opiates • Benzodiazepines • Phencyclidine • Methadone • Cotinine • Alcohol (Breathalyzer)	• Hepatitis B surface antigen • Hepatitis C antibody • Human immunodeficiency virus 1 & 2 antibodies

VS will be monitored according to the Time and Events Schedule for the SAD study (Table 1), MAD study (Table 2), and CSF Capture Study (Table 3) and will include blood pressure, pulse rate, respiration, and temperature. The PI or designee will verify the eligibility of each Subject with out-of-range VS and document approval prior to dosing.

Systemic blood pressure and pulse will be continuously monitored throughout drug administration and for 2 hours after the conclusion of study drug infusion. Monitoring of these parameters will be accomplished with a correct-sized finger cuff placed on either the index or middle finger and the Nexfin noninvasive continuous blood pressure monitor.

The ECG measurements, collected in triplicate, will be monitored using a 12-lead ECG signal according to the Time and Events Schedule for the SAD study (Table 1), MAD study (Table 2) and CSF Capture study (Table 3). During the collection of the baseline ECGs, Subjects should be in a quiet setting without distractions (eg, television or cell phones). Subjects should rest in a recumbent position for at least 5 minutes before ECG collection and should refrain from talking or moving arms or legs. Baseline ECG recordings should be taken at a time independent of blood sampling or VS measurements.

Subjects will be instructed to tell the study physician and/or research personnel of any AEs that occur at any time during the study. Subjects will be monitored for AEs from dose administration through the end-of-study.

For both the SAD and MAD parts, each Subject's level of alertness/sedation and mental state will be examined using 4 standard tests (ie, the MOAA/S, the POMS, the C-SSRS and the CADSS). The MOAA/S is a scale developed to measure the level of alertness in subjects who may be sedated (see Appendix #1). The POMS is a psychological rating scale used to assess transient, distinct mood states (see Appendix #2). The C-SSRS is a low-burden measure of the spectrum of suicidal ideation and behavior that was developed to assess severity and track suicidal events through any treatment (see Appendix #3). The CADSS is an instrument for the measurement of present-state dissociative symptoms (see Appendix #4). For the CSF Capture study, only the POMS and C-SSRS surveys will be administered.

These measures will be completed according to the Time and Events Schedule for the SAD part (Table 1), MAD part (Table 2) and CSF Capture part (Table 3).

Medical emergency personnel trained in advanced cardiac life support will be on site to monitor Subjects during the confinement period in the research center. Emergency medical equipment including but not limited to intubation equipment and pulse oximetry shall be maintained on site to administer appropriate medical care if required. Procedures will be completed as specified in this protocol unless contraindicated due to a reported AE.

12.9 ADVERSE EVENTS MONITORING AND REPORTING

Subjects will be monitored for any AEs from study drug administration until the end-of-study. These will include a full assessment of abuse-related AEs. The Investigator or designee will review each event. The Investigator or designee will assess its relationship to the study drug. Each sign or symptom will be graded for severity and the date and time of onset, cessation, and resolution will be recorded. Treatment of any adverse reactions will be evaluated and managed by the PI, a designated physician or appropriate emergency responders, as appropriate. All nonserious AEs will be reported on a regular basis, or as specified by the Sponsor.

An AE is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can be any unfavorable or unintended sign, symptom, or disease temporally associated with the use of a drug, without judgement to causality. An AE can arise from any use of the drug and from any route of administration, formulation, or dose, including an overdose.

A life-threatening AE/life-threatening suspected adverse reaction, in the view of either the Investigator or Sponsor, places the Subject at immediate risk of death. It does not include an adverse reaction that, if it had occurred in a more severe form, may have caused death.

A serious adverse event (SAE) or serious suspected adverse reaction in the view of either the Sponsor or the PI, results in any of the following outcomes.

1. Death. The death was the outcome of the AE. Date will be included, if known.
2. Life-threatening AE. The Subject was at substantial risk of dying at the time of the AE, or continued use of the Clinical Trial Material may have resulted in the death of the patient.
3. Inpatient hospitalization or prolongation of existing hospitalization. Emergency room visits that don't result in admission to the hospital should be evaluated for other serious outcomes.
4. Disability or permanent damage. A substantial disruption in a person's ability to conduct normal life function. For example, the AE resulted in a significant, persistent, or permanent change, impairment, damage, or disruption in the patient's body function, body structure, physical activities, and/or quality of life.
5. Congenital anomaly/birth defect. Exposure to Clinical Trial Material prior to conception or pregnancy that may have resulted in adverse outcomes for the child.
6. Other serious and important medical events. Events that do not fall into the above categories but may jeopardize the patient and may require medical or surgical intervention to prevent other outcomes. For example, allergic bronchospasms requiring emergency room treatment, seizures, or convulsions

that do not result in hospitalization, or the development of drug dependence or drug abuse.

All serious adverse event (SAE) reporting will adhere to the U.S. Code of Federal Regulations (21 CFR Part 312.32) for IND drugs. The PI will immediately report any SAEs to the sponsor, whether or not these are considered study intervention-related, including those listed in the protocol or investigator brochure; any such report must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. The PI or designee must send all SAEs on a MedWatch form to the Sponsor, copying the REGSupportORSC@nih.gov inbox at the time of awareness (within 24 hours for deaths, within 48 hours for all other SAEs). Additional information to include in the MedWatch Form 3500A is as follows:

- IND Number
- Protocol Number
- Principal Investigator's Name
- Event Attribution

The sponsor or designee will be responsible for determining if an SAE is reportable to the FDA as an IND Safety Report, i.e. the event is considered a serious unexpected suspected adverse reaction (SUSAR). The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) or other applicable regulatory agency, of any unexpected fatal or life-threatening suspected AE as soon as possible, but in no case later than seven calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify the FDA and all participating investigators of an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

All SAEs will be followed until satisfactory resolution. All AEs will be documented on appropriate study records, including case report forms and AE tracking logs. All AEs will be provided in aggregate to the sponsor upon request and no less than once a year.

An unexpected AE is defined as an AE or suspected adverse reaction that is not listed on the PI's Brochure or is not listed at the severity indicated. If the IB is not available, an unexpected AE is one that is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

The PI or designee will notify the appropriate sponsor contact immediately after SAE detection, observation, or report of occurrence, regardless of relationship to test article.

Serious AE reports should contain the following details.

A. Study name/number.
B. Study Drug.
C. Investigator details (name, phone, e-mail).
D. Subject numbers.

E. Subject initials.
F. Subject Demographics.
G. Clinical Event.
    a. Description.
    b. Date of Onset.
    c. Treatment (Drug, dose, form).
    d. AE relationship to study drug.
    e. Action taken regarding study drug in direct relationship to the AE.
H. If the AE was fatal or life threatening.
I. If applicable, cause of death (including whether or not the death is related to study drug).
J. If applicable, autopsy findings (if available).

In the event of an AE or SAE, the following safety measurements will be performed and recorded.

A. Sensory test (pinprick and vibration)
B. Motor examination, reflexes and assessment of coordination (neurological exam)
C. Vital signs
D. Additional blood and/or CSF samples may be obtained, at the discretion of the investigator.
E. Day 30 (+/- 3 days) phone follow up.

Any new SAE that occurs within one month after the last dosing in the study and is considered to be related to the investigational product should be recorded and reported immediately to the Sponsor. The person responsible for this study shall take care that this study has been carried out in accordance with pharmacovigilance local regulations.

All serious AE reporting will adhere to the U.S. Code of Federal Regulations (21 CFR Part 312.32) for IND drugs (15-Day alerts). The IRB will be notified of the alert reports per FDA regulations. All AEs including SAEs will be followed to resolution when possible. All AEs and treatment administered will be recorded on the case report form. The sponsor will be responsible for reporting and processing any SAEs to the FDA or other applicable regulatory agency.

Abuse-related AEs will be reported separately and include a case narrative. Case narratives will include the time of onset and duration of the event, dose of drug taken, severity, and outcomes. A pharmacokinetic analysis will also be performed to assess if an abuse-related AE correlates with drug exposure.

The relationship between the AE and investigational product (IP) will be determined by the PI based on his or her clinical judgement and the following definitions.

Related: The AE follows a reasonable temporal sequence from the study product administration and cannot be reasonably explained by the Subject's clinical state or other

factors. The AE follows a reasonable temporal sequence from the study product administration and represents a known reaction to the drug under study or other drugs in its class or is predicted by the known pharmacological properties of the drug.

Not Related: The AE does not follow a reasonable temporal sequence from study product administration or can be reasonably explained by the Subject's clinical state or other factors.

Pregnancies: Pregnancies will be documented and followed until birth or another outcome. Pregnancies will not be reported as AEs. However, if at any time the pregnancy outcome falls under the scope and definition of a SAE, it will be reported as such.

12.10 QUANTITATIVE ELECTROENCEPHALOGRAM (qEEG) MONITORING AND REPORTING

Scalp-recorded EEG will be collected on all Subjects in the SAD study for approximately 1 hour immediately preinfusion, for 1 hour after the start of the infusion and for a subsequent hour following a 1 hour break (ie, collecting EEG data during hours 2→3 after the start of the infusion; total of 3 hours recording after the start of dosing). During this time, there will be three, 10-minute intervals specifically dedicated to collecting 'clean' resting-state (5 minutes) and visual-evoked-potential (5 minutes). The remainder of the session will entail passive recording of the EEG during other study activities.

High-definition EEG will be acquired through a 64-channel Brain Products actiCHamp systems using active electrodes positioned according to an equidistant spacing, covering the whole head from slightly above the eyebrows to below the inion. The EEG leads will include horizontal and vertical electrooculography, which will be used to identify and remove periods of ocular artifact during postprocessing of the data. The EEG leads will be placed in a nylon fabric EasyCap. Prior to recording data, a thorough impedance check of the electrodes will be done to ensure high-quality recording. During the drug infusion, subjects will remain in a recumbent position with their head slightly raised. Approximately 40 minutes prior to infusion, as well as at approximately 40 and 160 minutes after the start of the infusion, participants will perform 10 minutes of dedicated EEG activities. To begin, the subject will be cued to undergo a 5-minute routine in which they stay comfortably in a recumbent position with their eyes closed and relax. This interval will allow for capture of "clean intervals" from which to derive time-frequency, and spectral power measures. Immediately following this, subjects will perform a brief, 5-minute visual evoked potential paradigm in which they passively view a contrast-reversing checkerboard stimulus alternating pseudo-randomly at approximately 0.5 Hz.

Subjects will spend the bulk of the remainder of the EEG recording time in a recumbent position. In the event subjects must leave the bed, the recording file will be paused, the EEG head cap will remain on the participant's head while cables that connect to the amplifier will be ejected and clipped to the participant's shirt to allow free movement. Once the participant returns, the cables will be inserted back into the amplifier, a brief impedance check will be done to ensure high-quality recording, and recording will then continue. Additional details of the EEG are in the MOP.

The National Institutes of Health Experimental Therapeutics and Pathophysiology Branch will receive the collected data. The data will undergo epoch-by-epoch visual artifact correction/rejection. Quantitative EEG analysis will only be carried out if a minimum of 30 seconds of waking, artifact free data are available for any resting-state scan. The data will undergo Laplacian transformation to produce current density estimates. The current density data will undergo spectral analysis via Fast Fourier Transform (FFT) with boxcar windowing to generate power estimates in the following frequency bands: delta (2-7Hz), theta (7-9Hz), alpha (9-12Hz), beta (12-30Hz), and gamma (30-60Hz). This will be carried out with the LORETA software. At each voxel (n=2394; voxel resolution=7 mm3), current density will be computed as the squared magnitude of the intracerebral current density within each of the 5 frequency bands (unit: amperes per square meter, A/m^2). Current density estimates will be intensity-normalized to unity and log-transformed before statistical analyses. This will be carried out in 2-second segments and averaged over the available artifact-free, waking data. These data will be used to estimate resting-state EEG current density. The primary qEEG outcome will be EEG gamma current density. Resting state EEG current density estimates in other frequency bands will be analyzed to evaluate the specificity of possible findings. Exploratory analysis for patterns of EEG activity that are predictors and correlates of outcome will be carried out using EEG power in all bands derived from all the electrodes, employing principal components analysis.

12.11 BLOOD AND CSF DRAWS FOR CLINICAL LABORATORY AND PHARMACOKINETIC TESTING

Blood and CSF draws for serial PK and clinical laboratory testing will be collected at defined timepoints within the SAD, MAD and CSF Capture parts according to the schedule of assessments and procedures (Tables 1, 2 and 3, respectively) and as described in section 8.6.

Blood will be stored and shipped to a LabCorp (Covance) central laboratory who will perform PK analysis of (2R,6R)-Hydroxynorketamine using a LC/MS/MS method to capture the following parameters: C_{max}, t_{max}, C_{min}, $AUC_{0-\infty}$, AUC_{0-Tau}, CL, V_z, CL_R, $t_{1/2}$. CSF samples will be analyzed at NIH using an alternate LC/MS/MS method assessing total (2R,6R)-Hydroxynorketamine concentrations at 3 collection timepoints (predose, 1-hr postdose and 8-hr postdose).

Subjects must have consented to participate before the screening blood draw. Blood draws collected for PK *and* clinical laboratory testing will be comprised of a total of approximately 16 mL from each Subject. Blood draws collected *only* for clinical laboratory testing will be comprised of a total of approximately 11 mL from each Subject. Blood draws collected for *only* PK testing will be comprised of a total of approximately 5 mL from each Subject. The maximum amount of blood draw (cumulatively) for any Subject will be approximately 219 mL.

Blood draws collected for clinical laboratory testing will include 4.5 mL for chemistry panel, 4 mL for hematology panel, 2.7 mL for coagulation panel, and 8.5 mL for serology

panel (see Table 9-11). Blood draws collected for PK will include approximately 5 mL for primary and repeat analysis of the drug substance [(2R,6R)-Hydroxynorketamine] and remaining samples will be stored.

CSF draws will be comprised of a total of approximately 10 mL from each subject and will consist of 3.5 mL for both metabolomics and proteomics and 3 mL for PK (Table 12). Subjects will have a spinal catheter placed for sample collection. Spinal catheter placement, sample collection and storage details will be provided in the MOP.

Table 9: Blood draw volumes and uses for SAD study

Sample	Total Volume (mL)	Clin. Lab. Volume (mL)	PK Volume (mL)
Screening Draw	20	20	NA
Day -1 Draw	11	11	NA
Day 1 Draw (preinfusion)	16	11	5
Day 1 Draw (end-of-infusion)	5	NA	5
Day 1 Draw (1-hour after the start of the infusion)	5	NA	5
Day 1 Draw (2-hour after the start of the infusion)	5	NA	5
Day 1 Draw (4-hour after the start of the infusion)	5	NA	5
Day 1 Draw (8-hour after the start of the infusion)	5	NA	5
Day 1 Draw (12-hour after the start of the infusion)	5	NA	5
Day 2 Draw (24-hour after the start of the infusion)	16	11	5
Day 3 Draw (48-hour after the start of the infusion)	16	11	5
Total Blood Draw	109		

Table 10: Blood draw volumes and uses for MAD study

Sample	Total Volume (mL)	Clin. Lab. Volume (mL)	PK Volume (mL)
Screening Draw	20	20	NA
Day -1 Draw	11	11	NA
Days 1, 4, 8, 11 Draw (preinfusion)	64 (4 × 16)	44 (4 × 11)	20 (4 × 5)

Days 1, 4, 8, 11 Draw (end-of-infusion)	20 (4 × 5)	NA	20 (4 × 5)
Days 1, 11 Draw (1 hour after the start of the infusion)	10 (2 × 5)	NA	10 (2 × 5)
Days 1, 11 Draw (2 hours after the start of the infusion)	10 (2 × 5)	NA	10 (2 × 5)
Days 1, 11 Draw (4 hours after the start of the infusion)	10 (2 × 5)	NA	10 (2 × 5)
Days 1, 11 Draw (8 hours after the start of the infusion)	10 (2 × 5)	NA	10 (2 × 5)
Days 1, 11 Draw (12 hours after the start of the infusion)	10 (2 × 5)	NA	10 (2 × 5)
Days 2, 11 Draw (24 hours after the start of the infusion)	32 (2 x 16)	22 (2 x 11)	10 (2 x 5)
Total Blood Draw	197		

Table 11: Blood draw volumes and uses for CSF Capture study

Sample	Total Volume (mL)	Clin. Lab. Volume (mL)	PK Volume (mL)
Screening Draw	20	20	NA
Day -1 Draw	11	11	NA
Day 1 Draw (preinfusion)	16	11	5
Day 1 Draw (end-of-infusion)	5	NA	5
Day 1 Draw (1-hour after the start of the infusion)	5	NA	5
Day 1 Draw (2-hour after the start of the infusion)	5	NA	5
Day 1 Draw (4-hour after the start of the infusion)	5	NA	5
Day 1 Draw (8-hour after the start of the infusion)	5	NA	5
Day 1 Draw (12-hour after the start of the infusion)	5	NA	5
Day 2 Draw (24-hour after the start of the infusion)	16	11	5
Total Blood Draw	93		

Table 12: CSF draw volumes and uses for CSF Capture study

Sample	Total Volume (mL)	Proteomics Volume (mL)	Metabolomics Volume (mL)	PK Volume (mL)
Day 1 Draw (preinfusion)	10	3.5	3.5	3
Day 1 Draw (end-of-infusion)	10	3.5	3.5	3
Day 1 Draw (8-hour after the start of the infusion)	10	3.5	3.5	3
Total CSF Draw	30	10.5	10.5	9

12.12 BLOOD AND CSF DRAW SAMPLE COLLECTION, HANDLING, SHIPPING, AND RECEIVING

Blood and CSF draws collected for clinical laboratory analysis and for PK will be collected, labeled, inventoried, stored, and shipped as described in the detailed in the MOP.

12.13 URINE COLLECTION, HANDLING, SHIPPING, AND RECEIVING

Urine samples collected for clinical laboratory analysis and for PK will be collected, labeled, inventoried, stored, and shipped as described in the detailed in the MOP.

12.14 PHARMACOKINETIC ANALYTICAL PROCEDURES

All blood samples will be processed to plasma for analysis. Analytical procedures will conform to a predetermined bioanalytical method.

12.15 PHARMACOKINETIC PARAMETERS

Blood samples will be analyzed for (2R,6R)-Hydroxynorketamine levels using an LC/MS/MS analytical method to determine plasma concentration levels in order to calculate the following PK parameters: C_{max}, t_{max}, C_{min}, $AUC_{0-\infty}$, AUC_{0-Tau}, CL, V_z, CL_R, $t_{1/2}$.

12.16 SUBJECT RELEASE, FOLLOW-UP, AND STUDY CLOSE

All Subjects will be released from study confinement according to the Time and Events Schedule for the SAD study (Table 1), MAD study (Table 2) and CSF Capture study (Table 3) following a final consultation with the PI or a designee. All Subjects are required to conform to all follow-up visits or phone calls according to the Time and Events Schedule for the SAD study (Table 1), MAD study (Table 2) and CSF Capture study (Table 3). A Subject will be considered to have completed the study if he or she completes the final blood draw collected for clinical laboratory analysis and for PK.

13.0 STATISTICAL ANALYSIS

Detailed summaries of the statistical analysis plan for 1) all safety data; 2) all PK; and 3) all qEEG data will be prospectively described in a Statistical Analysis Plan section of the MOP.

13.1 SAFETY DATA

All investigational product and protocol procedure related AEs will be listed, and if the frequency of events allows, safety data will be summarized using descriptive methodology.

Each incidence of investigational product and protocol procedure related AEs will be presented by severity and by association with investigational product as perceived by the PI. Symptoms/AEs reported to occur prior to study entry will be distinguished from those reported as new or increased in severity during the study. All AEs and symptoms will be classified by the most suitable term using the MedDRA and summarized by SOC and preferred term, by severity, by relationship to study drug and study procedure, and by study drug dose.

The number of investigational product-related SAEs will be reported.

Safety parameters that will be assessed include clinical lab parameters, VS, and ECG parameters. The parameters will be listed and summarized using standard descriptive statistics. Additional analysis will be performed if warranted upon review of the data.

13.2 PHARMACOKINETICS DATA

Pharmacokinetic parameter estimates will be calculated by standard noncompartmental methods of analysis.

The following PK parameters will be calculated as appropriate and if possible depending on actual samples collected: C_{max}, t_{max}, C_{min} at the end of the dosing interval after each dose in the MAD study, area under the plasma concentration-time curve from 0 to time of last measurable sample ($AUC_{0\text{-}last}$) and area under the plasma concentration-time curve from 0 to infinity ($AUC_{0\text{-}\infty}$) in the SAD study, area under the plasma concentration-time curve during the dosing interval ($AUC_{0\text{-}Tau}$) after the first dose and the last dose in the MAD study, CL, CL_R, V_z, $t_{1/2}$, and accumulation ratio for C_{max} (RC_{max}) and $AUC_{(0\text{-}\infty)}$ [$RAUC_{(0\text{-}\infty)}$].

Renal clearance will be calculated as the ratio of amount excreted/AUC. This will be compared to the unbound glomerular filtration rate, which is estimated using creatinine clearance.

Dose proportionality will be evaluated using a regression analysis model to assess the linear relationship between the exposure parameters (C_{max} and $AUC_{0\text{-}\infty}$ or $AUC_{0\text{-}last}$ from the SAD study; C_{max} and $AUC_{0\text{-}Tau}$ from the MAD study) and dose as a covariate after

log-transformation (Ln) of the data, according to the equation Ln (exposure parameter) = $\beta 0 + \beta 1 \times$ Ln (dose); dose proportionality will be established if the estimated values of the slope ($\beta 1$) are close to 1 and the 95% confidence intervals for the dose-dependent parameters include the value of 1 (12).

An assessment of steady state will be performed using trough concentrations (C_{min}) from days 4, 7, 10, and 12 in the MAD study. The ratio of geometric mean of C_{min} on each corresponding day, divided by the geometric mean of the pooled C_{min} from the following days will be calculated according to established methods (13, 14). The first assessment timepoint with a calculated C_{min} ratio that does not statistically differ from a value of 1 will be defined as the timepoint at which steady state is achieved (13, 14).

An interim PK analysis is planned after cohorts 1 and 2 of the SAD study to assess drug exposures. Additional interim PK analyses may be performed based on the results of the interim analyses performed following cohorts 1 and 2. Details of the interim PK analysis will be described in the PK Analysis Plan**.**

13.3 qEEG DATA

Quantitative EEG (qEEG) data will be obtained for all study subjects in the SAD cohorts. This data will be collected over approximately 3 hours with predefined resting-state and visual evoked potential intervals. Analysis of the EEG data will include preliminary artifact correction using PCA-based techniques to identify biological artifacts that are not attributed to cortical activity. This will include scrutiny of the component spectral and spatial profiles, ultimately eliminating components that present with nontypical cortical activity. The qEEG parameters that will be studied include the ones discussed below. Other qEEG parameters may be evaluated postacquisition and will be described in the Statistical Analysis Plan.

<u>Relative spectral power</u>
An FFT analysis will be performed on the raw EEG. From this FFT analysis, the absolute and relative power spectrum will be determined across all frequency bands (delta, theta, alpha, beta and gamma).

<u>Visual evoked time-locked data</u>
Analysis of sensory processing will be conducted through consideration of the visual evoked potential to a contrast reversing checkerboard. Time-locked, event-related potential will be calculated on the continuous EEG collected during the visual stimulation allowing for high temporal resolution of signals from the primary and secondary visual cortices. Past research has linked these potential to gain control mechanisms in the brain, and analysis of the amplitude and latency effects of infusion will be considered in this context.

Source localization

Frequency-specific and time-locked source localization will be performed using sLORETA within regions-of-interest specifying Default Mode, Central Executive, and Somatosensory networks.

14.0 ADMINISTRATIVE CONSIDERATIONS AND REQUIREMENTS

14.1 BASIC PRINCIPLES

This research will be carried out in accordance with the protocol, the ICH Guideline for Good Clinical Practice: Consolidated Guidance (E6), and applicable regulatory requirement(s) including clinical research guidelines established by the Basic Principles defined in the U.S. 21 CFR Parts 50, 56, and 312, and the Principles enunciated in the Declaration of Helsinki (revised version Fortaleza 2013).

14.2 INSTITUTIONAL REVIEW BOARD, ETHICAL COMPLIANCE, INVESTIGATOR RESPONSIBILTIES, AND SPONSOR RESPONSIBILITIES

This study is being conducted to evaluate the safe-dose ranges and PK of (2R,6R)-Hydroxynorketamine after slow-IV administration to healthy Subjects. The results of this study will provide useful information on dose and exposure of (2R,6R)-Hydroxynorketamine by this mode of administration.

The primary ethical concerns of this study are that this study will be performed in healthy Subjects who will receive no benefit from participation in the study, except for financial compensation for the time and inconveniences that may arise from participation in the study.

As with all clinical studies, there are risks associated with the clinical procedures. To avoid unnecessary risks, methods including the dosing strategy and blood sample collection scheme were designed to minimize the number of venipunctures required to complete the study. Further, the total volume of blood draws in support of clinical laboratory testing and PK evaluations collected from each Subject during the study does not exceed acceptable amount of blood to be collected over the defined time periods as recommended by standards set by the American Red Cross.

Potential Subjects will be fully informed of the risks and requirements of the study and, during the study, Subjects will be given any new information that may affect their decision to continue participation. They will be told that their consent to participate in the study is voluntary and may be withdrawn at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only Subjects who are fully able to understand the risks, benefits, and potential AEs of the study, and provide their consent voluntarily will be enrolled.

Any woman who is pregnant, breast feeding, or planning to become pregnant during the study will be excluded from participating in the study. Potential female Subjects must be postmenopausal, sterile, abstinent, or, if heterosexually active, be practicing a highly effective method of birth control before entry and throughout the study. Women, who are not heterosexually active at Screening, must agree to utilize a highly effective method of birth control if they become heterosexually active during their participation in the study.

The PI is responsible for ensuring that the clinical study is performed in accordance with the protocol, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory and country-specific requirements.

The GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human Subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study Subjects are protected, consistent with the Principals that originated in the Declaration of Helsinki and that the clinical study data are credible.

Before the start of the study, the PI (or sponsor where required) will provide the IEC/IRB with current and complete copies of the following documents:

- Final protocol and, if applicable, amendments.
- Sponsor-approved informed consent form (and any other written materials to be provided to the Subjects).
- Investigator's Brochure (or equivalent information) and amendments/addenda
- Sponsor-approved Subject recruiting materials.
- Information on compensation for study-related injuries or payment to Subjects for participation in the study, if applicable.
- Investigator's curriculum vitae or equivalent information (unless not required, as documented by the IEC/IRB).
- Information regarding funding, name of the sponsor, institutional affiliations, other potential conflicts of interest, and incentives for Subjects.
- Any other documents that the IEC/IRB requests to fulfill its obligation.

This study will be undertaken only after the IEC/IRB has given full approval of the final protocol, amendments (if any), the informed consent form, applicable recruiting materials, and Subject compensation programs, and the sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents being approved.

During the study, the PI (or sponsor where required) will send the following documents and updates to the IEC/IRB for their review and approval, where appropriate:

- Protocol amendments.
- Revision(s) to informed consent form and any other written materials to be provided to Subjects.
- If applicable, new or revised Subject recruiting materials approved by the sponsor.
- Revisions to compensation for study-related injuries or payment to Subjects for participation in the study, if applicable.
- New edition(s) of the Investigator's Brochure and amendments/addenda.
- Summaries of the status of the study at intervals stipulated in guidelines of the IEC/IRB (at least annually).

- Reports of AEs that are serious, unlisted/unexpected, and associated with the investigational drug.
- New information that may adversely affect the safety of the Subjects or the conduct of the study.
- Deviations from or changes to the protocol to eliminate immediate hazards to the Subjects.
- Report of deaths of Subjects under the Investigator's care.
- Notification if a new Investigator is responsible for the study at the site.
- Annual Safety Report and Line Listings, where applicable.
- Any other requirements of the IEC/IRB.

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for Subjects, data, or study conduct), the amendment and applicable informed consent form revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s).

At least once a year, the IEC/IRB will be asked to review and reapprove this clinical study. The reapproval should be documented in writing (excluding the ones that are purely administrative, with no consequences for Subjects, data, or study conduct).

At the end of the study, the Investigator (or sponsor where required) will notify the IEC/IRB about the study completion.

14.3 PRIVACY OF PERSONAL DATA

The collection and processing of personal data from Subjects enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of study Subjects confidential.

The informed consent obtained from the Subject includes explicit consent for the processing of personal data and for the Investigator to allow direct access to his or her original medical records for study-related monitoring, audit, IEC/IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

The Subject has the right to request through the Investigator access to his or her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

14.4 PROTOCOL AMENDMENTS

Neither the PI nor the Sponsor will modify this protocol without a formal amendment. All protocol amendments must be issued by the PI or the Sponsor and signed and dated by the PI. Protocol amendments must not be implemented without prior IEC/IRB approval, or when the relevant competent authority has raised any grounds for nonacceptance, except when necessary to eliminate immediate hazards to the Subjects, in which case the amendment must be promptly submitted to the IEC/IRB and relevant competent authority. Documentation of amendment approval by the Investigator and IEC/IRB must be provided to the sponsor or its designee. When the change(s) involve(s) only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified.

During the study, in situations where a departure from the protocol is unavoidable, the PI or other physician in attendance will contact the appropriate sponsor representative. Except in emergency situations, this contact should be made before implementing any departure from the protocol. In all cases, contact with the Sponsor must be made as soon as possible to discuss the situation and agree on an appropriate course of action. The data recorded in the CRF and source documents will reflect any departure from the protocol, and the source documents will describe this departure and the circumstances requiring it.

14.5 STUDY MONITORING

The Sponsor will be allowed to perform on-site monitoring visits without restriction. The monitor will record dates of the visits in a study site visit log that will be kept at the site. The Sponsor monitoring activities during visits will consist of reviews of procedures and data integrity. Direct access to source documentation (medical records) is allowed for the purpose of verifying that the data recorded in the Sponsors database are consistent with the original source data. Findings from comparative reviews will be discussed with the PI. The Sponsor expects that, during monitoring visits, the relevant investigational staff will be available, the source documentation will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor will meet with the Investigator on a regular basis during the study to provide feedback on the study conduct.

Representatives of the Sponsor's clinical quality assurance department may visit the site at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection and comparison with the Sponsors database. Subject privacy must, however, be respected. The PI and staff are responsible for being present and available for consultation during routinely scheduled site audit visits conducted by the sponsor or its designees.

Similar auditing procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The PI should immediately notify the sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

14.6 STUDY COMPLETION OR TERMINATION

The Sponsor reserves the right to terminate the study at any time for any reason at the sole discretion of the Sponsor. The PI may terminate the study at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of an investigational site by the sponsor or Investigator may include, but are not limited to:

- Failure of the PI to comply with the protocol, the requirements of the IEC/IRB or local health authorities, the sponsor's procedures, or GCP guidelines.
- Inadequate recruitment of Subjects by the PI.
- Discontinuation of further drug development.

The study is considered completed with the last visit for the last Subject participating in the study. The final data from the investigational site will be sent to the Sponsor after completion of the final Subject visit at that site, in the time frame specified in the Clinical Trial Agreement.

14.7 REGULATORY DOCUMENTATION

This protocol and any amendment(s) must be submitted to the appropriate regulatory authorities. A study may not be initiated until all regulatory requirements are met.

14.8 SUBJECT IDENTIFICATION, ENROLLMENT LOG, AND SCREENING LOG

The PI agrees to complete a Subject identification and enrollment log to permit easy identification of each Subject during and after the study. This document will be reviewed by the sponsor site contact for completeness.

The Subject identification and enrollment log will be treated as confidential and will be filed by the Investigator in the study file. To ensure Subject confidentiality, no copy will be made. All reports and communications relating to the study will identify Subjects by assigned number.

The PI must also complete a Subject screening log, which reports on all Subjects who were seen to determine eligibility for inclusion in the study.

14.9 DATA CAPTURE, DATA QUALITY CONTROL, AND REPORTING

Steps to be taken to ensure the accuracy and reliability of data include review of protocol procedures with the PI and associated personnel before the study, periodic monitoring visits by the Sponsor, and direct transmission of clinical laboratory data, ECG data, and EEG data from central laboratories into the Sponsor’s database. Written instructions will be provided for electronic transfer of data to the Sponsor’s database. The Sponsor will

review for accuracy and completeness during scheduled teleconferences, on-site monitoring visits and after transmission to the sponsor; any discrepancies will be resolved with the PI or designee, as appropriate. After upload of the data into the clinical study database, they will be verified for accuracy and consistency with the data sources.

14.10 REGISTRATION OF CLINICAL STUDIES AND DISCLOSURE OF RESULTS

The sponsor will register and/or disclose the existence of, and the results of clinical studies as required by law.

14.11 USE OF INFORMATION, DATA, AND PUBLICATION

All information generated as a result of this study, are considered confidential and remain the sole property of the Sponsor. Following study termination/conclusion, the PI and the Sponsor will evaluate key data outcomes and collaborate on the writing, submission and publication of a peer-reviewed manuscript detailing all outcomes of the study within 12 months of the study conclusion. Authorship of publications resulting from this study will be based on the guidelines on authorship, such as those described in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, which state that the named authors must have made a significant contribution to the design of the study or analysis and interpretation of the data, provided critical review of the paper, and given final approval of the final version.

14.12 REIMBURSEMENTS, INDEMNITY, AND INSURANCE

There is no cost to subjects for the research tests, procedures, and study product while taking part in this study. Procedures and treatment for clinical care may be billed to the subject, subject's insurance, or third party. Subjects may be compensated for their participation in this study. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

If it is determined by the site PI that an injury occurred to a subject as a direct result of the tests or treatments that are done for this study, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this study. Immediate medical treatment may be provided by the participating site.

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Appendix 1: MOAA's

Responsiveness scores of the Modified Observer's Assessment of Alertness/sedation Scale.

Response	Score
Responds readily to name spoken in normal tone	5
Lethargic response to name spoken in normal tone	4
Responds only after name is called loudly of repeatedly	3
Responds only after mild prodding or shaking	2
Does not respond to mild prodding or shaking	1
Does not respond to noxious stimulus	0

Appendix 2: POMS

Abbreviated POMS (Revised Version)

Name:_____ Date:

Below is a list of words that describe feelings people have. Please **CIRCLE THE NUMBER THAT BEST DESCRIBES HOW YOU FEEL RIGHT NOW**.

	Not At All	A Little	Moderately	Quite a lot	Extremely
Tense	0	1	2	3	4
Angry	0	1	2	3	4
Worn Out	0	1	2	3	4
Unhappy	0	1	2	3	4
Proud	0	1	2	3	4
Lively	0	1	2	3	4
Confused	0	1	2	3	4
Sad	0	1	2	3	4
Active	0	1	2	3	4
On-edge	0	1	2	3	4
Grouchy	0	1	2	3	4
Ashamed	0	1	2	3	4
Energetic	0	1	2	3	4
Hopeless	0	1	2	3	4
Uneasy	0	1	2	3	4
Restless	0	1	2	3	4
Unable to concentrate	0	1	2	3	4
Fatigued	0	1	2	3	4
Competent	0	1	2	3	4
Annoyed	0	1	2	3	4
Discouraged	0	1	2	3	4
Resentful	0	1	2	3	4
Nervous	0	1	2	3	4
Miserable	0	1	2	3	4

PLEASE CONTINUE WITH THE ITEMS ON THE NEXT PAGE

	Not At All	A Little	Moderately	Quite a lot	Extremely
Confident	0	1	2	3	4
Bitter	0	1	2	3	4
Exhausted	0	1	2	3	4
Anxious	0	1	2	3	4
Helpless	0	1	2	3	4
Weary	0	1	2	3	4
Satisfied	0	1	2	3	4
Bewildered	0	1	2	3	4
Furious	0	1	2	3	4
Full of Pep	0	1	2	3	4
Worthless	0	1	2	3	4
Forgetful	0	1	2	3	4
Vigorous	0	1	2	3	4
Uncertain about things	0	1	2	3	4
Bushed	0	1	2	3	4
Embarrassed	0	1	2	3	4

THANK YOU FOR YOUR COOPERATION

PLEASE BE SURE YOU HAVE ANSWERED EVERY ITEM

Citation:
Grove, J.R., & Prapavessis, H. (1992). Preliminary evidence for the reliability and validity of an abbreviated Profile of Mood States. *International Journal of Sport Psychology, 23*, 93-109.

*** SCORING KEY ***

Scores for the seven subscales in the abbreviated POMS are calculated by summing the numerical ratings for items that contribute to each subscale. The correspondence between items and subscales is shown below.

Item	**Scale**	Not At All	A Little	Moderate	Quite a lot	Extremely
Tense	**TEN**	0	1	2	3	4
Angry	**ANG**	0	1	2	3	4
Worn Out	**FAT**	0	1	2	3	4
Unhappy	**DEP**	0	1	2	3	4
Proud	**ERA**	0	1	2	3	4
Lively	**VIG**	0	1	2	3	4
Confused	**CON**	0	1	2	3	4
Sad	**DEP**	0	1	2	3	4
Active	**VIG**	0	1	2	3	4
On-edge	**TEN**	0	1	2	3	4
Grouchy	**ANG**	0	1	2	3	4
Ashamed	**ERA**	Reverse-score this item [0 = 4, 1 = 3, 2 = 2, 3 = 1, 4 = 0]				
Energetic	**VIG**	0	1	2	3	4
Hopeless	**DEP**	0	1	2	3	4
Uneasy	**TEN**	0	1	2	3	4
Restless	**TEN**	0	1	2	3	4
Can’t concentrate	**CON**	0	1	2	3	4
Fatigued	**FAT**	0	1	2	3	4
Competent	**ERA**	0	1	2	3	4
Annoyed	**ANG**	0	1	2	3	4
Discouraged	**DEP**	0	1	2	3	4
Resentful	**ANG**	0	1	2	3	4
Nervous	**TEN**	0	1	2	3	4
Miserable	**DEP**	0	1	2	3	4

Item	Scale	Not At All	A Little	Moderate	Quite a lot	Extremely
Confident	**ERA**	0	1	2	3	4
Bitter	**ANG**	0	1	2	3	4
Exhausted	**FAT**	0	1	2	3	4
Anxious	**TEN**	0	1	2	3	4
Helpless	**DEP**	0	1	2	3	4
Weary	**FAT**	0	1	2	3	4
Satisfied	**ERA**	0	1	2	3	4
Bewildered	**CON**	0	1	2	3	4
Furious	**ANG**	0	1	2	3	4
Full of Pep	**VIG**	0	1	2	3	4
Worthless	**DEP**	0	1	2	3	4
Forgetful	**CON**	0	1	2	3	4
Vigorous	**VIG**	0	1	2	3	4
Uncertain…	**CON**	0	1	2	3	4
Bushed	**FAT**	0	1	2	3	4
Embarrassed	**ERA**	Reverse-score this item [0 = 4, 1 = 3, 2 = 2, 3 = 1, 4 = 0]				

TEN = Tension	Note that 2 of the items on the Esteem-related Affect (ERA) subscale are reverse-scored prior to being combined with the other items. **Total Mood Disturbance (TMD)** is calculated by summing the totals for the negative subscales and then subtracting the totals for the positive subscales: TMD = [TEN+DEP+ANG+FAT+CON] – [VIG+ERA]. A constant (e.g., 100) can be added to the TMD formula in order to eliminate negative scores.
ANG = Anger	
FAT = Fatigue	
DEP = Depression	
ERA = Esteem-related Affect	
VIG = Vigour	
CON = Confusion	

Appendix 3: C-CSSRS

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Baseline/Screening Version

Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in ***The Columbia Suicide History Form****, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.]* Standardized Evaluation in Clinical Practice*, pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

SUICIDAL IDEATION		
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	**Lifetime: Time He/She Felt Most Suicidal**	**Past Months**
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. ***Have you wished you were dead or wished you could go to sleep and not wake up?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
2. Non-Specific Active Suicidal Thoughts General non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. ***Have you actually had any thoughts of killing yourself?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g. thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it…and I would never go through with it."* ***Have you been thinking about how you might do this?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having <u>some intent to act on such thoughts</u>, as opposed to *"I have the thoughts but I definitely will not do anything about them."* ***Have you had these thoughts and had some intention of acting on them?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. ***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
INTENSITY OF IDEATION		

The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). Ask about time he/she was feeling the most suicidal. Lifetime - ***Most Severe Ideation:*** _____ (***Type # (1-5)***) _____ (***Description of Ideation***) Past X Months - ***Most Severe Ideation:*** _____ (***Type # (1-5)***) _____ (***Description of Ideation***)	Most Severe	Most Severe
Frequency ***How many times have you had these thoughts?*** (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	____	____
Duration ***When you have the thoughts how long do they last?*** (1) Fleeting - few seconds or minutes (2) Less than 1 hour/some of the time (3) 1-4 hours/a lot of time (4) 4-8 hours/most of day (5) More than 8 hours/persistent or continuous	____	____
Controllability ***Could/can you stop thinking about killing yourself or wanting to die if you want to?*** (1) Easily able to control thoughts (2) Can control thoughts with little difficulty (3) Can control thoughts with some difficulty (4) Can control thoughts with a lot of difficulty (5) Unable to control thoughts (0) Does not attempt to control thoughts	____	____
Deterrents ***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?*** (1) Deterrents definitely stopped you from attempting suicide (2) Deterrents probably stopped you (3) Uncertain that deterrents stopped you (4) Deterrents most likely did not stop you (5) Deterrents definitely did not stop you (0) Does not apply	____	____
Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (2) Mostly to get attention, revenge or a reaction from others (3) Equally to get attention, revenge or a reaction from others on and to end/stop the pain (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (5) Completely to end or stop the pain (you couldn't go living with the pain or how you were feeling) (0) Does not apply	____	____

 C-SSRS—Baseline/Screening (Version 1/14/09) Page 1 of 2

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	**Lifetime**	**Past Years**
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act.* Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you_____as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you_____?*** ***Or Did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** *(*Self-Injurious Behavior without suicidal intent) If yes, describe: **Has subject engaged in Non-Suicidal Self-Injurious Behavior?**	**Yes No** ☐ ☐ Total # of Attempts _____ **Yes No** ☐ ☐	**Yes No** ☐ ☐ Total # of Attempts _____ **Yes No** ☐ ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred).* Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	**Yes No** ☐ ☐ Total # of interrupted	**Yes No** ☐ ☐ Total # of interrupted

Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:			**Yes** ☐ **No** ☐ Total # of aborted	**Yes** ☐ **No** ☐ Total # of aborted
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:			**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
Suicidal Behavior: Suicidal behavior was present during the assessment period?			**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
Answer for Actual Attempts Only	Most Recent Attempt Date:	Most Lethal Attempt Date:	Initial/First Attempt Date:	
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code*	*Enter Code*	*Enter Code*	
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code*	*Enter Code*	*Enter Code*	

 C-SSRS—Baseline/Screening (Version 1/14/09) Page 2 of 2

Appendix 4: CADSS

The Clinician Administered Dissociative States Scale (CADSS)

Name_____ ID_____ Date_____

Subjective Items:

1. Do things seem to be moving in slow motion?
0= Not at all.
1= Mild, things seem slightly slowed down, but not very noticeable.
2= Moderate, things are moving about twice as slow as normally.
3= Severe, things are moving so slowly that they are barely moving.
4= Extreme, things are moving so slowly, I have the perception that everything has come to a stop, as if time is standing still.

2. Do things seem to be unreal to you, as if you are in a dream?
0= Not at all.
1= Mild, things seem a little unreal, but I'm well aware of where I'm at.
2= Moderate, things seem dreamlike, although I know I am awake.
3= Severe, things seem very dreamlike, although I know that I am here, I have the feeling like I might be asleep.
4= Extreme, I feel like nothing is real, like I should pinch myself to wake up, or ask someone if this is a dream.

3. Do you have some experience that separates you from what is happening; for instance, do you feel as if you are in a movie or a play, or as if you are a robot?
0= Not at all.
1= Mild, I feel a little bit separated from what is happening, but I am basically here.
2= Moderate, I feel somewhat separated from what is going on, or I feel as if I am in a movie or a play.
3= Severe, I feel extremely separated from what is happening, but I can understand what people are saying.
4= Extreme, I feel as if everyone around me is talking a foreign language, so that I cannot understand what they are saying, or I feel as if I am on the outside looking in, or like I am a robot or a machine.

4. Do you feel as if you are looking at things from outside of your body?
0= Not at all.
1= Mild, I feel somewhat disconnected from myself, but I am basically all together.
2= Moderate, I feel like I am just outside of my body, but not looking down upon myself from far above.
3= Severe, I feel like I am twenty feet or more away from my body, looking down from above.
4= Extreme, I feel as if I am hundreds of feet above myself, looking down at myself

and everyone else here.

5. Do you feel as if you are watching the situation as an observer or a spectator?
0= Not at all.
1= Mild, I feel slightly detached from what is going on, but I am basically here.
2= Moderate, I feel somewhat removed as an observer or a spectator, but I am definitely in this room.
3= Severe, I feel very much as if I am an observer or a spectator, but I am still here in this room.
4= Extreme, I feel completely removed from what is happening, as if I am not a part of this experience in any way, but totally removed from what is happening, as an observer or a spectator.

6. Do you feel disconnected from your own body?
0= Not at all.
1= Mild, I feel a little bit disconnected from myself, but I am basically all here.
2= Moderate, I feel somewhat detached from my own body, but I am basically all together.
3= Severe, I feel detached from my own body, but not far removed from my body, and I feel as if it is me there.
4= Extreme, I feel like I am completely out of my body, as if I am looking at my own body from a long way off, as if there is another person there.

7. Does your sense of your own body feel changed: for instance, does your own body feel unusually large or unusually small?
0= Not at all.
1= Mild, I have a vague feeling that something about my body has changed, but I can't say exactly what it is.
2= Moderate, I feel like my body has increased or decreased in size slightly, or that it feels somewhat as if it is not my body.
3= Severe, I feel as if my body has increased to twice its normal size, or decreased to twice its normal size, or I very much feel as if this is not my body.
4= Extreme, I feel as if my body has swelled up to at least ten times its normal size, or as if it is ten times as small, or as if my arms have become like toothpicks.

8. Do people seem motionless, dead, or mechanical?
0= Not at all.
1= Mild, people seem a little bit more motionless, dead, or mechanical than would be normal.
2= Moderate, people seem to be at least twice as motionless or mechanical than would be normal.
3= Severe, people seem to be barely moving, or barely alive, or very mechanical.
4= Extreme, it's as if everyone were frozen or completely like machines.

9. Do objects look different than you would expect?
0= Not at all.
1= Mild, things seem slightly different than normal, although it is barely perceptible.
2= Moderate, things are somewhat distorted, but I have no problems recognizing things around me.
3= Severe, things are much more distorted or unreal than normal, but I am able to recognize things in the room.
4= Extreme, like everything is distorted, not real, I feel like I cannot recognize anything, everything is alien or strange.

10. Do colors seem to be diminished in intensity?
0= Not at all.
1= Mild, things seem slightly paler than usual if I think about it.
2= Moderate, colors are somewhat diminished, but still recognizable.
3= Severe, colors are extremely pale, in no way as vivid as they usually are.
4= Extreme, as if everything is in black and white, or all the colors have been washed out.

11. Do you see things as if you were in a tunnel, or looking through a wide angle photographic lens?
0= Not at all.
1= Mild, I feel a little bit like I am looking through a tunnel, or a wide angle lens.
2= Moderate, the periphery of my vision is blacked out, but I still have most of my visual field, or things are somewhat like a wide angle lens.
3= Severe, it seems as if I'm looking through a tunnel, or through a wide angle lens, but I can see everything clearly.
4= Extreme, as if I'm looking through a pair of binoculars backwards, where everything around the periphery is blacked out, and I can see a little point of light at the end of a tunnel, with little tiny people and objects, or I am seeing things as if through a wide lens and things are incredibly expanded.

12. Does this interview [assessment, questionnaire] seem to be taking much longer than you would have expected?
0= Not at all.
1= Mild, it seems as if this interview has gone on for at least twice as long as the true elapsed time.
2= Moderate, it seems as if this interview has gone on for at least two hours.
3= Severe, it seems as if at least ten hours have gone on since the start of the interview.
4= Extreme, it seems as if time is standing still, so that we have been here at this point in time forever.

13. Do things seem to be happening very quickly, as if there is a lifetime in a moment?
0= Not at all.

1= Mild, things are happening slightly faster than normal.
2= Moderate, things seem to be happening at least twice as fast as normal.
3= Severe, things seem to be happening at least 10 times faster than normal.
4= Extreme, as if this whole experience has happened at once, or as if there is a lifetime in a moment.

14. Have there been things which have happened during this interview [assessment] that now you can't account for?
0= Not at all.
1= Mild, there may have been things which happened which now I can't account for, but nothing pronounced.
2= Moderate, at least once there were things which happened which now I can't account for.
3= Severe, at least twice I have lost several minutes of time, so that now there are things I cannot account for.
4= Extreme, large pieces of time are missing, of ten minutes or more, so that I am confused about what has happened.

15. Have you spaced out, or in some other way lost track of what was going on during this experience?
0= Not at all.
1= Mild, I have had some episodes of losing track of what is going on, but I have followed everything for the most part.
2= Moderate, I have lost at least a minute of time, or have completely lost track of what is going on now.
3= Severe, I have lost several segments of time of one minute or more.
4= Extreme, I have lost large segments of time of at least 15 minutes or more.

16. Have sounds almost disappeared or become much stronger than you would have expected?
0= Not at all.
1= Mild, things are either a little quieter than normal, or a little louder than normal, but it is not very noticeable.
2= Moderate, things have become about twice as soft as normal, or twice as loud as normal.
3= Severe, things have become very quiet, as if everyone is whispering, or things have become very loud (although not deafening).
4= Extreme, things have become completely silent, or sounds are so loud that it is deafening, and I feel as if I am going to break my eardrums.

17. Do things seem very real, as if there is a special sense of clarity?
0= Not at all.
1= Mild, things seem to be a little bit more real than normal.
2= Moderate, things seem to be more real than normal.

3= Severe, things seem to be very real or have a special sense of clarity.
4= Extreme, things seem to have an incredible sense of realness or clarity.

18. Does it seem as if you are looking at the world through a fog, so that people and objects appear far away or unclear?
0= Not at all.
1= Mild, things seem somewhat foggy and unclear, or I do have the feeling that things are far away, but there is not a major effect on how I perceive things around me.
2= Moderate, things seem very foggy and unclear, or things seem like they are far away, but I can identify the interviewer and objects in the room easily.
3= Severe, I can barely see things around me, such as the interviewer and the objects in the room.
4= Extreme, I cannot make anything out around me.

19. Do colors seem much brighter than you would have expected?
0= Not at all.
1= Mild, colors seem a little bit brighter than normal, but not more than twice as bright.
2= Moderate, colors seem brighter, about twice as bright as normal.
3= Severe, colors seem very bright, at least five times as bright as normal.
4= Extreme, colors seem extremely bright, almost fluorescent, at least 10 times as bright as normal.

20. Do you feel confused about who you really are?
0= Not at all.
1= Mild, I feel a little bit confused about who I am.
2= Moderate, I feel confused about who I am, but I basically know who I am.
3= Severe, I feel very confused about who I am, and at times I wonder if I am a person, or if I am many people.
4= Extreme, I feel as if there were two or more sides to myself.

21. Do you feel like there are different parts of yourself which do not fit together?
0= Not at all.
1= Mild, I feel like there are different sides of myself, but they're basically part of myself.
2= Moderate, I feel like I have different parts which don't quite fit together.
3= Severe, there are two or more sides to myself which have unique characteristics.
4= Extreme, I have two or more parts to myself with unique personality characteristics.

22. Do you have gaps in your memory?
0= Not at all.
1= Mild, there are some recent things which I cannot remember.

2= Moderate, there have been a few gaps in my memory which lasted a few minutes.
3= Severe, there have been large gaps in my memory which lasted for more than a few minutes.
4= Extreme, I cannot piece together what is happening from one moment to the next due to large gaps in my memory.

23. Do you feel like you have more than one identity?
0= Not at all.
1= Mild, I feel like there is more to me than my personality, but it's basically part of my identity.
2= Moderate, I feel like I have more than one personality, but the personalities are not really distinct.
3= Severe, I have two or more personalities, although they are not fully developed as distinct entities.
4= Extreme, I have two or more personalities which are distinct and have their own names and other unique characteristics