CLINICAL STUDY PROTOCOL
Interventional Drug or Biologic

Reducing Adolescent Suicide Risk: Safety, Efficacy, and Connectome Phenotypes of Intravenous Ketamine

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PRINCIPAL INVESTIGATOR:
Name: Michael Bloch, MD, MS
Department: Child Study Center
Telephone Number: (203)745-9921
Email Address: Michael.bloch@yale.edu

IND Number: 152866
IND Holder: Michael Bloch, MD, MS
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Study Phase (1, 2, 3, or 4-postmarketing): Phase 2

Contents

STATEMENT OF COMPLIANCE

SIGNATURE OF PRINCIPAL INVESTIGATOR

Protocol Title: Reducing Adolescent Suicide Risk: Safety, Efficacy, and Connectome Phenotypes of Intravenous Ketamine

Version and Date: _____

The signatory agrees to the content of the reflected version of the clinical study protocol. He/she will conduct the study as presented and in compliance with all applicable regulations including those outlined in International Council for Harmonization (ICH) Good Clinical Practice (GCP) and the Code of Federal Regulations.

Name of Investigator: _____

Affiliation: _____

Signature:_____Date: _____

Signed copies of this signature page are stored in the sponsor's study file and investigator site file.

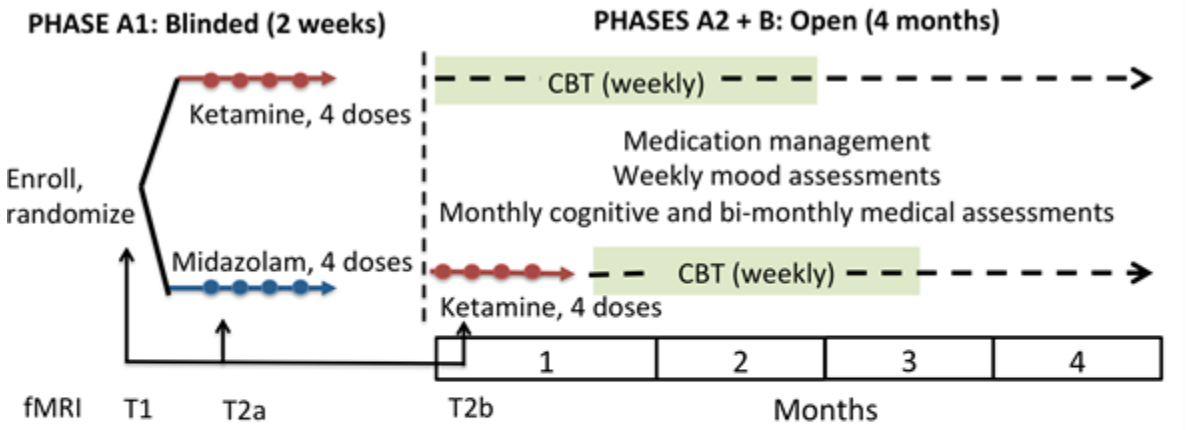
1 . Protocol Summary

This document is a protocol for a human research study. The purpose of this protocol is to ensure that this study is to be conducted according to ICH GCP guidelines, and according to CFR 21 Part 312, other applicable government regulations and Institutional research policies and procedures.

1.1 Synopsis

Primary Objective The primary objective of this Phase 2 study is to determine whether ketamine rapidly reduces suicidal ideation (as measured by the C-SSRS, recent ideation subscale) compared to an active control, midazolam, in adolescents at high suicide risk (meeting criteria for SRI-resistant depression (having failed at least one antidepressant)AND having a suicidal event within the 120 days prior to enrollment).
Secondary Objectives The secondary objectives of this study are to closely monitor the safety (cardiovascular function, bladder health, and cognitive function) and tolerability of a conservative repeat dosing ketamine paradigm and to identify functional connectome signatures predictive of efficacy or modified by treatment. Exploratory objectives include describing the trajectory of mental health outcomes (suicidality, depressive symptoms, mental health resource utilization) over 4 months in ketamine responders and non-responders.
Study Duration 7 years, participant duration is 18 weeks (20 weeks for those who cross over to open ketamine)
Study Design Parallel, double-blind, placebo-controlled RCT with option to cross-over to active treatment in open phase
Number of Study Sites 1) Yale University/Yale New Haven Hospital
Study Population Adolescents (13 to 17 years old, inclusive) with SRI-resistant depression AND a history of a suicidal event within 120 days prior to enrollment. .A suicide event is defined as either a suicide attempt (i.e. act of potentially self-injurious behavior with explicit or inferred intent to die –OR– degree of suicidal ideation requiring an emergency evaluation or a transition to higher level of care (e.g. intensive outpatient program, partial hospital program, inpatient)). Participants will be recruited through the Yale New Haven Health System through inpatient, intensive outpatient, emergency, and subspecialty clinical services. We do not plan to advertise in the community for this study.
Number of Participants N = 40-66 (Last subject enrollment date is planned for 8/31/26)
Primary Outcome Variables Columbia-Suicide Severity Rating Scale (C-SSRS), recent ideation subscale, 48-hours after receiving first blinded treatment
Secondary and Exploratory Outcome Variables (if applicable) Montgomery Asberg Depression Rating Scale; Children's Depression Rating Scale-Revised; Clinical Global Impression; Systematic Assessment for Treatment Emergent Effects; Cogstate battery; Pre- and post-treatment functional connectome (derived from multimodal combination of task-based and resting state fMRI); mental health utilization (number of emergency, intensive outpatient, partial hospital, or inpatient visits during open phase)

1.2 Schema



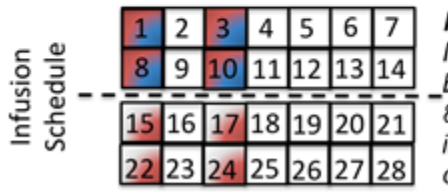


Fig 1: Study Design. *2-week double blind phase (Phase A1; N=33 per group) followed by a 4-month open extension. Bottom panel: infusion schedule of blinded treatments (D1, 3, 8 & 10). The primary outcome is on D3 (48-hours after first infusion). Open treatments in midazolam non-responders are on D15, 17, 22, 24 (Phase A2). Their medication management and CBT (Phase B) are shifted 2 forward two weeks. fMRI occurs at 3 timepoints: T1 (pre-treatment), T2a (Day 3, prior to infusion 2), and T2b (Day 17, prior to open infusion 2 on the bottom line, or 3 days into open treatment on the top trajectory.*

1.3 Schedules of Activities

The total number of study visits from Baseline through study completion is 24 for those who progress directly from phase A1 to B (i.e. those who are either randomized to ketamine or who have a significant clinical response to midazolam) and is 30 for those who complete phases A1, A2 (open ketamine), and B. Please see the 2 separate tables of study events that follow.

Study Schedule for Double Blind Treatment (Phase A1)—All participants

			Phase A1					
	Visit No.	1	2	3	4	5	6	7
Procedures	Screening	D0 (Baseline)	D1	D2	D3	D8	D10	D11
Blinded Intervention – Phase A1 [a]			X		X	X	X	
Permission and Assent	X							
Diagnostic Interview	X							
MINI-KID	X							
Physical Exam	X							
Demographics Form	X							
Diagnosis & Treatment History Form	X							
Family History Form	X							
Medications Form	X							
MRI Safety Questionnaire	X							
Vital signs	X		X		X	X	X	
Lab tests [b]	X							
ECG	X		X					
Urine toxicology	X		X			X		
Urine pregnancy test [c]			X			X		
Safety Planning Session		X						

			Phase A1					
	Visit No.	1	2	3	4	5	6	7
Procedures	Screening	D0 (Baseline)	D1	D2	D3	D8	D10	D11
MRI Imaging		X			X			
AE assessment (SAFTEE-SI)			X	X[d]	X	X	X	X
PK Blood draw [e]			X					
Clinical questionnaires								
C-SSRS	X	X	X		X	X	X	X
MADRS		X	X	X [d]	X	X	X	X
CGI		X	X		X	X	X	X
CDRS-R	X	X						X
PSC		X						X
TEASAP		X			X			X
MASC		X						X
CADSS		X	X		X	X	X	X
BHS		X						X
ASQ		X						X
CTQ		X						
Edinburgh Handedness Inventory		X						
Wechsler Abbreviated Scale of Intelligence		X						
Cogstate neurocognitive battery		X						X
Guess form			X					
Breaking the Blind [f]								X

a. Subjects will be randomly assigned to receive either 0.5 mg/kg IV ketamine OR 0.045 mg/kg IV midazolam. Both medications will be infused over 40 minutes Subjects will not receive more than 40 mg ketamine or 3.6 mg midazolam

b. Labs will include: CBC with differential, complete metabolic panel (including electrolytes, LFTs, BUN, creatinine, and glucose), TFTs, a serum pregnancy test for WOCBP, and routine urinalysis

c. WOCBP will have a weekly urine pregnancy test prior to the first treatment of the week

d. These assessments will occur by phone

e. Blood for pharmacokinetics will be drawn pre-infusion; and 40, 80, 110, and 230 minutes post-infusion start. Total blood volume collected will not exceed 30 mL/day

f. After breaking the blind, participants will be separated into two groups, with separate schedules: Open Schedule #1: Subjects assigned to midazolam who did not respondin Phase A1 will proceed to Phase A2; Open Schedule #2: All other subjects will proceed directly to Phase B

Study Schedule for Open Treatment (Phase A2)— Only Open Schedule #1 participants

		Phase A2					
	Visit No.	8	9	10	11	12	13
Procedures	Study Day	D15	D16	D17	D22	D24	D25
Open Intervention – Phase A2[a]		X		X	X	X	
Vital signs		X		X	X	X	
ECG		X					
Urine toxicology		X			X		
Urine pregnancy test [b]		X			X		
MRI Imaging				X			
AE assessment (SAFTEE-SI)		X	X [d]	X	X	X	X
PK Blood draw [c]		X					
Clinical questionnaires							
C-SSRS		X		X	X	X	X
MADRS		X	X [d]	X	X	X	X
CGI		X		X	X	X	X
CDRS-R							X
PSC							X
TEASAP				X			X
MASC							X
CADSS		X		X	X	X	X
BHS							X
ASQ							X
Cogstate neurocognitive battery							X

a. Subjects will receive 0.5 mg/kg IV ketamine over 40 minutes. Subjects will not receive more than 40 mg ketamine.
b. WOCBP will have a weekly urine pregnancy test prior to the first treatment of the week
c. Blood for pharmacokinetics will be drawn pre-infusion; and 40, 80, 110, and 230 minutes post-infusion start. Total blood volume collected will not exceed 30 mL/day
d. These assessments will occur by phone

Study Schedule for Open Follow-up (Phase B)—All Participants (Open schedule #1 subjects are two weeks delayed relative to Open schedule #2 subjects due to their participation in Phase A2)

		Phase B									
Open Schedule #2 (A1 -> B)	Visit #	8	9-10	11	12-14	15	18[c]	19 [c]	22[c]	23 [c]	24
	Study Day*	D17	D24, D31	D38	D45, D52, D59	D66	D73, D80, D87	D94	D101, D108, D115	D122	End of Study D126
Open Schedule #1 (A1 -> A2 -> B)	Visit #	14	15-16	17	18-20	21	22-24[c]	25 [c]	26-28[c]	29 [c]	30
	Study Day*	D31	D38, D45	D52	D59, D66, D73	D80	D87, D94, D101	D108	D115, D122, D129	D136	End of Study D140
CBT		X	X	X	X	X					
Medication Management		X	X	X	X	X		X		X	
ECG						X				X	
Lab tests [a,d]						X				X	
Urine toxicology						X				X	
Physical Exam and											X
Vital Signs											X
Clinical questionnaires											
C-SSRS		X	X	X	X	X	X	X	X	X	X
MADRS		X	X	X	X	X	X	X	X	X	X
CGI		X	X	X	X	X	X	X	X	X	X
CDRS-R				X		X		X		X	
PSC				X		X		X		X	
TEASAP				X		X		X		X	
MASC				X		X		X		X	
CADSS				X		X		X		X	
BHS				X		X		X		X	
ASQ				X		X		X		X	
Cogstate Neurocognitive				X		X		X		X	
MRI Imaging		X [b]									
AE assessment (SAFTEE-GI)		X	X		X		X		X		
AE assessment (SAFTEE-SI)				X		X		X		X	X
Discharge Discussion											X

		Phase B									
Open Schedule #2 (A1 -> B)	Visit #	8	9-10	11	12-14	15	18[c]	19 [c]	22[c]	23 [c]	24
	Study Day*	D17	D24, D31	D38	D45, D52, D59	D66	D73, D80, D87	D94	D101, D108, D115	D122	End of Study D126
Open Schedule #1 (A1 -> A2 -> B)	Visit #	14	15-16	17	18-20	21	22-24 [c]	25 [c]	26-28 [c]	29 [c]	30
	Study Day*	D31	D38, D45	D52	D59, D66, D73	D80	D87, D94, D101	D108	D115, D122, D129	D136	End of Study D140

*For the open phase, study visits may be scheduled +/- 2 days for weekly visits and +/- 3 days for monthly batteries.

a. Labs will include: CBC with differential, complete metabolic panel (including electrolytes, LFTs, BUN, creatinine, and glucose), TFTs, and routine urinalysis

b. This MRI scan will only be administered for subjects on Open Schedule #2 (proceeded directly from Phase A1 to Phase B). Subjects on Open Schedule #1 will have had their Day 17 MRI during phase A2

c. These weekly mood and AE assessments can occur by phone if the participant is clinically stable during the final 8 weeks

d. Any spontaneous reports of abdominal pain, increased urinary frequency, urge incontinence, or other urinary or gastrointestinal symptoms can prompt an early medical and laboratory evaluation, if necessary

2 Introduction

2.1 Study Rationale

1) Suicide is the second leading cause of death in young people, and major depressive disorder increases the risk of adolescent death by suicide 30-fold. There have been no major updates to evidence-based pediatric prescribing recommendations since the TORDIA trial completed 13 years ago (recommending switching to a different selective serotonin reuptake inhibitor (SSRI) and adding CBT) and there are no evidence-based rapid-acting pharmacologic interventions to reduce suicide risk.

This evidence gap results in increased off label prescribing of untested medications in high-risk adolescents, ranging from second generation antipsychotics to ketamine. Careful, developmentally informed research on the safety, efficacy, and response predictors to ketamine is urgently needed.

The main purpose of the study is to examine the safety, efficacy, response predictors, and post-treatment trajectory of adolescents with SRI-resistant depression and high suicide risk following a highly conservative repeat dosing ketamine infusion paradigm (four infusions of 0.5mg/kg each over two weeks) compared to an active placebo, midazolam. Those who are randomized to midazolam and remain ill have the option to cross-over to ketamine in the open phase. All participants will be followed closely for four months post-treatment and treated with standard of care depression treatment (medication management and cognitive behavioral therapy).

Brain-based predictors of anti-suicidal responses will be assessed via connectome predictive modeling (CPM), examining functional brain circuits via fMRI before and after treatment. Given the unregulated use of ketamine in the community at widely varying doses and frequencies, the safety data gathered from this highly conservative repeat dosing paradigm is critical to inform the field about potential risks. Efficacy data at rapid, short-term, and intermediate-term (4 month) timepoints will be critical to determining whether a larger study is warranted in this population. The assessment of brain-based predictors of response through the integration of functional neuroimaging adds an important measure of biological engagement that will inform subsequent studies and stands to contribute towards the goal of personalized medicine (i.e. determining not only if a treatment works, but in whom).

2.2 Background

2.2.1 Preclinical Experience

Ketamine is an FDA-approved anesthetic agent that is commonly used to induce surgical anesthesia due to its low incidence of significant respiratory depression and hypotension. Its anesthetic effects are thought to be directly related to non-competitive inhibition of NMDA receptors. The majority of NMDA receptors in the forebrain consist of heterotetramers of the subunits NR1, NR2A, and NR2B; these subunits combine to form a cation channel, permeable to both sodium and calcium, that is dually gated by voltage and glutamate. Ketamine binds non-selectively to all common NMDA receptor subtypes at a site within the open channel and thereby blocks the entry of calcium. Ketamine's antidepressant actions have been hypothesized to relate to selective inhibition of NMDA receptors on inhibitory interneurons in prefrontal cortex[1], as well as NMDA-receptor independent actions of the metabolite hydroxynorketamine (HNK) stimulatory actions at AMPA receptors[2]. Intravenous ketamine has a typical onset of action within 1–2 minutes and a short elimination half-life (2-4 hours)[3], with effects rapidly dissipating following termination of the infusion. Ketamine undergoes hepatic metabolism to norketamine via cytochrome P450 enzymes, with further metabolism of norketamine to secondary metabolites of hydroxynorketamines and dehydronorketamine[3] (see also 2.1.2.a, Fig 1).

Ketamine has increasingly been used in adult psychiatric populations at lower and slower doses than used in anesthesia, with increasing evidence for it efficacy as a rapid antidepressant[4]. Esketamine, the s-enantiomer of ketamine, (trade name Spravato) was recently FDA-approved for treatment resistant depression when given in conjunction with an oral antidepressant, for treatment-resistant depression.

Ketamine has a wide therapeutic window and has been used safely in Pediatrics for over 50 years for sedation prior to medical procedures and dentistry[5-7]. Ketamine is in fact used more frequently in Pediatrics than in adult populations, typically at doses of 1mg/kg – 4.5mg/kg IV over 60 seconds when used as an anesthetic agent. While treatment resistant depression and suicide are significant problems in the pediatric population, experience with ketamine and esketamine for psychiatric use in this population is limited. A small open-label trial[8] and a few case reports[9,10] have described the successful use of ketamine in pediatric patients with refractory psychiatric conditions (see also, Clinical Experience, 2.1.2), however it is not FDA-approved for this purpose. Studies of Spravato in pediatric patients with psychiatric disorders are ongoing. Preclinical data suggests that ketamine may reverse depressive phenotypes in adolescent rats[11]. While the anesthesia literature suggests that single doses of ketamine in the clinical range are clearly tolerated in adolescent populations, animal studies of repeat or high-dose ketamine exposure in animals suggest that it has the potential

for neurotoxicity (irreversible neuronal apoptosis, Olney lesions, cognitive deficits)[12-14], particularly at early developmental stages. The toxicity threshold of repeat ketamine exposure is not known in adult or pediatric populations, and thus repeat dosing must be approached with caution and appropriate monitoring.

2.2.2 Clinical Experience

2.2.2.1 Ketamine single dose adolescent study—tolerability, pharmacokinetics

We recently completed a randomized, midazolam-controlled single-dose crossover trial of ketamine in adolescents (13 to 17 years) with treatment-resistant depression (TRD) (N=17) (NCT02579928). Participants had a primary DSM-5 diagnosis of Major Depressive Disorder (MDD) and a Children's Depression Rating Scale-Revised (CDRS-R) >40. Adolescents also must have failed at least one prior 8-week trial of a standard antidepressant medication (e.g. SSRI, SNRI) to meet TRD criteria. Exclusions included a lifetime history of psychotic disorders or mania, autism spectrum disorder, intellectual disability, substance use disorder, or active suicidal or homicidal ideation on presentation requiring inpatient hospitalization. Participants were required to remain on stable dosing of their current psychiatric medication regimen for the four weeks prior to the first infusion, and during the four-week trial itself. All participants underwent a physical examination and laboratory screening, and electrocardiography (ECG), and female participants received urine pregnancy testing. Treatments were randomly assigned by the pharmacy, with participants receiving a single infusion of either ketamine (0.5 mg/kg over 40 minutes) or midazolam (0.045 mg/kg) on Day 1, and the alternate compound 2 weeks later. Midazolam was chosen as an active placebo in keeping with its similar PK profile and precedent[15]. Only the pharmacy was aware of drug identity, and all study personnel, including investigators,

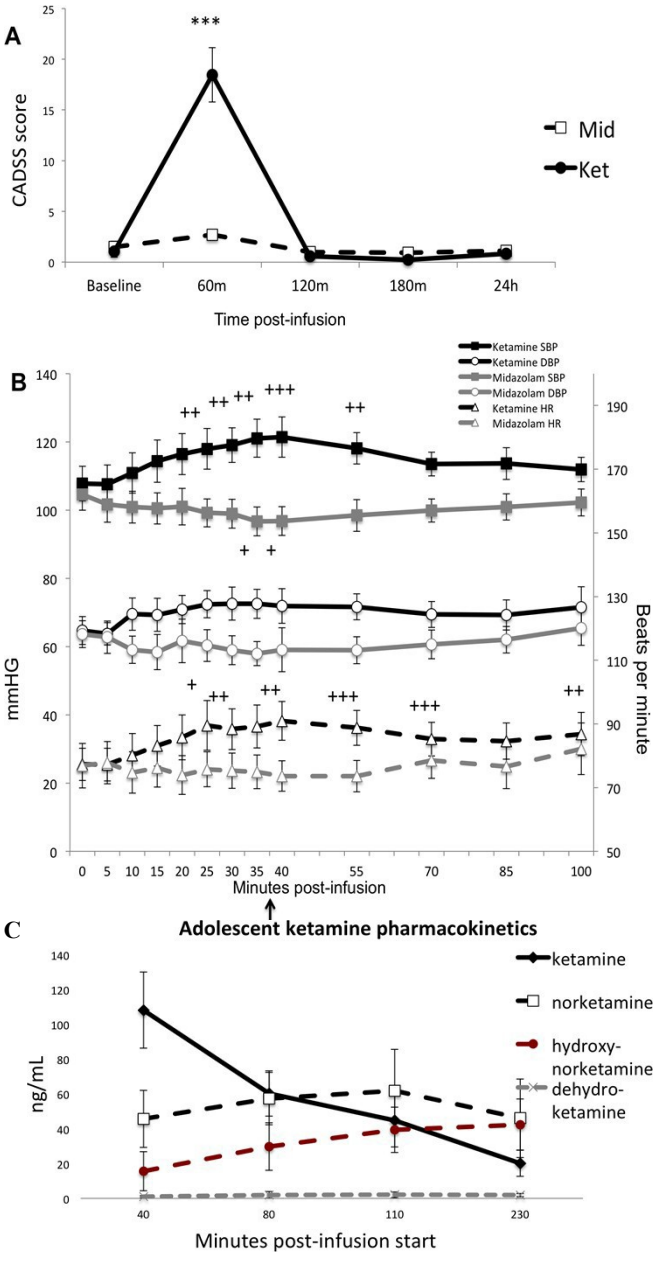


Fig 2: Infusion side effects and PK ***(A)*** CADSS scores are higher at 1 hour compared to midazolam. (B) Ketamine transiently increases systolic blood pressure (SBP) and heart rate relative to baseline readings. Arrow indicates the end of the infusion. (C) Serum levels of ketamine and it's three principle metabolites are shown at four post-infusion timepoints*+p < 0.05 ++p < 0.01, +++p < 0.001 timepoint versus baseline; ***p<0.001 ketamine versus midazolam*

raters, anesthesiologists, subjects, and data analysts, were blinded to randomization order. To further maintain blinding, efficacy raters were not present during infusions. On infusion days, two intravenous catheters were placed in each arm, one for medication infusion and the other for PK blood draws. An ECG was performed, and pulse, blood pressure, and pulse oximetry were checked every 5 minutes during the infusion, and every 15 thereafter. Acute dissociative side effects were assessed by the Clinician-Administered Dissociative States Scale (CADSS)[16]. A different trained rater, absent during the infusion and blinded to the intra-infusion ratings, administered pre-infusion baseline and post-infusion ratings, up to 14 days. The primary outcome was depression severity (Montgomery-Asberg Depression Rating Scale (MADRS)[17]) 24 hours post-infusion.

Of the 26 evaluated adolescents, 19 consented to the trial (7 did not meet criteria), and 17 received the first infusion (one subject withdrew due to an undisclosed medical condition and the other had a panic attack prior to starting the infusion pump and decided to withdraw). Of the remaining 17, 16 completed both infusions (one subject improved after the first infusion (later determined to have been ketamine) and dropped out of the trial in order to receive community ketamine). The study sample had significant depressive symptoms (average screening CDRS of 63.2
(±17.1) and MADRS of 33.1 (±9.3)) and a relatively chronic disorder (average duration of current episode of 21 months (± 18.8), with a median of 12 months). The average participant age was 15.5 years, and all ages between 13 to 17 years old were represented. All participants had failed at least one antidepressant trial, but the sample on average had failed 3.24 (± 1.9) prior antidepressant treatments and 6.1 (± 5.5) total psychiatric medications.

Ketamine and midazolam treatments were well-tolerated and there were no serious adverse events. Dissociative symptoms were observed with ketamine treatment, although they were time-limited (**Fig 2A**) and well tolerated. No significant dissociative symptoms were experienced in the days following the treatment. Aside from the participant who withdrew just prior to receiving infusion 1, no participants experienced significant dysphoria or panic, and no participants required emergent diazepam use. Hemodynamic data showed significant effects of ketamine and midazolam (**Fig 2B**), but there were no serious hemodynamic events. Ketamine raised systolic blood pressure (SBP) up to 55 minutes after infusion start (i.e. 15 minutes following the termination of the infusion), and minimally raised diastolic blood pressure (DBP). Four subjects (of 17) met criteria for

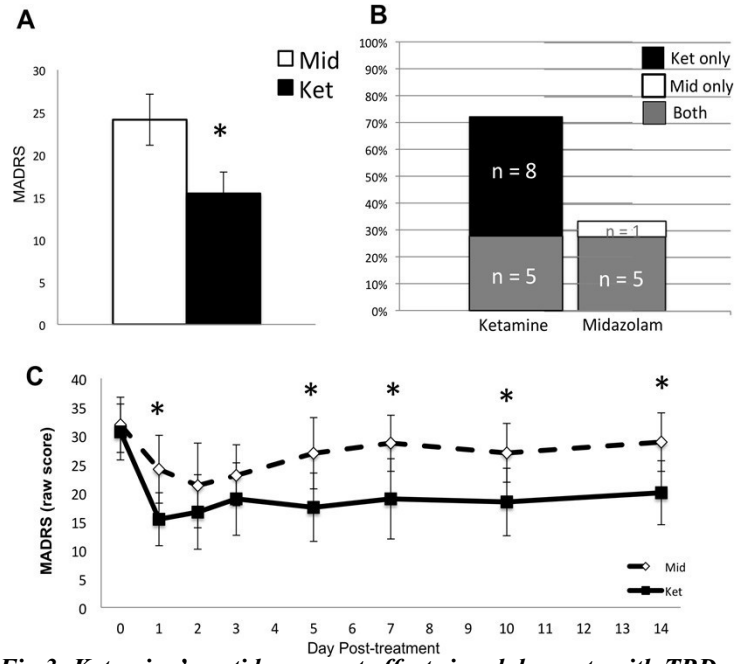


***Fig 3: Ketamine's antidepressant effects in adolescents with TRD**. **(A)** Ketamine reduced depressive symptoms relative to midazolam, as measured via MADRS scores, 24 hours following infusion, paired t-test (n=16). **(B)** 76% of the sample had a significant clinical response to ketamine (i.e. a greater than 50% reduction in MADRS within the first three days following treatment). 35% of the sample had a significant antidepressant response to midazolam. These groups share 5 participants who had a response to both. **(C)** Timecourse depicting actual average MADRS scores after ketamine or midazolam: in pairwise comparisons, ketamine significantly reduces depressive symptoms relative to midazolam over fourteen days, with significant separation at Days 1, 5, 6, 10, and 14 (independent t-tests at each data point), *p<0.05 ketamine vs midazolam*

adolescent Stage 2 hypertension (140/90)[18] during the ketamine infusion, but none persisted past the end of the infusion and none exceeded 150/95. Ketamine also raised heart rate (HR) with an increase from baseline up to 100 minutes post-infusion start, although no participant had a HR exceeding 120 beats per minute.

PK data were obtained and processed through our collaboration with Dr. Zarate (NIMH), including serum ketamine (K), norketamine (NK), hydroxynorketamine (HNK), and dehydronorketamine (DHNK) at baseline, 40m, 80m, 100m, and 230m post-infusion start (**Fig 2C**). While there is no adult comparison group built into this study design, the ranges of ketamine and NK are similar to those reported in adult PK studies using the same dose, timepoint, and analysis lab[19]. When compared with this published adult data, adolescents show 1.5 times greater HNK concentrations and five-fold lower concentrations of DNHK at all timepoints. These findings are intriguing given the purported NMDA-independent therapeutic actions of HNK[2], but require verification in a sample directly comparing adolescents and adults.

2.2.2.2 Ketamine single dose adolescent study—efficacy

The primary outcome for this study (NCT02579928) was the MADRS at Day 1, comparing ketamine to midazolam. Ketamine significantly reduced MADRS scores compared to midazolam at Day 1 following infusion (midazolam: 24.13 ±12.08, 95% CI: 18.21 to 30.04, versus ketamine: 15.44 ±10.07, 95% CI: 10.51 to 20.37, mean difference of -8.69 ±15.08, 95% CI: -16.72 to -0.65, df=15, p= 0.036) (**Fig 3A**). There were no significant carryover effects observed in the trial. In the sixteen participants who completed both phases of the study, the average MADRS score at pre-infusion baseline was 31.88 (±9.82, 95% CI: 27.06 to 36.69) for midazolam and 30.56 (±10.63, 95% CI: 25.36 to 35.77) for ketamine. The mean difference between treatment arms at baseline was -1.31 (±8.73, 95% CI -7.89 to 5.27, df=15, p=0.68). Individual participants were assessed for significant clinical responses to ketamine or midazolam, which were defined as a greater than 50% reduction in MADRS score within three days of treatment (**Fig 3B**). Participants were significantly more likely to respond to ketamine than midazolam (McNemar χ^2=4.0, df=1, p=0.046). 77% of the sample had a response to ketamine, which comprised 8 participants responding only to ketamine, and 5 participants demonstrating a response to both ketamine and midazolam. 35% of the sample responded to midazolam, comprised of the 5 participants who responded to both infusions, and a single participant who responded only to midazolam and not to ketamine. Three participants did not respond to either infusion. **Fig 3C** displays the time-course data, showing that a significant separation of ketamine from midazolam persists until 14 days (the latest timepoint).

2.2.2.3 Neuroimaging of depressed adolescents treated with ketamine

A small subset of participants from the above trial participated in resting state functional MRI imaging, imaged at 3 timepoints in an early pilot (N=3; baseline, 24-hours after infusion 1 and 24-hours after infusion 2). Imaging consisted of a structural scan (3D, T1, T2) and a resting-state run in order to examine resting state functional connectivity (FC) in these subjects. FC both between and within pre-identified canonical networks were compared (default mode, salience, sensorimotor, subcortical, frontoparietal, and medial frontal). Of the three subjects, two had a significant clinicalresponse to

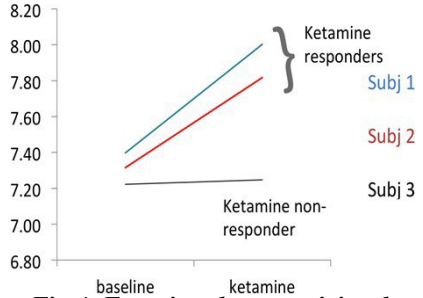


Fig 4. Functional connectivity changes post-ketamine in adolescents.

ketamine, while the third did not. Ketamine responders showed increased within-subcortical network functional connectivity whereas the ketamine non-responder did not. (**Fig 4**). This data demonstrates that imaging adolescents in a ketamine clinical trial at rapid timepoints is feasible.

2.2.2.4 Adolescent repeat ketamine dosing experience

The antidepressant and anti-suicidal effects of a single dose of ketamine are ephemeral[4], and repeat dosing strategies have been employed to extend the duration of wellness in adults[20,21]. Based on our case report showing rapid, potent antidepressant and anti-suicidal effects of ketamine in a severely ill teenage boy with a repeat dosing paradigm[10], we obtained pilot funding for a repeat dosing study (NCT03889756). This pilot study recruited a more severe depression phenotype, requiring 2 prior failed antidepressant trials, and did not have a restriction on suicidal ideation as the initial crossover trial did. The design was a 2-phase design: a 3-week parallel, midazolam-controlled double blind phase followed by a 6 month open phase in which participants assigned to midazolam who remained depressed could receive open ketamine and all participants received standard of care depression treatment. This design also incorporated a limited number of symptom triggered-maintenance ketamine infusions during the open phase in those who responded to ketamine. We have run 3 participants through this protocol, all 3 of whom completed the blinded phase, and two of three completed the open phase. Two did not respond to ketamine, but improved in the open phase, and one responded to ketamine (antidepressant and anti-suicidal responses (suicidal ideation measured via MADRS item 10 and SSI-5)) and relapsed in the fourth month.

2.2.3 Background/prevalence of research topic

Youth suicide: Suicide is now the second leading cause of death in young people in the United States (10 to 34 years)[22] and is globally the leading cause of death in female adolescents (15 to 19 years)[23]. Suicide is a complex behavior that represents a convergence of genetic[24], biological, psychological, social, and cultural factors[25], and methods to reduce teen suicide are a top mental health priority[26]. A significant challenge to the task is that many, if not most, of the risk factors associated with youth suicide are either fixed (genetic or biological factors, perfectionism, low socioeconomic status, LGBTQ status, parental separation, divorce, or death, and parental psychiatric disorder)[25] or historical (personal or family history of a suicide attempt[27], early adverse experiences including physical or sexual abuse[28]). Thus, the identification of modifiable risk factors is essential. Major depressive disorder increases the risk of adolescent suicide by 30-fold[29] and is associated with hopelessness, another potent suicide risk factor[30]. Suicide risk is further increased with treatment-resistance[31] and in the months following discharge from an inpatient psychiatric unit[32]. *This work is significant as it tests a novel, rapid-acting anti-suicidal intervention in high-risk adolescents.*

Pediatric patients deserve evidence-based care: Pharmacologic anti-suicidal interventions for pediatric patients do not currently exist, and evidence-based medications for pediatric TRD are similarly scarce[33]. The sole strong recommendation for Child Psychiatrists for adolescents with TRD comes from the Treatment of Resistant Depression in Adolescents trial (TORDIA (N=334)[34]), which completed over a decade ago. There have been no major NIMH-sponsored trials for pediatric TRD since, and once practitioners have followed the TORDIA recommendation to switch to a different selective serotonin reuptake inhibitor (SSRI) and add cognitive behavioral therapy (CBT), they are stranded in non-evidence based territory[33]. Lack of evidence has not prevented children from becoming depressed, nor has it

stopped Child Psychiatrists from attempting to help them. The result is that our highest risk children are increasing exposed to interventions with uncertain efficacy and higher harm potential (e.g. the escalated use of atypical antipsychotics[35]). The adolescent brain is a unique pharmacologic substrate, with active maturation of monoaminergic[36] and glutamatergic[37] systems thought to underlie mood disorders and suicide[38,39], highlighting the need for pediatric-specific evidence. *This work is significant as it will provide the first evidence for a pharmacologic treatment beyond TORDIA recommendations in youth with TRD and high suicide risk.*

Pediatric ketamine: community use without data or safety monitoring: Clinical communities are now well aware that ketamine, a glutamatergic modulator, rapidly reduces depression and suicidal thinking in adults with TRD[40]. Consistent with the sad reality of the pediatric evidence base[33], promising interventions in adults will be used off-label in Child Psychiatry practice if they are available[35], and ketamine is no exception[41,42]. While there is case report data[10] and a small open-label trial[8] suggesting potential utility in pediatric depression and suicidality, placebo-controlled assessments are critical to separate genuine pharmacologic from non-specific effects, to allow for an informed risk/benefit analysis. While we have recently demonstrated that a single dose of ketamine is superior to an active placebo, midazolam, in adolescents with TRD (**see Clinical Experience, 2.2.2**), repeat dosing presents additional safety concerns in pediatric patients. While repeat dosing appears to extend the duration of wellness in adults[20], animal studies raise specific neurotoxicity concerns regarding high-dose exposures in developing populations[13,14], in addition to age-neutral concerns regarding cardiovascular, hepatobiliary, urothelial, neurocognitive, and abuse potential risks raised by human studies of heavy recreational ketamine users[43-45]. The use of off-label ketamine in pediatric communities occurs without oversight, regulation, or the evidence based needed to determine dosing and safety monitoring strategies. *This work is significant in providing safety, efficacy, and symptom trajectory data for a conservative repeat dosing ketamine paradigm in adolescents.*

Clinical need for predictive models of treatment response: Psychiatry suffers from a lack of predictive power in choosing treatments for patients. Indicators that predict the presence, quality, and durability of antidepressant[46] and anti-suicidal responses[47] and that generalize to novel cohorts of patients remain elusive. For example, a promising finding of ketamine enhancing whole brain connectivity[48], recently failed to replicate in a novel dataset[31]. Replication and validation are inherent to the term “predicts”, which is too often used loosely as a synonym for “correlates with”. True prediction means that a finding in one data set will also hold for novel subject, and evaluation of markers using either cross-validation or external data is needed for clinical utility [49,50]. *Establishing predictive models is necessary to translate biological findings into clinical tools.*

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Connectome-Based Predictive Modeling (CPM) as a promising data-driven approach:
Functional connectivity analyses generated from fMRI data have produced useful data about brain wiring in health and disease across the lifespan, and the NIH's investment in the initial Human Connectome Project[51] indicates the importance of this modality. Despite the excitement, we lack clinically oriented tools that can take advantage of this connectivity

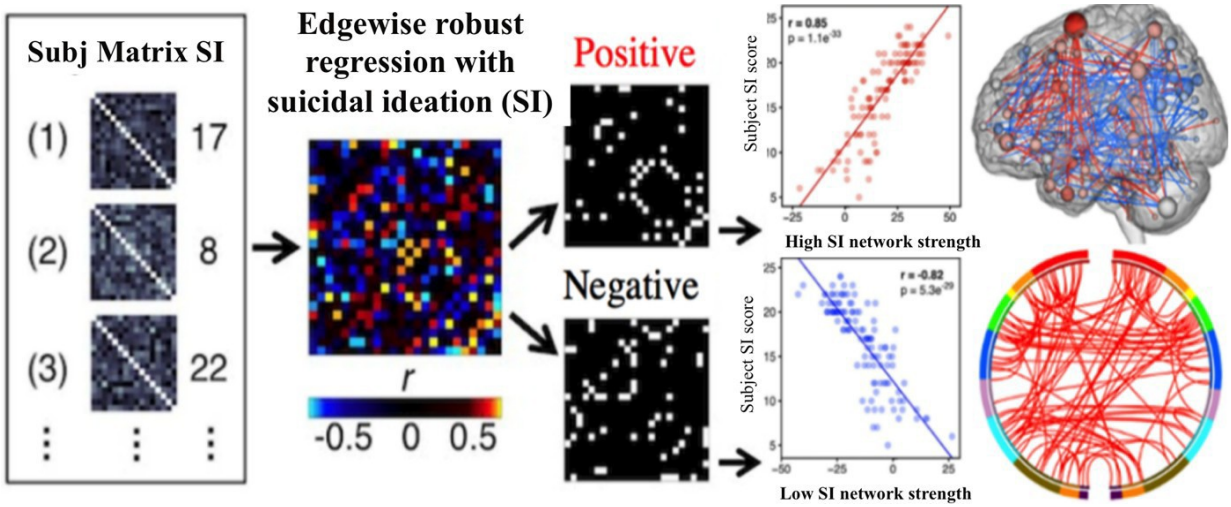

Fig. 5: Connectome-based Predictive Modeling (CPM) Schematic: *In CPM, atlases and connectivity data are used to generate subject-specific connectivity matrices, then edge strength (the elements of the connectivity matrix) can be regressed with a subject score (suicidal ideation in this case, labeled SI) and a predictive model relating connectivity to SI can be built. These models then identify the relevant functional phenotypes (essentially the edges and their strength) associated with SI. The models are validated (internally) through cross-validation and tested for significance. Derived networks (the functional phenotypes) are complex and span the whole brain.*

information. We have developed connectome-based predictive modeling (CPM) as a data-driven approach to relate brain functional organization to behavior [52-57]. There are several critical components to this computational technique that enhance individual differences and improve generalizability in heterogenous populations, which are necessary for a tool with any clinical utility. First, as a step towards individualized medicine, we utilize individualized functional brain atlases that are specific to both the patient and their brain state (from one task to another and at rest), which improve model prediction[58]. Second, our methods rely on perturbing cognitive circuits across multiple Research Domain Criteria (RDoC) domains using tasks. Just as a heart can appear normal at rest, stressing the heart may reveal important pathology. We have showing that "stressing" brain circuits significantly improves the ability to detect individual differences, particularly compared to rest[59]. Finally, modeling methods that are designed to generalize across multiple behaviors and diagnostic groups are needed[60]. For example, while task conditions are likely better at generating models of behaviors related to the circuits they perturb, it is unlikely that a single task can be developed that is optimal for complex dimensions of interest, like suicidal ideation. Instead, methods that use data from several tasks, tweaking the brain circuits along multiple dimensions, are thought to span the functional space sufficiently, such that the optimal rotation can be found to maximally separate subjects along the ideation axis, leading to better predictive models that generalize across heterogeneous groups of patients, as we have shown in autism[61], ADHD[53], and cocaine use disorder[62]. *This work is significant as it integrates a predictive brain-based approach[63] into a clinical trial design in order to identify the functional phenotypes, or fingerprints[64], predictive of post-treatment suicidal ideation in high-risk adolescents, and how those are modified with a rapid-acting treatment. The data generated here will be internally validated*

with cross-validation, and will provide a strong foundation for external validation in larger subsequent trials and independent data sets.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

(1) **Ketamine:** Ketamine has a wide margin of safety and is usually given in doses of 1mg/kg – 4.5mg/kg IV over 60 seconds when used a sole anesthetic agent. It has been used routinely in pediatrics for over 40 years, typically at higher doses than proposed in the current study. Ketamine has been used to treat treatment-refractory depression in adults at Yale for over 20 years, with robust evidence for medical and psychiatric safety. There are several potential risks associated with ketamine use. These include **1) Cardiovascular**: elevated blood pressure and pulse rate (relatively common and are dose-dependent) and very rare changes in cardiac rhythm. **2) Respiration:** respiratory rate is frequently elevated; however, with high dose administration severe respiratory depression and apnea have been reported (<1.5% of transient apneic events at anesthetic doses in pediatric emergency room settings. Ketamine also has rarely been associated with laryngospasm**. 3) Eyes**: ketamine has been associated with slight elevations in intraocular pressure**. 4) Gastrointestinal**: Anorexia, nausea and vomiting have been observed, however this is usually not severe. Vomiting has been seen in <4% of children in emergency room sedation settings. **5) Neurological:** enhanced skeletal-muscular tone resulting in tonic clonic movements have rarely been observed with acute administration**. 6) Hepatobiliary:** While not listed as an acute risk in ketamine's safety insert, case series data in people who recreational abuse ketamine have shown laboratory signs of hepatic inflammation. **7) Urinary**: in people who recreationally abuse ketamine, bladder inflammation and cystitis have been reported, which appear to be both dose and frequency dependent. **8) General:** Anaphylaxis, local pain at injection site and transient rash have been described at the case report level. **9) Psychological:** ketamine has been associated with a variety of transient symptoms including, but not limited to anxiety, dysphoria, disorientation, insomnia, flashbacks, hallucinations, and psychotic episodes. Emergence reactions have occurred in approximately 12% of subjects given anesthetic doses of ketamine. These symptoms usually last no more than a few hours. However, recurrences have taken place up to 24 hours after the anesthetic dose administration. Recovery agitation after ketamine iv sedation in the pediatric emergency room has been seen in <1.5% of children. It is also believed that the incidence of the psychological disturbances is reduced with the use of lower doses. No residual adverse psychological effects are known to have resulted from the medical use of ketamine**. 10) Substance abuse/dependence:** Ketamine has been reported as a drug of abuse. Reports suggest that Ketamine dependence and tolerance are possible following prolonged administration. A withdrawal syndrome with psychotic features has been described following discontinuation of long-term ketamine use. Therefore, ketamine should be prescribed and administered with caution. It is unclear whether exposure to ketamine in the laboratory can result in ketamine use or abuse. All participants are encouraged not to participate if they have concerns about the possibility of ketamine abuse. Also, they are asked to contact us immediately if they become aware of a desire to use or abuse ketamine. All participants are advised that we would refer them to an appropriate treatment facility if necessary. In our experience doing research with ketamine, we are unaware of individuals abusing ketamine as a result of study participation. **Unknown risks of repeated dosing:** Not all of the risks of repeat ketamine dosing may be known, despite this paradigm being conservative.

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(2) **Midazolam:** Midazolam is used frequently in the pediatric emergency room and in sedation settings, either as a sole agent for anxiolysis, or in combination with other sedatives and anesthetics for more complex or prolonged procedures. It is typically given at doses of 0.5-0.5mg/kg over 2 minutes, depending on the intended level of sedation (mild versus deep sedation). It has been used routinely in Pediatrics since the 1990's, typically at higher doses than those described in this study. There are several potential risks associated with midazolam use**: 1) Cardiovascular:** decrease in blood pressure (hypotension in <3% of pediatrics patients at sedation dosing ref) and very rarely, changes in cardiac rhythm**. 2) Respiration**: apnea, cough, hiccups, decreased tidal volume and respiratory rate (transient apnea has been reported in <3% of pediatric patients at sedation dosing); with high dose administration severe respiratory depression, airway obstructions, apnea, and respiratory arrest have been reported**. 3) Eyes**: may cause nystagmus **4) Gastrointestinal**: can be associated with nausea and vomiting (<3% of adult patients). **5) Neurological**: may cause drowsiness, headache, over-sedation; like all benzodiazepines, midazolam has amnestic properties, more notable as dose increases **6) General**: Anaphylaxis, local pain at injection site and transient rash have been reported. **7) Psychological**: midazolam can be associated with paradoxical agitation (particularly in the elderly or in very young children), however is estimated at <1% of patients; there are also rare reports of emergence delirium, euphoria, and hallucinations, although these are associated with higher dosing that proposed here, Additionally, fast-acting reversal medications are available (flumazenil) that can terminate midazolam's effects should the patient experience side effects during the infusion**. 8) Substance abuse/dependence**: As with all benzodiazepines, physical and psychological dependence is associated with prolonged use. The four-dose infusion paradigm proposed in the study is not expected to produce any physiological dependence. As all benzodiazepines have some abuse potential, midazolam should be prescribed and administered with caution. Midazolam is used frequently in pediatric medical settings, at higher doses administered over shorter periods of time, and this use has not been linked with subsequent addition problems. That said, all participants are encouraged not to participate if they have concerns about the possibility of midazolam abuse. Also, they are asked to contact us immediately if they become aware of a desire to use or abuse midazolam. All participants are advised that we would refer them to an appropriate treatment facility if necessary.

(3) **MRI:** Magnetic resonance (MR) is a technique that uses magnetism and radio waves, not x-rays, to take pictures and measure chemicals of different parts of the body. The United States FDA has set guidelines for magnet strength and exposure to radio waves, and we carefully observe those guidelines. Participants will be watched closely throughout the MR study. Some people may feel uncomfortable or anxious. If this happens to a participant, he or she may ask to stop the study at any time and will be taken out of the MR scanner. On rare occasions, some people might feel dizzy, get an upset stomach, have a metallic taste or feel tingling sensations or muscle twitches. These sensations usually go away quickly we ask participants to tell the research staff if they occur.

There are some risks with an MR study for certain people. If subjects have a pacemaker or other metal objects inside the body, they may not be in this portion of the study because the strong magnets in the MR scanner might cause harm. Another risk is the possibility of metal objects being pulled into the magnet and hitting the subject. To lower this risk, all people involved with the study must remove all metal from their clothing and all metal objects from their pockets. We also ask all people involved with the study to walk through a detector designed to detect metal objects. No metal can be brought into the magnet room at any time. Also, once the participant is in the magnet, the door to the room will be closed so

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that no one from outside accidentally goes near the magnet. We ask subjects to read and carefully answer the questions on the MR Safety Questionnaire related to personal safety.

This MR study is for research purposes only and is not in any way a complete health care imaging examination. The scans performed in this study are not designed to find abnormalities. The principal investigator, the lab, the MR technologist, and the Magnetic Resonance Research Center are not qualified to interpret the MR scans and are not responsible for providing a health care evaluation of the images. If a worrisome finding is seen on a participant scan, a radiologist or another physician will be asked to review the relevant images. Based on his or her recommendation (if any), the principal investigator or consulting physician will contact the participant and their parent to inform them of the finding and recommend seeking medical advice as a precautionary measure. The decision for additional examination or treatment would lie only with the participant/parent and their physician. The investigators, the consulting physician, the Magnetic Resonance Research Center, and Yale University are not responsible for any examination or treatment received based on these findings. The images collected in this study are not a health care MR exam and for that reason, they will not be routinely made available for health care purposes.

(4) **Blood drawing/intravenous placement:** Bruising or thrombosis can occur with placement of the intravenous line. A total of 70cc will be drawn over 2 weeks (30cc at baseline, 40cc on first infusion day (Day 1)), which equates to 1.2cc/kg – 2.0cc/kg (based on average weights of 13-17yo adolescents). For those who cross over to open ketamine at the end of week two, an additional 40cc will be drawn on the first open infusion day (i.e. a total of 110cc over the first 3 study weeks, equating to 1.7cc/kg - 2.4cc/kg based on average male and female adolescent weights). This amount drawn is considerably less than the 9.5cc/kg over 8-week requirement set by the review board. The risks of blood draws include brief pain at the time of needle insertion, bruising, swelling at needle site and rarely, fainting or infection.

(5) **Psychiatric evaluation, rating scales and questionnaires:** These are all non-invasive, should add no risk, and have been used without difficulty or adverse events in previous studies with a similar population. The major disadvantage is the time taken to completethem.

(6) **Clinical Deterioration:** There is a risk that a participant may experience an increase of suicidality or depressive symptoms due to the natural course of the illness, poor response to ketamine administration, or receipt of active placebo (midazolam) during the blinded phase (these patients will be offered ketamine treatment after Day 11). Because subjects will be asked to refrain from changing any psychotropic medication over the course of the blinded phase of the study, clinical progress will be monitored closely with frequent assessments, ratings scale, and contact with clinic personnel. The following are criteria for evaluation and possible pharmacological and/or non-pharmacological treatments: (1) an increase of 25% in C-SSRS or MADRS score at any time over the course of treatment, and (2) new-onset of suicidal ideation or an increase in suicidal ideation. In the event that a subject is judged to remain significantly depressed and/or at increased risk for suicidality at the end of the study, we will help make appropriate referrals to outpatient providers, intensive outpatient programs or inpatient psychiatric hospitals as clinically indicated. Investigators may continue close monitoring of significantly “at-risk” subjects until such referrals are provided and available to the study participant. Additionally, the standard of care treatment for a child or adolescent who insufficiently responds to initial antidepressant therapy is to try another antidepressant or add an additional medication. By participating in this trial, the child or adolescent would be delaying the standard of care change in their medication by 2-4 weeks.

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(7) **Confidentiality:** All information that is collected in connection with this study will remain confidential and will only be disclosed as required by U.S. or state law. This studyis

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covered under an NIH Certificate of Confidentiality, which is important given our periodic urine drug testing. Every foreseeable precaution to protect confidentiality will be taken. Nevertheless, despite such precautions, there is a potential risk for a loss of confidentiality due to reasons beyond our control (e.g., computer hacking).

(8) Remote Assessments: Like online shopping, videoconferencing technology has some privacy and security risks. It is possible that information could be intercepted by unauthorized people (hacked) or otherwise shared by accident. This risk can't be completely eliminated, however Yale has approved the use of an encrypted version of zoom for videoconferencing sessions because the appointments take place over a secure encrypted network. When meeting with the team remotely for study-related activities, participants should be in a private space.
(9)

RISK MITIGATION PLANS:

1. Permission and Assent. At the point of enrollment into the study, a researcher will describe the protocol and specific procedures, as well as risks and benefits associated with the study participation, to the participant and his or her parent(s). The researcher will answer any questions the participant and their family might have regarding the protocol. Families are also encouraged to discuss the study with their outpatient psychiatrist. Once the family has had time to fully digest the procedures, risks, and benefits, the researcher will ask the participant to sign and IRB-approved assent form and their parents or guardians to sign an IRB-approved permission form agreeing to participate in the study. If there are two parents or guardians, both most provide permission and each must sign a separate permission form. Following the permission, at the beginning of the visit, the subject will be briefed on the specific procedures to be administered and will have an opportunity to ask additional questions. The researchers will remind subjects and their families that participation is voluntary and that the protocol can be stopped at any time with no obligation to continue and no penalties whatsoever. Refusal to participate will not affect the subject's relationship with any clinics at Yale or the hospital or their ability to seek medical care. Participants and families are given a copy of the signed permission forms. Original paper forms will be stored in a separate locked file cabinet in a locked office. These forms are not stored with any data or other PHI, and they do not have subject ID numbers on them. In the case of electronic consenting, originals will be stored in Part 11-complaint RedCap.

2. Protection Against Risks Enumerated Above

(1) Ketamine Administration: The dose of ketamine established in prior research (0.5 mg/kg over 40 minutes) will be used in this study to minimize risks. The maximum total single dose allowed in this study will be 40mg, corresponding to a weight of 80kg, as suggested by the FDA. In order to minimize the acute ketamine risks described above, vital signs will be monitored regularly throughout and for the first hour following the ketamine infusion. An ACLS-trained physician will be present for all infusions, with access to rescue equipment. Subjects will be monitored for at least 2 hours following ketamine infusion by Dr. Bloch or another study physician. A study doctor (Principal Investigator (PI) of the trial) will be present at all times during the infusion and recovery. In the event that a research subject has a significant psychiatric event requiring hospitalization, they will be treated on the adolescent unit (LV2) at Yale Psychiatric Hospital (YPH). Emergent medical care would be provided at Yale-New Haven Hospital. All participants will be asked not to engage in demanding work for the first 3 days after the ketamine infusion. If participants develop

psychiatric symptoms, we may admit them to the hospital. Hospitalization may be involuntary if patients are in imminent danger of harming themselves or others.

The permission forms will provide a description of what participants may experience during the intravenous ketamine infusion at a dose of 0.5 mg/kg over the course of 40 minutes, so that they will be well prepared for possible responses to ketamine. Participants will be told that some people have reported mildly decreased concentration or a “hangover” on the day after ketamine infusion. A research nurse will be present throughout the study to monitor the patient’s response and note any changes in physical or mental state. A research

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clinician will be present throughout the study to offer support and to help clarify the progress of the test day in case the medication causes feelings of confusion. the study PI and the study psychologist will also be available. The physician would be informed in case of any significant changes in the patient's physical or mental state. The trained research nurse will also offer support and provide consistent "reality testing" for individuals experiencing confusion or transient psychosis. Oral diazepam (5mg) will be kept available to control markedly distressing behavioral effects of ketamine, should they emerge. Oral ondansetron (4-8mg) will be kept available to control any significant nausea or vomiting.

All participants will be asked to contact the study team at any time if any unpleasant effects occur. All participants will be given a card, which provides contact information for the study PI and study staff. All participants have close scheduled contact with study physicians and raters (twice weekly during acute blinded phase; weekly in open phase). Participants will be instructed and encouraged to contact the treatment team between scheduled meetings should their distress worsen. In order to enroll in the study, patients must not have a lifetime history of substance abuse or dependence, thereby reducing the risk of ketamine substance abuse/dependence by study participants.

(2) Midazolam Administration: The weight-based midazolam dosing established in prior ketamine trials in adults (0.045mg/kg) will be used to minimize risks, as this is considered a very low dose compared to the sedation literature. The maximum total dose allowed in this study will be 3.6mg per infusion, corresponding to a weight of 80kg. Midazolam has one of the shortest half-lives of all of the benzodiazepines, and thus adverse events relating to the drug typically are not observed more than 30 minutes following drug administration. In order to minimize the risks of midazolam described above, subjects will be medically screened prior to enrollment in the study, and participants will have identical hemodynamic monitoring as described above for ketamine infusions.

(3) MRI risks: All subjects will be screened for any contraindications to MRI scanning using the Yale University MRI Safety Questionnaire. Additionally, all participants will walk through a ferromagnetic detector prior to entering the MRI. MRI procedures will only be performed by trained personnel of the Magnetic Resonance Resource Center. Any subject who is distressed in the MR scanner and asks to terminate an MR imaging session will be removed immediately. During the permission process, we will ensure the subject understands that they may withdraw from the study at any time. If anything unusual in the MR scans is noticed by the research staff, the images will be shown to a neuroradiologist, Dr. Robert Fulbright, who will decide if the subject should be informed and a follow-up clinical exam recommended. A clinical follow-up will not be provided by the research program and must be pursued independently via the subject's health care provider.

(4) Blood drawing/intravenous placement: The risks of blood draws and intravenous line placements are rare, and when these are done under sterile conditions by trained personnel the occurrence is even more remote. Numbing spray and distraction techniques will be offered prior to offset the discomfort of iv placement, as is commonly done in Pediatric medical settings. On the first infusion day, when pharmacokinetic labs are drawn, blood draws will occur off of the non-infusing iv in order to prevent the discomfort of repeated needle sticks. A total of 70cc will be drawn over 3 weeks (30cc at baseline, 40cc on first infusion day (Day 1)). For those who cross over to open ketamine at the end of week two, an additional 40cc will be drawn on the first open infusion day (i.e. a total of 110cc over the first 3 study weeks). This amount ranges from 1.6cc/kg for a 45kg adolescent to 1.1cc/kg fora

65kg adolescent (weight range of 45kg – 65kg for 13-17yo girls and boys). This amount is well below the maximum of 9.5cc/kg over 8 weeks.

(5) Psychiatric evaluation, rating scales, and questionnaires: In order to minimize risks associated with the psychiatric ratings and ensure the accuracy of reporting, these measures will be administered by trained research staff and supervised by a study physician. Participants will be informed that they do not need to answer any question on the rating scales or questionnaires that make them feel uncomfortable. Participants will also be informed they can take a break if they become tired from any of the questions or ratings.

(6) Clinical deterioration: Given the high-risk population in this study, safety planning and frequent risk assessment are critical. A safety planning module is included in the baseline visit, which will be conducted in collaboration with our study CBT therapist. The safety planning module is described in more detail in Section 6.1, Study Intervention Administration, Baseline Visit. In brief, the safety plan module focuses on the development of an individualized safety plan that includes support from multiple individuals and includes internal and external coping strategies to be implemented should the participant experience suicidal urges. A copy of the plan is provided to the participant, parents, and uploaded into their EPIC medical record.

At each visit, risk assessment is an important component of interactions between participants and staff. During the double-blind phase, a credentialed clinician meets with the participant before discharge from each study visit to make a clinical determination of risk of imminent harm to self or others. Determination of risk involves inquiring about the presence and intensity of suicidal thinking, differentiating between passive and active thoughts, evaluating the presence of risk and protective factors, and inquiring about plans, access, and intent. In the follow-up phase of the trial, a risk assessment and review of safety plan is conducted at each clinical visit, as is our standard clinical practice. The assessment occurs during the CBT session during the first 8 weeks, and during the medication management check-in during the last 8 weeks once CBT has ended.

In the event that a participant is deemed to be an imminent safety risk to themselves or others while at a study visit, they will be transported to the Emergency Room for evaluation. For times in between visits, participants and parents will be given a phone number for 24-access to a clinical staff member. For any calls to this number, risk assessments are conducted over the phone, with appropriate referral to emergency services if warranted. Any no shows to study appointments will be vigorously pursued.

If a participant shows significant worsening of symptoms, he or she will be offered removal from the study and evaluated for clinically appropriate pharmacological and non-pharmacological treatments by a clinic psychiatrist in collaboration with their community psychiatric provider. If a patient is doing poorly during the blinded phase, a thorough discussion will take place between the subject, his or her guardian, study physicians and outpatient treaters as to whether subject is able to continue until Day 11, at which time they would be offered open ketamine treatment on Day 15 (assuming that they were receiving midazolam during the blinded phase). Patients will be informed that the decision to initiate a course of psychotropic medication will not affect their eligibility to participate in future studies, to receive treatment at the Yale Child Study Center or Yale New Haven Hospital, or to receive treatment on a private basis from a referring clinician. In the event that a subject is judged to remain significantly depressed and/or at increased risk for suicidality at the end of

the study, we will help make appropriate referrals to intensive outpatient programs or inpatient psychiatric hospitals as clinically indicated, in collaboration with their community psychiatric provider(s). Investigators may continue close monitoring of significantly "at risk" subjects until such referrals are provided and available to the study participant.

Another risk for mental illness and clinical deterioration is the experience of abuse (for example parental abuse of a child or intimate partner violence). As mandated reporters, any suspicion of abuse of a minor must be reported to the Department of Children and Families (DCF) in Connecticut. In the event that abuse is suspected, any participant would be provided a list of additional resources and possible referrals. While every participant must have an outpatient mental health provider in place in order to participate, we will provide additional information for trauma focused programs (e.g. the Yale Childhood Violent Trauma Center). We will also provide a list of additional support resources (e.g. DCF and the state of Connecticut mental health services (211) provide a number of support groups for intimate partner violence, sexual assault and incest, and overall mental health).

(7) Confidentiality risks: Private identifiable information will be collected (name, date of birth, age, telephone number, address, medical and psychiatric history, diagnoses, laboratory tests, and psychiatric rating scores) but will be kept confidential and will not be divulged in any publication emanating from this work. Please see section **10.1.2**, for the protections in place to mitigate confidentiality risks. The Sponsor-Investigator is responsible for monitoring the data and conducting performance safety reviews every six months. The Sponsor-Investigator, the Yale the Human Investigation Committee (HIC)/IRB, and the NIMH DSMB have the authority to stop or modify the study. The Sponsor-Investigator will evaluate any adverse events and determine whether they affect the Risk/Benefit ratio of the study and whether modifications to the protocol or permission/assent forms are required. A summary of all adverse events will be reported to the IRB, at a minimum, when annual re-approval of the protocol is sought, and to the DSMB. The summary will include the number of subjects enrolled and a summary of graded adverse events to date.

(8) Remote Assessments: Subjects will be encouraged to complete assessments in person whenever feasible and safe. When remote sessions take place, it is possible that information could be intercepted by unauthorized people (hacked) or otherwise shared by accident. This risk can't be completely eliminated, however Yale has approved the use of an encrypted version of zoom for videoconferencing sessions because the appointments take place over a secure encrypted network.

When video sessions are conducted, they will be performed on an encrypted zoom platform. Video sessions can be conducted on the subjects' cell phone, tablet or personal computer enabled with a camera/microphone and internet connection.
Subjects will be instructed to use their home computer or personal device, and not a shared or work device, and use a home (private) Wi-Fi network, and not free(public) Wi-Fi for your internet connection. All zoom appointment links will be sent by email to the subject with password protected links.

2.3.2 Known Potential Benefits

Ketamine arm: While there may be no direct benefit from a subject's participation in this study, the success of ketamine as a rapid-acting antidepressant in adult patients, as well as our promising preliminary data in pediatric subjects, suggest that adolescent subjects may receive

a significant benefit. Given the current design, all subjects will be exposed to the potentially beneficial intervention.

Midazolam arm: While the active placebo medication is not expected to significantly relieve depressive symptoms, the high frequency of contact with a child psychiatrist and research staff may provide a benefit to subjects. Patients with have scheduled contact with Child Psychiatry twice weekly during the blinded phase, when they may be receiving the active placebo. They will also have 24/7 access to a Child Psychiatrist for any patient-initiated contact. Midazolam is a benzodiazepine that is also utilized to ameliorate anxiety symptoms in many patients. All patients who receive midazolam during the blinded phase, and remain significantly depressed or suicidal at Day 11, will be offered active ketamine treatment.

Brain imaging: No direct benefits to the subject are to be expected.

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Societal Benefits: The potential benefits to society of these investigations are considerable. Youth suicide and treatment-resistant depression continue to be a major public health problem with tragic cost to the individual, the family, and the community. The present study may improve our understanding of depression and suicidality by providing a pharmacologic rationale for developing novel treatments. The neuroimaging component of this study will provide knowledge on the complex networks that predict suicidal ideation after receiving ketamine, and how those networks change with treatment. The knowledge gained in this study could benefit patients and society at large because we may learn important information about brain organization and how it relates to behavior, and could facilitate future research on identifying which individuals might respond to this pharmacologic anti-suicidal intervention, one of the main goals of personalized medicine.

2.3.3 Assessment of Potential Risks and Benefits

We judge this study to fall into the following Pediatric risk category:
45 CFR 46.405: Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects
21 CFR 50.52: Clinical investigations involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects

Ketamine arm: Given the efficacy of ketamine as a rapidly-acting antidepressant and anxiolytic in adults and in our preliminary data in pediatric patients with treatment-resistant depression, ketamine also may alleviate symptoms of depression and anxiety in adolescents.

Midazolam arm: Midazolam has been previously used for short-term anxiolysis in both children and adults, so it may reduce anxiety symptoms temporarily (for minutes to hours). While the active placebo medication is not expected to alleviate symptoms of depression or anxiety over the longer term, the high level of contact with Pediatric Psychiatry (scheduled contact 7 out of the 14 days post-infusion), as well as 24/7 access to Child Psychiatry may directly benefit the subjects.

3 Objectives & Endpoints

3.1 Overall objectives

The objectives of this 7-year, single site, clinical trial are (1) to compare the efficacy, safety, and tolerability of a fixed dose of intravenous ketamine (0.5mg/kg over 40 minutes) compared to an active placebo, midazolam (0.045mg/kg over 40 minutes) in adolescents (13 to 17 years old; N=20-33 per group) at high suicide risk, (2) to identify connectome signatures associated with suicidal ideation post-treatment using CPM, and (3) to define the trajectory of suicidal thinking and mood symptoms in both ketamine responders and non-responders when experimental treatment is followed by standard of care (medication management based on the Children's Texas Depression algorithm[65] plus 8 weeks of cognitive behavioral therapy (CBT)). We define adolescents at high suicide risk as those with SRI-resistant depression (defined as having remained depressed despite an SRI trial at ≥ 6 weeks at therapeutic dosing) AND having a suicidal event within the 120 days prior to enrollment (suicide events include suicide attempts, as well as high levels of suicidal ideation that require an emergency evaluation or a transition to a higher level of care (e.g. intensive outpatient program, partial hospital program, inpatient)[66].

3.2 Primary Objective

The primary objective of this study is to determine whether ketamine reduces suicidal ideation (as measured via the C-SSRS, recent ideation scale) relative to an active placebo, midazolam, **48-hours after first administration** in adolescents with SRI- resistant depression at high suicide risk.

3.3 Secondary Objectives

The secondary objectives of this study are to closely monitor the safety (cardiovascular function, bladder health, laboratory values (complete blood count and complete metabolic panel), and cognitive function) and tolerability of a conservative repeat dosing ketamine paradigm and to identify functional connectome signatures predictive of efficacy or modified by treatment.

3.4 Exploratory Objectives

To describe the trajectory of important mental health outcomes (suicidality, depressive symptoms, mental health resource utilization) over 4 months in ketamine responders and non-responders.

3.5 Outcome Variables

3.5.1 Primary Outcome Variables

We have chosen the C-SSRS, recent ideation subscale, as our primary outcome measure, which will be measured at 48-hours after the first blinded infusion (and prior to the administration of the second blinded infusion). The C-SSRS is a well-validated instrument in adolescent populations[67] and adapts well to pediatric clinical trials[68]. This outcome will assess the most severe level of ideation experienced since the last visit (i.e., since Study Day 1, when the participant received the first blinded treatment).

3.5.2 Secondary Outcome Variables

Clinical secondary outcomes: We have chosen to use the MADRS[17] as the depression outcome measure for the rapid timepoint (48 hours) and to measure both the MADRS and the CDRS-R[69] at the short-term timepoint (Day 11). This choice is based on our experience using these scales in pediatric trials of rapid-acting agents (2.1.2) and adult ketamine studies that demonstrate that the MADRS shows increased sensitivity to the acute changes[70] as compared to the Hamilton Depression Rating Scale (on which the CDRS-R is based [69]).

Safety outcomes: Vital signs are recorded at each infusion visit and a physical exam, laboratory screening (liver function tests, renal function tests, thyroid function test, and urinalysis), and electrocardiogram (ECG) are conducted at baseline. In addition to baseline medical clearance, participants will receive an ECG, liver function tests, renal function tests (blood urea nitrogen and creatinine), and urinalysis half-way through the open phase and at the study conclusion. Any spontaneous reports of abdominal pain, increased urinary frequency, urge incontinence, or other urinary or gastrointestinal symptoms will prompt an early medical and laboratory evaluation. Adverse events will be systematically assessed at every visit during phases A1 and A2 using the Systematic Assessment for Treatment Emergent Events (SAFTEE-SI)[71], which examines in a systematic fashion all possible treatment-emergent side effects and probes specific adverse symptoms. Any adverse reactions to the medication or protocol will be carefully explored and documented. Documentation of any spontaneously reported side effects or adverse events is completed at every visit using the case report form. In phase B, adverse events will be assessed by the

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SAFTEE-GI on weekly visits, and systematically by the SAFTEE-SI with monthly batteries. Participants will be provided an adverse event log to track the development of any signs or symptoms of substance abuse, and will undergo repeat urine toxicology screening (weekly during ketamine treatment; halfway through the open phase, and end of open phase). The Cogstate battery will be used to track neurocognitive function during this study, which assesses attention, working memory, psychomotor function, associative learning, and executive functioning.

Neuroimaging outcomes: All MRI-eligible participants will be imaged at 3 timepoints: (T1) Baseline, (T2a) Day 3, and (T2b) Day 17 (see Fig 5). A series of 4 tasks designed to enhance neurocognitive differences across individuals will directly tap into specific fundamental cognitive processes, based on the Behavioral Assessment Methods for RDoC Constructs report[72]. See section 6.2.5, Biomarkers, for a description of tasks and acquisition protocols. Subject performance on all tasks will be recorded via button presses during the scan and pre-treatment versus post-treatment behavior will be analyzed. Finally, one run of resting-state will be collected at the start of the experiment and a structural scan will be completed at the end (3D, T1- and T2-weighted).

3.5.3 Exploratory Outcome Variables

There is no post-market surveillance data of ketamine in any psychiatric population and the symptom trajectory after ketamine treatment, when followed by CBT and medication management, is not known. All participants will have weekly assessments of mood, suicidality, and adverse events (MADRS, C-SSRS, CGI, SAFTEE-SI) and monthly mood and cognitive batteries (**Fig 1**). We will also collect data regarding engagement with the mental health system (e.g. any visits to the emergency room for psychiatric purposes, any change in the level of care). At the end of the blinded phase, participants will be assessed for treatment response (response is defined as a 50% reduction in C-SSRS (suicidality) or MADRS (depression)). All participants meeting criteria for a response (ketamine or midazolam) will be tracked for time to relapse.

4 Study Design

4.1 Overall Design

The project is composed of two phases (**Fig 1**): The **first phase** (Phase A1) will consist of a **double-blind, midazolam-controlled parallel design trial** to evaluate the safety and efficacy of a conservative multiple IV infusion ketamine paradigm (four infusions over two weeks, each 0.5mg/kg over 40 minutes) compared to midazolam (four infusions over two weeks, each 0.045mg/kg over 40 minutes). Investigators will be blind to the treatment assignment during this phase, and raters absent from the infusion paradigm will perform blinded clinical efficacy ratings. The **second phase** will consist of an **open arm extension phase**, in which those who received midazolam will be offered active treatment if they remain suicidal or depressed (Phase A2), and all participants will be followed closely for four months while being treated with medication management and an 8-week course of CBT (Phase B). For study participants taking psychotropic medications (i.e., antidepressants, antipsychotics, mood stabilizers, anticonvulsants, or any other CNS drug used to treat symptoms of MDD), they will remain on those medications at fixed doses during the blinded phase (or through the 2nd week of the open phase if they go on to receive open ketamine). Medication management and CBT

will begin post-ketamine (or post-midazolam in those that have a significant response to the active placebo) treatment. Those who did not respond to ketamine will be followed at the same frequency and treated with medication management and 8 weeks of weekly CBT.

4.1.1 Study Date Range and Duration

The total expected study date range is 7 years to enroll 40-66 adolescent subjects. There are two possible study durations for individual participants: (1) 4 months and 2 weeks (i.e.18 weeks = 126 days) for those who are (a) initially randomized to ketamine OR (b) are initially randomized to midazolam and have clinical improvement; or (2) 4 months and 4 weeks (i.e. 20 weeks = 140 days) for those who are initially randomized to midazolam, do not have a significant clinical response, and opt to receive open ketamine.

4.1.2 Number of Study Sites

This is a single site clinical trial that will take place at Yale University, New Haven, CT. Clinical assessments will primarily occur at the Child Study Center at the Yale School of Medicine, neuroimaging will take place at the Magnetic Resonance Resource Center (MRRC) at the Yale School of Medicine, and infusion treatments will take place at the Yale Psychiatric Hospital at Yale New Haven Hospital. All three of these locations are within 0.5 miles of each other.

4.1.3 Number of Participants

We aim to enroll 40-66 adolescents over the course of 7 years (see Sample Size Determination in 9.2 for further justification)). Based on our prior experience, we expect to screen roughly 90 potential participants to enroll the target number of 40-66 by August 31, 2026.

4.2 Scientific Rationale for Study Design

(1) Ketamine vs. esketamine: The pediatric landscape differs substantially from the adult landscape on the relative uses of ketamine and esketamine. In adults, dose response data for esketamine has been more thoroughly described[73] compared to ketamine[74,75]. In pediatrics, dose response data are not available for either compound (the current Janssen pediatric esketamine study includes a dose response, but those data are not yet available). There is also considerable weight variability in the adolescent population given the growth that occurs across the age range of interest (13 to 17 years old), and thus the weight-based dosing of intravenous ketamine is desirable. A potential limitation of ketamine versus esketamine is its intravenous mode of administration, however our adolescents have tolerated both single and repeat dosing paradigms ([10] and 2.2.2) well. Finally, ketamine is what is being used off-label in our pediatric communities[41,42], and gathering the safety data that will be produced here is of utmost importance.

(2) Repeat dose vs. single dose design**:** Our primary efficacy endpoint is 48 hours following the administration of a first ketamine dose compared to midazolam, which may raise questions about the need for repeated dosing. In our first trial, while we maintained separation from placebo on antidepressant measures out to 14 days, the longest timepoint assessed, the patients who remained in contact all subsequently relapsed. Repeat dosing is thought to extend the duration of wellness in adults with no differences detected between twice-weekly versus thrice-weekly dosing[20]. We have demonstrated that adolescents tolerate repeat dosing ([10] and 2.2.2) and have proposed a conservative repeat dosing paradigm here (four ketamine doses over 2 weeks). We have opted to refine our protocol from 2.2.2.4 by

condensing the blinded phase from three to two weeks, and the open phase from six months to four. There is no standardized dosing protocol in adolescents, and we have opted to reduce the amount of time participants are exposed to the placebo intervention. The antidepressant efficacy of a single ketamine dose (14 days in our small sample, section 2.2.2) is longer than that typically reported in adult studies (little clinical benefit after day five[74]), and it is not known what duration of response to expect in adolescents with repeat dosing. While it is tempting to speculate that the enhanced plasticity potential of the developing brain[76] may enhance ketamine's plasticity inducing effects[77] compared to adults, there is simply no data at this point, and the results generated here will inform the design of a larger, more definitive trial. As roughly 2/3 adult participants who relapsed off esketamine did so by 4 months[73], we feel that this is a reasonable timeframe for the open phase and will enhance retention.

4.3 Justification for Dose

As a dose response for ketamine's psychiatric effects in pediatrics does not exist, the use of a single dose (0.5mg/kg over 40 minutes) is a potential criticism. We have chosen this design given (1) our promising efficacy signal at this dose in adolescents (**Fig 3**), (2) our PK data showing blood levels of ketamine and metabolites in line with the ranges published in adult studies (**Fig 2**), and (3) the adult study suggesting 0.5mg/kg as superior to 0.1mg/kg, 0.2mg/kg, and 1.0mg/kg doses[74]. We believe that our measures of biological engagement (connectome phenotypes predictive of ketamine response, ketamine modulation of connectome phenotypes, and the pre-/post-treatment behavioral data we will obtain from the reward, working memory, response inhibition, and social perception tasks used in building the CPM models) will provide important measures of target engagement that can be leveraged in designing a larger, more definitive trial, that will potentially include a dose response.

4.4 End of Study Definition

We plan to enroll subjects for this trial until our original enrollment target has been met (66 participants) or until August 31, 2026, whichever arrives first. The date of August 31, 2026 was chosen because it allows for us to enroll at least 40 subjects given our current recruitment pace under the amended study inclusion/exclusion criteria.

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5 Study Population

Study Population: Adolescents (13 -17 years old) with "high suicide risk", operationally defined as having both SRI-resistant depression (having failed at least one antidepressant) AND having had a suicidal event within the 120 days prior to enrollment (i.e. a suicide attempt OR suicidal ideation requiring an emergency room visit or escalation of care (intensive outpatient, partial hospital program, and inpatient unit).
Selection rationale: Here we have defined "high suicide risk" as the combination of SRI-resistant depression and having had a suicide event within 120 days of enrollment, an adaption of the criteria of the Treatment of Adolescent Suicide Attempters (TASA) study[78]. While TASA required only a depressive disorder (not necessarily a treatment resistant disorder), we feel that SRI-resistance is an important selection criterion as it (1) increases the risk for suicide[31], (2) is associated with reduced placebo responses in clinical trials of mood disorders[79], and (3) provides a stronger ethical justification for use of an experimental medication where the risks are not clearly defined. SRI-resistant populations may also have higher degrees of hopelessness[80], which is also a potent risk factor for suicide[30]. We are further enriching our population for suicidal ideation by including only those patients with a suicide event in the 120 days prior to enrollment. As defined by the Columbia Classification Algorithm of Suicide Assessment, a suicide event includes bona fide suicide attempts and degrees of suicidal thinking that necessitate emergency services or higher levels of psychiatric care[81]. Patients with high ideation that require emergency services often make attempts on subsequent follow-up and are similar to patients presenting with an actual attempt on a wide range of clinical features in cross-sectional and longitudinal studies[82,83]. Even passive ideation about wanting to be dead is a risk for completed suicide[84].

5.1 Inclusion Criteria

Eligibility will be determined by the PI or by an appropriately trained designee, using the below requirements and a participant and parent interview.

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

2) Ages 13-17 years, inclusive
3) Meet DSM-5 criteria for Major Depressive Disorder by structured interview (MINI-KID+)
4) Children's Depression Rating Scale, Revised (CDRS-R) score ≥45 at screening
5) Continued clinically significant depressive symptoms despite an SRI trial (e.g. SSRI or SNRI) of adequate dose and duration, meaning at least 6 weeks at therapeutic dosing, including at least 4 weeks of stable dosing
6) Suicide event within the past 120 days (i.e. a suicide attempt (defined as an act of potentially self-injurious behavior with explicit or inferred intent to die) –OR– degree of suicidal ideation requiring an emergency evaluation or a transition to higher level of care (e.g. intensive outpatient program, partial hospital program, inpatient)
7) Columbia Suicide Severity Rating Scale ideation score of ≥ 1 at screening
8) Medically and neurologically healthy on the basis of physical examination, medical history, and the clinical judgement of the evaluating physician.
9) Parents able to provide written informed permission and adolescents must additionally provide assent.
10) Stated willingness to comply with all study procedures and availability for the duration of the study

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11) Provision of signed and dated parental permission and adolescent assent form. If there are two parents or guardians, both must provide permission and each must sign a separate permission form.

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1) History of psychotic disorder, manic episode, or autism spectrum disorder diagnosed by MINI-KID
2) History of substance dependence diagnosis by MINI-KID (excluding tobacco) or active substance use (including current alcohol use or positive urine toxicology)
3) Intellectual disability (IQ<70) per medical history
4) Pregnancy (urine pregnancy tests on the day of the first infusion of each treatment week for menstruating girls) or lactation
5) Prior participation in a ketamine study, prior clinical psychiatric treatment with ketamine, or prior recreational use of ketamine
6) Pre-existing cardiovascular disease or untreated or unstable hypertension
7) Body weight greater than 80 kgs
8) Currently taking benzodiazepines or other medications that may cause respiratory depression, or lamotrigine, which is hypothesized to interfere with ketamine's mechanism of action
9) Inability to provide written parental permissions and adolescent assent according to the Yale Human Investigation Committee (HIC) guidelines in English.

For participation in the fMRI scans only (participants with contraindications or intolerance to fMRI may still participate in all other portions of the trial, providing they meet all other inclusion/exclusion criteria):

10) Any contraindication to MRI including severe claustrophobia, or metal in the body (including mental dental braces)

5.3 Lifestyle Considerations

During the study, participants are asked to:

1. Take proper pregnancy precautions as follows:
    - **Women:** During the treatment phase and for at least 30 days after the last dose of study medication, contraception is required for female participants of childbearing potential (i.e. have had their first menstrual cycle and are not surgically sterile). Sexual abstinence is strongly recommended; however

      heterosexually active female subjects must practice an acceptable form of contraception.

        - Acceptable methods of contraception include the following:
        - Hormonal contraception being taken for at least 1 month prior to screening.
        - Intrauterine device (IUD).

- Condom with spermicide (cream, spray, foam, gel, suppository, orpolymer film).
- Diaphragm with spermicide (with or without condom).
- Cervical cap with spermicide (with or without condom).
- Vaginal sponge impregnated with spermicide used with a condom.
- Sexual abstinence is strongly recommended and subjects who practice total abstinence from sexual intercourse as their preferred lifestyle are not required to use additional contraception. For each subject, the reliability of sexual abstinence will be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject.

- **Men:** During the treatment phase and for at least 90 days after the last dose of study medication, contraception is required for male participants of childbearing potential (i.e. have reached spermarche and have not been vasectomized for at least 3 months prior to screening). Sexual abstinence is strongly recommended; however, heterosexually active male subjects must:
  - Practice an acceptable method of contraception with his female partner from those listed above (see examples of contraception provided above for female subjects).
  - Use a condom if his partner is pregnant.
  - Agree not to donate sperm.

2. Not engage in demanding work for the first 3 days after the treatment infusions (i.e. during the weekdays of the 2-week blinded phase, and the 2-week open phase if they go on to receive open ketamine).
3. Not drive or operate heavy machinery 24 hours after an infusion.
4. Attend all scheduled appointments.
5. Refrain from using any illegal substances, including alcohol (urine toxicology tests are regularly administered to monitor any illicit substance use)

5.4 Screen Failures

Please see detailed screening procedures as described in section 6.1. Briefly, after an initial phone screen to rule out any clear exclusion from the study protocol, potential subjects will be scheduled for a screening visit at the Yale Child Study Center or undergo a screening visit through telepsychiatry assessment. At the screening visit, a member of our research team will discuss all aspects of the study: its purpose, the procedures that will be performed, any/all risks of the procedures, possible benefits, and possible alternative treatments. If the patient is considered eligible for the study and agrees to proceed, the patient and his/her parents/guardians will be asked to sign the assent and parental permission forms, respectively.

Once consented, the participant will undergo a standard clinical evaluation consisting of psychiatric history, physical, laboratory and mental status exams with one of the study doctors as described in detail in section 6.1, Screening. The purpose of the screening procedures is to ensure that the participants meets all inclusion/exclusion criteria. If the subject is potentially traveling a long distance for the study, screening may be conducted over two sessions where consenting, all the necessary assessments and psychiatric history is gathered remotely and then an in-person visit is scheduled at a later time to complete physical exam and laboratory assessments. Subjects who fail screening may re-screen at a later date in certain circumstances (e.g. if they fail screening due to taking a restricted

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medication (e.g. lamtrotrigine) but later discontinue this medication their outpatient providers and wish to re-screen).

5.5 Strategies for Recruitment and Retention

We will conduct outreach and education with our most likely recruitment sources, which include 3 inpatient units in the Yale system ((1) Winchester 1, 14 years old and younger, (2) Liberty Village 2, 14 to 17 years old, and (3) Washington Square 3 (16 to 25 years old)), the adolescent intensive outpatient unit, Pediatric Emergency services, and the in-home child services team. We have existing close relationships with all of these sites, and outreach will consist of a lecture describing the study and population of interest, and leaving fliers with inclusion and exclusion criteria, as well as study staff contact information. We do not plan to have any direct advertising or recruitment to patients or the public and expect the majority of patients to come through the referral of physician or mental health provider. Based on our prior experience with these studies, we expect that we may receive some direct patient inquiries by virtue of being listed on ClinicalTrials.gov. In order to potentially expand the geographic area for enrollment we will offer remote assessment and CBT treatment/medication management during the open-phase of the trial when clinically appropriate and at the preference of the family.

Enrollment in research will not disrupt, hold, or alter placement of the patient in the clinically appropriate level of care. Similarly, research participation will not result in a reduction of participant contact with trained pediatric mental health providers for monitoring and safety assessment.

This 2-phase study requires a significant amount of participant contact with the research team. In the first phase (2 weeks), participants have a half-day of baseline assessments and an hour-long fMRI scan. Then there are 4 treatment days where participants receive ketamine or midazolam, and an additional 1-hour fMRI scan prior to the second treatment. In our experience with a similarly designed ketamine study in adolescents, we have had 100% retention through the blinded phase. Our participants have tolerated the treatments well, and in general they express feeling like the treatments and interactions are of value.
In the open phase (4 months), participants who received placebo but remain significantly symptomatic will be offered open ketamine according to the schedule described above. There is one additional neuroimaging session in the first week of the open phase, and participants are asked to have labs drawn, an ECG, and urinalysis at the end of months 2 and 4 for safety monitoring. All participants are offered 8 weeks of weekly CBT and medication management. Participants have weekly mood assessments and monthly mood and cognitive batteries. While there are some retention risks with the open phase, particularly in those who do not respond to ketamine, we believe that the close attention, medication management, and CBT provided by the study is superior to what is generally provided in Child Psychiatry outpatient clinics, which have a severe shortage of providers.

To ensure that the inherent costs of participating (e.g. transportation) are not a significant barrier to joining the study if it is clinically appropriate to do so, compensation is provided to offset costs related to travel and time.

Participants will be paid $40 for completing the 2-week double blind phase. For those that meet criteria for crossing over to the open ketamine phase, they will receive an additional $40 for completing the 2-week open treatment phase. They will receive the following compensation in the open phase:

Completing all 4 visits and rating scales in Month 1: $80
Completing all 4 visits and ratings scales in Month 2: $80
Completing all 4 visits and rating scales in Month 3: $80

Completing all 5 visits and rating scales in Month 4: $80

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Payments will be made using a Bank of America pre-paid debit card. Subjects will be paid at the end of the last completed visit for that compensation block.

6 STUDY INTERVENTION

6.1 Study Intervention(s) Administration

Screening:

After an initial phone screen to rule out any clear exclusion from the study protocol, potential subjects will be scheduled for a screening visit at the Yale Child Study Center or undergo a screening visit through telepsychiatry assessment. At the screening visit, a member of our research team will discuss all aspects of the study: its purpose, the procedures that will be performed, any/all risks of the procedures, possible benefits, and possible alternative treatments. All potential participants will have the opportunity to discuss study participation without their parents or guardians present. If the patient is considered eligible for the study and agrees to enroll, the patient and his/her parents/guardians will be asked to sign the assent and parental permission forms, respectively.

Once consented, the participant will undergo a standard clinical evaluation consisting of psychiatric history, physical, laboratory and mental status exams with one of the study doctors. This assessment includes collection of detailed information about all prior psychiatric therapies, including dose, duration of treatment, side effects, and partial efficacy. The participant and his/her parent/guardian will also receive a clinical diagnostic interview using the Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID)[85]. Additionally, the participant and his/her parent/guardian will complete clinical ratings related to the participant's 1) depressive and suicidality symptoms and 2) symptoms of other commonly comorbid psychiatric conditions. A medical assessment including vital signs, physical exam, baseline serum labs (i.e. CBC with differential, complete metabolic panel (CMP) (including electrolytes, LFTs, BUN, creatinine and glucose), TFTs, a serum pregnancy test, and routine urinalysis) and urine drug screen will be completed prior to enrollment. Participants must be medically and neurologically healthy on the basis of physical examination, medical history, and the clinical judgement of the evaluating physician in order to participate. Examples of medical rule-outs include but are not limited to: unstable hypertension, recent severe neurologic injury, a history or current signs/symptoms of liver or renal insufficiency, and current diagnoses of cardiac, vascular, pulmonary, gastrointestinal, endocrine, neurologic, hematologic, rheumatologic, or metabolic disorders that are deemed clinically significant based on investigator judgement. If the subject is potentially traveling a long distance for the study, screening may be conducted over two sessions where consenting, all the necessary psychiatric assessments and history is gathered remotely and then an in-person visit is scheduled at a later time to complete medical assessment -- physical exam and laboratory assessments.

We will also request that participants and their families sign a release to speak with their current psychiatric care providers, in order to confirm the relevant aspects of the history, to answer any questions about the trial, and to facilitate ongoing collaboration. We will also send a written letter to these providers. We will describe the frequency of study contact (several days a week during the treatment period, then weekly during the follow-up phase). We will review how the study visits may integrate with the participant's ongoing mental health schedule. We will strongly encourage continuous contact between the outpatient and study teams, particularly if a participant reports potential side effects or worsening symptoms.

We will ensure that the outpatient team has the contact information for the study team, including the PI and the 24-hour clinical access number. The clinical assessment will take approximately 2 hours.

Female subjects of childbearing potential will require urine/serum pregnancy testing prior to enrollment in the protocol. Because full confidentiality regarding pregnancy cannot be entirely guaranteed, these testing requirements and the limited scope of confidentiality will be made known to all subjects during the permission and assent procedure. In this manner, young women who would not be comfortable with pregnancy testing or sharing the results of such testing can "opt out" of the study at the time of the initial assent, without having to declare specific reasons. If the pregnancy test is positive, the subject will not be able to participate in the protocol.

Additionally, in order to participate in this protocol, the pediatric subject will need to be asked questions about his/her prior and/or current illicit drug use and undergo drug testing (urine). Because drug use will exclude the minor from participating, the parent may ask why the child or adolescent was asked not to participate or to leave the study. Therefore, parents and/or minors who are uncomfortable with questions about drug use can "opt out" of the study at the time of initial permission, without having to declare specific reasons. If subjects choose not to enroll, then any previously collected drug test results will be destroyed.

Adolescent drug use information will not be shared with parents unless the study team feels that the minor is exhibiting behaviors that would pose an immediate threat to the minor or to others. The PI or co-investigator will ask the minor if the study team can share the drug testing results with parents. If the minor declines, the study team will refer the minor for evaluation based upon the clinical judgment of the Principal Investigator. In all cases, the safety and well-being of the minor will be protected. We will explicitly inform parents and minors, in the permission and assent documents, and orally with regard to these guidelines. As an NIH-funded study, drug use information is protected under a Certificate of Confidentiality.

If the adolescent participant meets all inclusion and exclusion criteria, they will be scheduled for Baseline and treatment visits.

Baseline (Study Day 0, Visit 1)

Baseline visits will generally be conducted on Mondays in order to facilitate flow into the established treatment procedures at Yale Psychiatric Hospital's Interventional Psychiatry Service (IPS), in which ketamine treatments are administered on Tuesdays and Thursdays. Baseline assessments include clinical questionnaires and neuroimaging. Participants will present to the Child Study Center to complete the clinical questionnaires and obtain a weight, then will walk with research staff to the MRRC (~5 minute walk) in order to complete the one-hour neuroimaging scan.

Clinical Questionnaires on the Baseline Day are as follows:

1) C-SSRS: assessment of suicidal ideation and behavior in clinical and research settings[66,86]
2) CDRS-R: a standardized rating scale that assesses depression severity in children and adolescents[69]
3) MADRS: a standardized rating scale that assesses depression severity in children and adolescents[17]
4) Pleasure Scale for Children (PSC)[87]: a standardized rating scale to assess anhedonia[88]
5) Treatment-Emergent Activation and Suicidality Assessment Profile (TEASAP): a rating scale designed to detect increased behavioral activation and suicidality[89]
6) Multidimensional Anxiety Scale for Children (MASC): a multidimensional assessment of anxiety in children and adolescents[90]
7) CADSS: interviewer administered items that evaluate dissociative symptoms [16]
8) Clinical Global Impressions (CGI): a widely used instrument used to assess overall severity

of illness and symptom improvement on 1-7 point scales[91],
9) Beck Hopelessness Scale (BHS): instrument to assess hopelessness[92]
10) Adolescent Stress Questionnaire (ASQ): a 56-item instrument for measuring current stressors across a range of domains [93]
11) Childhood Trauma Questionnaire (CTQ): a self-report screening measure for maltreatment histories[94]
12) Edinburgh Handedness Inventory
13) Wechsler Abbreviated Scale of Intelligence
14) Cogstate neurocognitive battery

The participant and their family will be introduced to the study CBT therapist. During this introduction they will complete a Safety Planning Module together. The safety planning module focuses on the development of an individualized safety plan that includes support from multiple individuals and includes internal and external coping strategies to be implemented should the participant experience suicidal urges. We emphasize with participants and their parents that the creation of the safety plan is a collaboration between the patient, therapist, and parents, and that all involved must understand steps for and assessment of safety.

The safety plan includes the following: identification of risk behaviors, identification of coping strategies, identification of family support and others to provide support, creation for plan of 'In case of emergency', identification of resources to call in crisis (including the 24-hour access number for a study clinician (e.g., doctor, therapist, or psychologist), and signature from child or adolescent, parent, and therapist (indicating all on board with plan). Copies of the completed plan will be provided for the participant, the participant's family, and uploaded into their EPIC medical record. We ask families to place the plan in an easily accessible locations (e.g. on the refrigerator, bedroom wall). If the adolescent wishes to use a smartphone, they may also take a picture of the plan using the phone's camera function.

The MRI scan will last approximately 1 hour and will include a combination of anatomical and functional (task and rest) scans. Subjects will perform 4 tasks (Table 1) during each scan session. The MRI scans will take place on one of the 3T Siemens scanners located at the Magnetic Resonance Research Center. A licensed MR technician will perform all MR scans, and all scans will use standard clinical sequences. A member of the research team will accompany the subjects to the MRRC and will stay with them for the duration of the scan.

Table 1: Continuous Performance fMRI tasks

Task	RDoC Domain	Domain Construct
Card Guessing Task	Positive Valence Domain	Reward Responsiveness
N-back Task	Cognitive Systems	Working Memory
Stop Signal Task	Cognitive Systems	Response Inhibition
Reading the Mind in the Eyes Task	Social Processes	Perception and Understanding of Others

All planned MR sequences are currently FDA-approved MR imaging sequences; none exceed the FDA Guidelines for 3T MR imaging systems. Tasks performed during the experiment sessions will include:

1. Card guessing task (e.g.,[95]): In this reward response task, subjects will be shown a card on a screen and will have to guess whether the number on the other side of the card is less than

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or greater than five. The card will then be flipped over and subjects will receive reward feedback based on their response.
2. N-back task[96,97]: Subjects will be asked to watch a set of pictures (or words) and push a button if a new picture (or word) is different than the previous item (1-back condition) or than the item that came two items before (2-back condition).
3. Response inhibition task/Stop-Signal (e.g.,[98]): Subjects will see items on the screen and either respond (go trial) or not respond (no-go trial), depending on the presented item.
4. Understanding mental states task: Subjects will be asked to infer the perspective of others, either by looking at photographs of their eyes and labeling the photographed subjects' emotions[99], or by responding to questions that require them to interpret social "hints" in vignettes[100].
5. Perception: Subjects will passively watch images on the screen or listen to sounds through headphones. Possible stimuli include flashing checkerboards, movie clips, beeps and tones, blank screens, etc.
6. Resting state run: Subjects will be asked to stay still with eyes open during the resting runs. There is no task involved.

Phase A1: Blinded Intervention (Visits 2 – 7):
Subjects will receive four infusions of either ketamine (0.5mg/kg IV) or midazolam (0.045mg/kg IV) over a two-week period. Infusions are delivered over 40 minutes while on continuous cardiac monitoring and pulse oximetry; they will be monitored for 2 hours post-infusion (or until pharmacokinetic blood draws are complete on Day 1, 230 minutes post-infusion)

First Blinded Infusion (Day 1, Visit 2)
The participant will be instructed to follow American Society of Anesthesiologists NPO guidelines the night before the infusion. These guidelines allow milk or a light meal 6 hours prior to the procedure and clear liquids up to 2 hours prior to the procedure. Subjects will present to the Child Study Center to complete pre-infusion questionnaires and receive a urine toxicology (and urine pregnancy, if applicable) test. Pre-infusion clinical measures are the C-SSRS, MADRS, and CGI.

The participant will then walk to the Interventional Psychiatry Service (IPS) at Yale Psychiatric Hospital with research staff (~ 5 minute walk). One hour prior to the infusion, two IVs will be placed, one to administer the infusion, and the second to facilitate blood collection for pharmacokinetic blood draws. The blinded infusion will be administered over a 40-minute period, either ketamine at a dose of 0.5mg/kg, or midazolam at a dose of 0.045 mg/kg, randomized by the Investigational Drug Service. Doses of both medications not to exceed a maximum total dose corresponding to a weight of 80kg.

Ketamine and midazolam administrations will be performed on the IPS service at YPH under ACLS-accredited physicians with experience with midazolam-controlled ketamine studies, including those conducted in pediatric populations. During the infusion, vital signs are recorded every 5 minutes. Once the infusion is complete, they are recorded every 15 minutes. The psychotomimetic side effects of ketamine or midazolam, and the mental status of the participant will also be monitored every hour for two hours following the infusion. Pharmacokinetic blood draw timepoints are pre-infusion, 40-minutes post-infusion start (i.e. as the infusion is completing), 80 minutes, 110 minutes, and 230 minutes. These timepoints were chosen for consistency with the published adult PK literature and with our previous pediatric ketamine work. Participants will be monitored through the 230-minute timepoint, at

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which time they will be released with a parent or guardian. Prior to visit discharge, research staff will use the SAFTEE-SI to systematically assess for potential adverse events. In addition, a credentialed clinician will review the participant's individualized safety plan and make a clinical determination of risk of imminent harm to self or others. Assessment of risk involves inquiring about the presence and intensity of suicidal thinking, differentiating between passive and active thoughts, evaluating the presence of risk and protective factors, and inquiring about plans, access, and intent. If a participant were deemed to be an imminent safety risk to themselves or others, they would be transported to the Emergency Room for further evaluation.

Remote follow-up (Day 2, Visit 3): Participants will be contacted by phone to follow-up regarding mood (MADRS) and any potential adverse events (SAFTEE-SI). A member of the research staff will speak with both the participant and a parent and will confirm return instructions for the following day. This day is purposefully designed to be a day with low scheduled interaction given the large number of procedures and interactions on Days 1 and 3. Participants will be reminded that study staff, study physicians, and the PI are all available should there be any questions, concerns, or problems. This assessment may be done either by telephone or through telepsychiatry video assessment.

Primary Outcome (48-hour C-SSRS), Neuroimaging, Second Blinded Infusion (Day 3, Visit 4): Participants will again be instructed to follow American Society of Anesthesiologists NPO guidelines the night before the infusion (milk or a light meal 6 hours prior to the procedure, and clear liquids up to 2 hours prior to the procedure). Participants will present to the Child Study Center to complete the pre-infusion questionnaires (C-SSRS, MADRS, CGI). Note that this administration of the C-SSRS is the primary outcome of the efficacy portion of the study. The timeframe for the clinical measures is "since last visit".

Participants will then walk to the MRRC with research staff (~5 minute walk) in order to complete the second neuroimaging scan. Tasks and rest conditions will be identical to those described above (Baseline visit). Following neuroimaging, participants will walk with research staff across the street to the Interventional Psychiatric Service (~1 minute walk). A single IV will be placed, as there are no pharmacokinetic blood draws on this or subsequent infusions. As above, the blinded infusion will be administered over a 40-minute period, either ketamine at a dose of 0.5mg/kg, or midazolam at a dose of 0.045 mg/kg, as previously determined by the Investigational Drug Service.

Ketamine and midazolam administrations will be performed on the IPS service at YPH under ACLS-accredited physicians with experience with midazolam-controlled ketamine studies, including those conducted in pediatric populations. During the infusion, vital signs are recorded every 5 minutes. Once the infusion is complete, they are recorded every 15 minutes. The psychotomimetic side effects of ketamine or midazolam, and the mental status of the participant will also be monitored every hour for two hours following the infusion. Prior to visit discharge, research staff will use the SAFTEE-SI to systematically assess for adverse events. As with all infusion visits, a credentialed clinician will make a clinical determination of risk of imminent harm to self or others. After two hours, if participants have returned to baseline mental and physical status, they will be discharged in the care of their parent or guardian.

Third and Fourth Blinded Infusions (Days 8 and 10, Visits 5 and 6):

Participants will present to the Child Study Center for pre-infusion questionnaires (Days 8 and 10) and urine toxicology (and urine pregnancy, as applicable) (Day 8), and will have been instructed to follow the same NPO guidelines as previous infusions. Infusion procedures for the third and fourth blinded infusions are identical to those described for the second blinded infusion.

Mood/Cognitive Battery, Breaking of the Blind (Day 11, Visit 7):
All participants will return on study day 11 (Visit 7) for assessment of the mood and cognitive measures described in the Schedule of Activities. Important secondary clinical outcomes will be collected on this date, including the MADRS and the CDRS-R. After all clinical measures are collected and response criteria are calculated, the PI will contact the Investigational Drug Service in order to break the blind. The Investigational Drug Service will provide the treatment assignment for the participant in writing via electronic communication.

The PI will discuss the clinical and treatment results with the participant and their family. In the event that the participant had been randomized to ketamine, they will proceed to the open phase (Phase B, which includes CBT and medication management as standard of care treatment) regardless of treatment response. In the event that the participant had been randomized to midazolam and they did not experience a treatment response, they will be offered open ketamine treatment (Phase A2) prior to progressing to Phase B. In the event the participant was randomized to midazolam and they did experience a significant treatment response, they will proceed to Phase B and will not be offered any open ketamine treatments.

Phase A2: Open Ketamine Intervention for Midazolam Non-responders (Visits 8 – 13):
Participants will follow an identical schedule to that described for the blinded phase (Phase A1) above, with the exception that the four infusions will be ketamine treatments (open treatment in which both the participant and the study physicians are aware of the treatment). In brief, the following days have the following treatments (please see detailed descriptions in Phase A1) for procedures each day:
First Open Ketamine Infusion (Day 15, Visit 8), includes pharmacokinetic blood draw
Phone Follow-up (Day 16, Visit 9)
Neuroimaging (Third Scan), Second Open Ketamine Infusion (Day 17, Visit 10)
Third Open Ketamine Infusion (Day 22, Visit 11)
Fourth Open Ketamine Infusion (Day 24, Visit 12)
Mood Cognitive Battery (Day 25, Visit 13)

Phase B: Open Phase with Standard of Care Depression Treatment
(Visits 8 – 24 for participants proceeding directly from Phase A1)
(Visits 14 – 30 for participants proceeding from Phase A2)

At this point in the trial, all subjects will have completed any trial-associated infusion procedures. All of the remaining clinical interventions (8 weeks of weekly CBT and medication management according to the modified Texas Children's Medication Algorithm) are considered standard of care depression treatments.

For the first 8-weeks (Visits 8-15 in those proceeding directly from A1; Visits 14 – 21 for those proceeding from A2), the general assessment schedule will be weekly meetings. These meetings will include a 45-minute CBT session, a 15-minute medication management visit, and a limited amount of clinical ratings (C-SSRS, MADRS,

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CGI, SAFTEE-GI). The study team aims to integrate study procedures and clinical care to facilitate ease of participation for study families and to rational information flow through the visits. Visits will begin with the study assessments, and those scores will be passed on to the CBT therapist and Child Psychiatrist. The CBT session will follow, and any important information will be relayed to the Psychiatrist conducting the medication check component of the visit. A risk assessment and review of the safety plan occurs at every visit, as is our standard clinical practice.

In the event that pandemic- or public health-related conditions preclude a participant from attending one of the above in-person sessions, the session may need to be conducted via telehealth. Any telehealth appointments must follow the requirement for the presence of a parent or guardian in the home during the telehealth session. When clinically appropriate, families may also choose to conduct these sessions remotely via video telehealth sessions. Sessions will only be conducted via telehealth when this is preferred by the family and judged to be clinically appropriate by both the CBT therapist and PI/study doctor.

In the group who progressed directly from phase A1, the third neuroimaging session (Day 17, Visit 8) will occur prior to the therapy and medication management session (see study schedule of activities). Every fourth week, a more extensive mood and cognitive battery will be completed. At the end of this 8-week period (Day 66, Visit 15 in those from A1, and Day 80, Visit 21 in those from A2), participants will have a medical surveillance battery consisting of an ECG, serum labs, urinalysis, and urine toxicology. The 8-week course of CBT is slated to end at this time.

For the final 8-weeks (Visits 16- 24 in those proceeding directly from A1; Visits 22- 30 in those proceeding from A2), weekly mood and AE check-ins can occur by telephone or via video telehealth visit if the participant is clinically stable. The monthly mood and cognitive batteries will still occur in person and can coincide with continued 15-minute medication management visits.

Participants may request to continue weekly visits in-person visits (rather than remote visits) during this time, and any phone assessments that are indicative of clinical deterioration will prompt an unscheduled, in-person visit. A final set of medical surveillance labs will be collected at the end of this 8-week period, as above (Day 122, Visit 23 in those from A1, and Day 136, Visit 29 in those from A2).
Weekly mood assessments are estimated to take approximately 30 minutes to complete and monthly mood and cognitive batteries are estimated to take 1.5-2 hours to complete.

Participants will have a final in-person visit on the day of study discharge (Day 126, Visit 24 in those progressing directly from A1; Day 140, Visit 30 in those progressing from A2).

On Study Visits
Please see the detailed description of study visits and procedures in section above and summarized on the Schedule of Activities (Section 1.3)

End of Study and Follow-up
The study has a significant follow-up phase built in following the acute blinded phase intervention (the 4-month open phase). The study is considered completed at the end of the 4-month open phase. During the follow-up phase, important safety, efficacy, and resource utilization measures are collected (see section 3.5, Outcome Variables). Participants who withdraw from the study early will be offered continued follow-up for mood and cognitive

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assessments if they are amenable to such. Participants may request copies of their lab results or clinical ratings if they would like a copy for their records or to share with their outpatient mental health provider. Any incidental or concerning findings revealed in the neuroimaging

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portion of the study will be reported to the patient, who will be encouraged to follow-up with a medical provider outside of the study.

6.1.1 Study Intervention Description

Ketamine (Ketalar) has FDA approval as a sole anesthetic agent for diagnostic and surgical procedures that do not require skeletal muscle relaxation. Ketamine has been increasingly used as a rapid-acting antidepressant in adult populations with treatment-resistant depression[4], but it is not FDA-approved for this indication. As of March 5th, 2019, the FDA granted approval to Spravato (esketamine) nasal spray, in conjunction with an oral antidepressant, for the treatment of depression in adults who have tried other antidepressant medicines but have not benefited from them (treatment-resistant depression). Esketamine is the s-enantiomer of ketamine. Because of the risk of serious adverse outcomes resulting from sedation and dissociation caused by Spravato administration, and the potential for abuse and misuse of the drug, it is only available through a restricted distribution system, under a Risk Evaluation and Mitigation Strategy (REMS). An open-label trial[8] and several case reports have described the successful use of ketamine in pediatric patients with refractory psychiatric conditions (see also, Clinical Experience, 2.1.2), however it is not FDA-approved for this purpose. Studies of Spravato in pediatric patients with psychiatric disorders are ongoing.

Ketamine hydrochloride injection USP 50 mg/ml, 10 ml vials, will be procured by the Investigational Drug Service (IDS), a specialized pharmacy at Yale (obtained from the manufacturer or supplier of FDA-approved ketamine that is currently in use by the Yale New Haven Hospital IDS). The medication dose used in the protocol (0.5mg/kg infused over 40 minutes) is substantially lower than doses that are used for anesthesia (1mg/kg – 4.5mg/kg IV over 60 seconds). The maximum number of ketamine infusions in this protocol is four, and the maximum total dose per infusion is 40mg per infusion (corresponding to a weight of 80kg). The PI holds a therapeutic license for administering controlled substances.

Midazolam HCl (Versed, the active placebo in this study) is FDA approved in children and adolescents for sedation, and is often used as an anxiolytic agent prior to induction of general anesthesia with other anesthetic agents, or as a sole sedating agent for minor procedures. Midazolam hydrochloride injection USP 5 mg/ml, 1 ml vials, will also be procured by the IDS (obtained from the manufacturer or supplier of FDA-approved midazolam that is currently in use by the Yale New Haven Hospital IDS) . The medication dose used in the protocol (0.045mg/kg infused over 40 minutes) is substantially lower than doses that are used for anesthesia (0.5mg - 2mg IV push, repeated every 2-3 minutes to a maximum of 10mg). The maximum number of midazolam infusions in this protocol is four, and the maximum total dose per infusion is 3.6mg (corresponding to a weight of 80kg). As with ketamine, the PI holds a therapeutic license for administering controlled substances.

6.1.2 Dosing and Administration

Infusions (Day 1, 3, 8, and 10, Visits 2 - 6): Subjects who, in the opinion of the PI, are eligible to continue with the protocol procedures (after the results of the screening/baseline measures and diagnostics are considered) will present to the Interventional Psychiatry Service (IPS) at Yale Psychiatric Hospital for the first infusion on Study Day 1. The participant will be instructed to follow American Society of Anesthesiologists NPO guidelines the night before the infusion. These guidelines allow milk or a light meal 6 hours prior to the procedure and clear liquids up to 2 hours prior to the procedure. One hour prior to the infusion, IV's will be placed, two IV's on days that include bloodwork (ketamine metabolites on Day 1 only)

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and one IV on infusion days without bloodwork (Day 3, 8, and 10). The infusion will be administered over a 40-minute period, either ketamine at a dose of 0.5mg/kg, or midazolam at a dose of 0.045 mg/kg (doses of both medications not to exceed a maximum total dose corresponding to a weight of 80kg).

Ketamine and midazolam administrations will be performed on the IPS service at YPH under ACLS-accredited physicians with experience with midazolam-controlled ketamine studies, including those conducted in pediatric populations. The participant's vital signs will be monitored every 5 minutes during the infusion and every 15 minutes thereafter up to two hours following the infusion. The psychotomimetic side effects of ketamine or midazolam, and the mental status of the participant will also be monitored every hour for two hours following the infusion. During this acute phase of the study, patients will receive four infusions, scheduled on Study Days 1, 3, 8, and 10 (corresponding to visits 2 – 6). All participants will return on Study Day 11 (Visit 7) for extensive mood and cognitive measures. After the mood and cognitive battery on Study Day 11, the blind will be broken to determine which arm the participant was randomized to. Those who were randomized to midazolam and remain symptomatic will be offered open ketamine, will be treated as described above on Study Days 15, 17, 22, and 24 (Visits 8-12), and will have a follow-up post-ketamine mood and cognitive battery on Study Day 25 (Visit 13).

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

Ketamine hydrochloride injection USP 50 mg/ml, 10 ml vials manufactured by Hikma pharmaceuticals will be acquired from wholesaler McKesson or from the manufacturer or supplier that is being used by the Yale New Haven Hospital Investigative Drug Service at the time of medication preparation.

Midazolam hydrochloride injection USP 1 mg/ml, 5 ml vials manufactured by Hikma pharmaceuticals will be acquired from wholesaler McKesson or from the manufacturer or supplier that is being used by the Yale New Haven Hospital IDS at the time of medication preparation.

6.2.2 Formulation, Appearance, Packaging, and Labeling

The formulation, appearance, packaging, and labeling of Ketamine and Midazolam can be found in the package inserts included in this application. Investigational drug accountability and management (purchase, storage, preparation, dispensing, and disposition) will be conducted by Yale-New Haven Health IDS Pharmacy.

6.2.3 Product Storage and Stability

Investigational drugs will be stored per package insert. All vials will be used as single dose vials.

Ketamine: Store at 20° to 25°C (68° to 77°F) [See USP Controlled Room Temperature]. Protect from light.

Midazolam: Store at 20°-25°C (68°-77°F), excursions permitted to 15°-30°C (59°-86°F) [See USP Controlled Room Temperature].

6.2.4 Preparation

Ketamine will be diluted in sodium chloride 0.9% to a total volume of 100mL,delivered intravenously at a rate of 150 mL/hr

Midazolam will be diluted in sodium chloride 0.9% to a total volume of 100mL,delivered intravenously at a rate of 150 mL/hr

6.3 Measures to Minimize Bias: Randomization and Blinding

Participants will be randomized to ketamine or midazolam in the double-blind phase by the Investigational Drug Service, 1:1. Only the Investigational Drug Service will know the identity of the experimental compound. Medication labels in the double-blind phase read "Ketamine or Midazolam", followed by "Investigational Study Drug". All medication infusion solutions are made to a total volume of 100mL so that infusion instructions are identical, protecting the blind for the nursing staff. Although this is a double-blind study, additional measures are being taken to protect the blind. Efficacy raters will not be present during the infusions, i.e. there will be separate raters for intra-infusion ratings (side effect ratings scales) and for efficacy.

The first phase of this trial is a double blind, randomized controlled trial. At the end of the blinded phase, there will be a scheduled breaking of the blind in order to proceed to the open phase of the trial. After the clinical ratings are complete on Study Day 11, the Investigational Drug Service will be contacted, and they will send a written electronic communication to the PI with the randomization information for that subject. This information is needed in order to determine whether the participant meets criteria to proceed to open ketamine administration in the following phase.

6.4 Study Intervention Compliance

The study intervention will be administered on site by medical staff in the presence of a member of the study team, therefore intervention compliance will be assured.

6.5 Concomitant Therapy

Participants are allowed to remain on their current psychiatric medications in this study. This design choice was made for several reasons. For patients with difficult-to-treat depression, a complete washout of medications is potentially problematic and may contribute to: (1) acute worsening of mood resulting in hospitalization or suicide; (2) inflation of baseline scores, which may bias response rates; and (3) a systematic selection bias due to elimination of participants who cannot tolerate drug withdrawal and who refuse to consider a washout. We also believe that continuing current medications approximates the "real world" conditions in which ketamine is given to pediatric patients off-label. We ask that patients refrain from any dose or agent changes during the blinded phase of the protocol (or the first two weeks of the open protocol if they qualify to receive open ketamine). The open phase of the protocol includes medication management based on the Texas children's medication algorithm[65], an adaptation that allows for antipsychotic augmentation.

There are, however, several medication restrictions (see exclusion criteria 5.2) to avoid any potential interactions with the investigation medications. These include a prohibition of

benzodiazepines or any other standing medications that have significant respiratory depressive activities. Lamotragrine (Lamictal) is also a restricted medication as its biological action is thought to potentially inhibit ketamine's antidepressant actions at the mTOR pathway.

6.5.1 Rescue Medicine

An ACLS-trained physician with access to airway equipment will be present throughout the infusions (although we believe that the likelihood of a serious event at the doses proposed in this study is low). Subjects will be monitored for at least 2 hours following ketamine or midazolam infusion by Dr. Bloch. A study doctor (Principal Investigator (PI) of the trial) will be present at all times during the infusion and recovery. In the event, that a research subject has a significant psychiatric event requiring hospitalization, they will be treated on the adolescent unit (LV2) at Yale Psychiatric Hospital (YPH). Emergent medical care would be provided at Yale-New Haven Hospital.
Oral diazepam (5mg) will be kept available to control markedly distressing behavioral effects of ketamine, should they emerge. Oral ondansetron (4-8mg) will be kept available to control any significant nausea or vomiting. Please also see section 2.3.1, Risk Mitigation Plans.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Patients may withdraw at any time or be dropped from the study, at the discretion of the PIs, should medical contraindications develop, if intolerable adverse reactions occur, if the patient becomes manic or psychotic, or if the patient worsens to such a degree that further participation puts the patient at risk (e.g., active suicidality). If a patient worsens during the blinded phase, a thorough discussion will occur with the patient, their family, their outpatient psychiatrist, and the treatment team to discuss the risks and benefits of continuing in the study. If the participant has been randomized to midazolam and is still symptomatic, then they are able to receive ketamine at the end of the blinded phase (two weeks maximum). The PI must assess subjects who decide to withdraw from the study to ensure their care is transitioned to the provider

If a participant needs to be hospitalized during the blinded phase, all efforts will be made to have them hospitalized within the Yale New Haven Hospital (YNHH) System. As soon as the study team is aware, they will reach out to the treating physician at the participant's location. The team will discuss the participant's clinical status and the main study requirements during the 2-week double blind phase (including avoiding benzodiazepines and refraining from making changes to standing medications). Study treatments occur at the Yale Psychiatric Hospital and the clinical units will be aware of the study through recruitment outreach, which we hope will facilitate working through any clinical or logistical challenges to meet the best interest of the patient. If a participant is hospitalized outside of the YNHH system, the study team will still make every effort to reach and collaborate with their clinical staff.

7.2 Participant Discontinuation/Withdrawal from the Study

Subjects may withdraw voluntarily at any time for any reason. An investigator may discontinue or withdraw a participant from the study for the following reasons:

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- Pregnancy
- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression that requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Study termination

The occurrence of a hospitalization or other suicide event per se is not on its own a sufficient reason for automatic removal. Any serious adverse event will prompt a review of the current risks and benefits of study participation with the participant, their family, and any other medical personnel involved in their care. Recommendations of whether to continue or to discontinue will be based on a consensus of what is believed to be in the best interest of the participant at that time.

The reason for participant discontinuation or withdrawal from the study will be recorded in the participant binder by the relevant clinician. Subjects who sign the informed assent form (with corresponding parental permissions) and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed assent form (with corresponding parental permissions), and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

7.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she does not respond to phone or email communications. After 2 weeks a letter will be sent to their address and they will be informed of their discontinuation from the study.
Any no-shows to study visits will be vigorously pursued. The following actions will be taken if a participant fails to return to the clinic for a required study visit:

• The site will attempt to contact the participant and their parent and reschedule the missed visit as soon as possible. Research staff will counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
• If the participant or parent are unable to be reached, a member of the study team will check the participant's electronic medical record to ascertain whether any new visits have occurred to psychiatric or medical services within the YNHH system.
• Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts will be documented in the participant's medical record or study file.

Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

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8 STUDY ASSESSMENTS AND PROCEDURES

8.1 Efficacy Assessments

Please also see section **3.5** Outcome Variables and the Schedule of Activities, which includes both efficacy and baseline assessments.

1) C-SSRS: assessment of suicidal ideation and behavior in clinical and research settings[66,86]- Primary outcome at Study Day 3; administered at baseline, prior to each infusion during blinded phase; administered weekly in open phase
2) CDRS-R: a standardized rating scale that assesses depression severity in children and adolescents[69]- administered at baseline, at Day 11, and in monthly mood and cognitive batteries
3) MADRS: a standardized rating scale that assesses depression severity in children and adolescents[17]- administered at baseline, prior to each infusion during the blinded phase; administered weekly in the open phase
4) Pleasure Scale for Children (PSC)[87]: a standardized rating scale to assess anhedonia[88]- administered at baseline, at Day 11, and in monthly mood and cognitive batteries
5) Treatment-Emergent Activation and Suicidality Assessment Profile (TEASAP): a rating scale designed to detect increased behavioral activation and suicidality[89]- administered at baseline, at Day 11, and in monthly mood and cognitive batteries
6) Multidimensional Anxiety Scale for Children (MASC): a multidimensional assessment of anxiety in children and adolescents[90]- administered at baseline, at Day 11, and in monthly mood and cognitive batteries
7) Clinical Global Impressions (CGI): a widely used instrument used to assess overall severity of illness and symptom improvement on 1-7 point scales[91]- administered at baseline, prior to each infusion during the blinded phase; administered weekly in the open phase
8) Beck Hopelessness Scale (BHS): instrument to assess hopelessness[92]- administered at baseline, at Day 11, and in monthly mood and cognitive batteries
9) Adolescent Stress Questionnaire (ASQ): a 56-item instrument for measuring current stressors across a range of domains[93]

8.2 Safety and Other Assessments

8.2.1 Safety

1) Vital signs are recorded at each infusion visit
2) Baseline: a physical exam, laboratory screening (liver function tests, renal function tests, thyroid function test, and urinalysis), and electrocardiogram (ECG) are conducted
3) Follow-up medical surveillance: participants will receive an ECG, liver function tests, renal function tests (blood urea nitrogen and creatinine), and urinalysis half-way through the open phase and at the study conclusion
4) Adverse reactions will be systematically assessed at visits as detailed in the Schedule of Activities. Staff will use the Systematic Assessment for Treatment Emergent Events (SAFTEE-GI or SAFTEE-SI)[71], as described in the Schedule of Activities, which examines in a systematic fashion all possible treatment-emergent side effects and probes specific adverse symptoms. Any reactions to the medication or protocol will be carefully explored and documented. Documentation of any spontaneously

reported or inquiry-elicited side effects or adverse events is completed at every visit using the case report form.

5) Any spontaneous reports of abdominal pain, increased urinary frequency, urge incontinence, or other urinary or gastrointestinal symptoms will prompt an early medical and laboratory evaluation. Participants will be provided an adverse event log to track the development of any signs or symptoms of substance abuse and will undergo repeat urine toxicology screening (weekly during blinded infusions or open ketamine treatment; halfway through the open phase, and end of open phase).
6) The Cogstate battery will be used to track neurocognitive function during this study, which assesses attention, working memory, psychomotor function, associative learning, and executive functioning; it is administered at baseline, Day 11, and in the monthly mood and cognitive batteries

8.2.2 Pharmacokinetics

On the first infusion day (Day 1) of the blinded phase, pharmacokinetic blood samples with be collected, to be analyzed by our collaborator, Dr. Carlos Zarate, at the NIMH. To facilitate participant comfort, two intravenous catheters will be placed on that day, one for the infusion and the other to draw off of for PK blood samples. Timepoints are as follows: pre-infusion, 40-minutes post infusion start, 80 minutes, 110 minutes, and 230 minutes. Total blood volume to be drawn that day is not to exceed 30mLs. In the event that a participant crosses over in the open phase to receive ketamine, there will be an identical procedure for the first infusion (Day 15).

8.2.3 Biomarkers/Neuroimaging Assessments

There are no serum biomarkers being collected as part of this study. Neuroimaging measures could, however, be considered biomarkers. We plan to scan participants at 3 timepoints: baseline, Day 3 (prior to second blinded infusion), and Day 17 (prior to second open infusion in those who crossover to open ketamine, and at a comparable timepoint to those receiving CBT and medication management in the open phase).

Total magnet time will be under 1 hour for each scanning session. ***Continuous performance tasks:*** A series of 4 tasks designed to enhance neurocognitive differences across individuals will directly tap into specific fundamental cognitive processes, based on the Behavioral Assessment Methods for RDoC Constructs report[72]. *Positive Valence*. To perturb reward circuitry, the (i) card guessing task will be used[95,101,102], which has been extensively validated, shows good construct validity, psychometric properties, and characterization of individual differences[72], and has been used in clinical populations[103-108]. *Cognitive Systems*. The constructs of working and declarative memory will be simultaneously probed using a version of the common, extensively validated (ii) N-back task [96,97,109]. The construct of response inhibition will be assessed using the (iii) stop-signal paradigm[98,110]. This task has been extensively validated[72], shown to have good construct validity and reliability [111,112], and used in clinical populations [110]. *Social Processes*. To probe perception and understanding of others, the (iv) Reading the Mind in the Eyes Test will be used[99]. This task can be used in clinical populations [113] and in adults without ceiling effects [99]. Its revised version has been shown to have good reliability and construct validity[113-115]. Subject performance on all tasks will be recorded via button presses during the scan and pre-treatment versus post-treatment behavior will be analyzed. Finally, one 6-minute run of passive movie-watching, one 6-minute run of

resting-state, and a structural scan will be completed (3D, T1- and T2-weighted).

Acquisition Protocol: The imaging protocol will match the Human Connectome Project (HCP) protocols as closely as possible. Imaging will be performed on a 3T Siemens Prisma, 32-channel head coil. T1-weighted anatomic slices (FLASH: 72 slices 2mm thick, TR=300ms, TE=2.47ms, FoV=220mm, matrix=192x192, Flip angle= 70º, bandwidth = 300Hz/pixel) aligned with the AC-PC providing whole-brain coverage including the cerebellum. Each of the 4-task runs and 1-resting-state run will be acquired over 6 minutes (total of 30 minutes of connectivity data) using multiband EPI (voxel size $2mm^3$)[116], 8 TRs to achieve steady-state, matrix 104x90, multiband=8, flip=52º, TE=33ms, TR=720ms, 72 slices, FOV=208x180mm (584 frames per run). Structural data will be obtained using 3D T1-weighted MPRAGE: thickness 0.7mm, matrix size=224×224, TR=2400ms, TE=2.14ms, Flip angle=8°, and a T2-weighted 3D SPACE, thickness 0.7mm, matrix size= 224×224, TR=3200ms, TE=56ms, Flip angle =variable, isotropic 0.7mm voxel resolution. The total MRI session is less than 60 minutes.

8.3 Adverse Events and Serious Adverse Events

8.3.1 Definition of Adverse Events (AE)

Adverse event (AE) means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.3.2 Definition of Serious Adverse Events(SAE)

An AE or suspected adverse reaction is considered "serious" (SAE) if, in the view the Sponsor-Investigator, it results in any of the following outcomes:

- death,
- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,
- a congenital anomaly/birth defect, or
- An important medical event that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 Classification of an Adverse Event

Severity

Adverse events will be graded according to [name grading scale, e.g. CTCAE v5.0]. For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- Mild – Events require minimal or no treatment and do not interfere with the participant’s daily activities.

- Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference withfunctioning.
- Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

Relationship to Investigational Product

All AEs must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- Definitely Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- Probably Related – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- Potentially Related – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- Unlikely to be related – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- Not Related – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

Expectedness

The Principal Investigator will determine the expectedness of each AE based on the listed adverse event section of the Reference Safety Information in the most recent US package insert.

An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information described in the US Package Insert.

8.3.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The principal investigator or other clinician seeing the participant will record all reportable events with start dates occurring any time after parental permission and assent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the participant will be asked about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3.5 Adverse Event Reporting

Adverse events will be reported to the FDA, Yale IRB, the DSMB, and NIMH (see Table 2 below for reporting requirements) in accordance with all local applicable laws and regulations.

8.3.6 Serious Adverse Event Reporting

The identification of SAEs will begin when a member of the research team reports an adverse event. The site PI or her designee will conduct prompt investigations of all reported adverse events. All SAEs will be documented on an SAE form, entered in the appropriate CRF, and communicated to the appropriate team members.

Reporting to the Yale IRB

SAEs will be reported to the Yale IRB in accordance with IRB Policy 710 (see also section 8.4, Unanticipated Problems). We will additionally report SAEs to the IRB that may be expected in the clinical population but are considered severe in nature (e.g. attempted suicide, psychiatric or medical hospitalization) regardless of their potential relationship to the study

interventions. All adverse events that involve risk but do not meet the *prompt* reporting requirements described in Policy 710 will be reported to the IRB in summary form at the time of continuing review.

Reporting to the FDA

For studies conducted under an IND, there are two types of Safety Reports submitted to FDA:

- 7-Calendar-Day FDA Telephone or Fax Report: The sponsor-investigator will directly notify the FDA, within 7 calendar days after initial receipt of the information, of any adverse event that is fatal or life-threatening, unexpected, and considered at least possibly related to the investigational product.

- 15-Calendar-Day FDA Written Report: The sponsor-investigator will directly notify the FDA within 15 calendar days after initial receipt of the information, of any serious adverse event (other than those that are fatal or life-threatening) that is unexpected and considered at least possibly related to the investigational product.

Serious Adverse Events which do not meet the criteria for expedited reporting will be reported to the FDA in the IND Annual Report.

Table 2

Reporting to	Description of Event	Timeframe	Other
Yale IRB	Adverse events or injuries that are serious, unexpected, and at least possibly related;	Within 5 calendar days of the Principal Investigator becoming aware of the event.	UPIRSO Reporting Form (710 FR 4)
	Adverse events or injuries that are non-serious, expected, or unrelated	At the time of continuing review.	Reported to the IRB in summary form; such summary may be a simple brief statement that events have occurred at the expected frequency and level of severity as previously documented
	Deaths not attributed to the research (e.g., from "natural causes," accidents, or underlying disease when the Principal Investigator has ruled out any connection between the study procedures and the subject's death)	At the time of continuing review.	Reported to the IRB in summary form; such summary may be a simple brief statement that events have occurred at the expected frequency and level of severity as previously documented
FDA	Any adverse event that is fatal or life-threatening, unexpected, and considered at least possibly related to the investigational product.	Within 7 calendar days after initial receipt of the information,	MedWatch Form FDA 3500
	Any serious adverse event (other than those that are fatal or life-threatening) that is unexpected and considered at	Within 15 calendar days after initial receipt of the information,	MedWatch Form FDA 3500

Reporting to	Description of Event	Timeframe	Other
	least possibly related to the investigational product.		
	Serious Adverse Events which do not meet the criteria for expedited reporting	IND Annual Report	
NIMH DSMB	Death Definitely, Probably, or Potentially Related	Immediately (within 5 business days)	The SAE should be reported as soon as possible to the NIMH DSMB, within 5 business days for deaths related to study participation. The SAE will also be included in the SAE section of the next DSMB Report.
	Serious Adverse events; Unexpected: and Definitely, Probably, or Potentially Related	DSMB Immediately (within 5 days)	The SAE should be reported as soon as possible to the NIMH DSMB, within 5 business days for unexpected serious adverse events related to study participation The SAE will also be included in the SAE section of the next DSMB Report.
	Serious Adverse events; Expected events: regardless of relation to study (related and unrelated)	DSMB Tri-Annually	The SAE will be listed in the SAE section of the DSMB Report (currently a tri-annual report). No need to notify the DSMB on an immediate basis.
	Serious Adverse events; Unexpected: and Unlikely or NOT Related	DSMB Tri-Annually	The SAE will be listed in the SAE section of the DSMB Report (currently a tri-annual report). No need to notify the DSMB on an immediate basis.
	Death Unlikely or NOT Related	DSMB Tri-Annually	The SAE will be listed in the SAE section of the DSMB Report (currently a tri-annual report). No need to notify the DSMB on an immediate basis.
NIMH Program Officer	Deaths related to study participation	Reported immediately (no later than within 5 business days) of the principal investigator first learning of the death	

Reporting to	Description of Event	Timeframe	Other
	Unexpected Serious Adverse Events related to study participation	10 business days of the study team becoming aware of the SAE.	All reports must be made in writing to the NIMH Program Official (PO). These reports should indicate that the monitoring entities (i.e., the PI and IRB, DSMB) and appropriate regulatory entities (e.g., OHRP, FDA) have been notified in accordance with the approved monitoring plan and federal regulations. Reports should be submitted to the monitoring entity (e.g., a DSMB or ISM) at least annually on a schedule determined by the monitoring entity's policy. Monitoring entities may require more frequent reporting.
	AEs and SAEs that are deemed expected and/or unrelated to the study,	Summary should be submitted to the NIMH PO with the annual progress report.	

8.3.7 Reporting Events to Participants

The AEs and SAEs that will be reported to participants are determined by the Yale IRB in accordance with Yale IRB Policy 710.

8.3.8 Events of Special Interest

Not applicable.

8.3.9 Reporting of Pregnancy

Female participants will receive a serum pregnancy test with baseline screening labs. Urine pregnancy tests will be administered at the start of the week (prior to any infusion) during the blinded phase and in the event of a cross-over to open ketamine. Because full confidentiality regarding pregnancy cannot be entirely guaranteed, these testing requirements and the limited scope of confidentiality will be made known to all subjects during the permission and assent procedures. In this manner, young women who would not be comfortable with pregnancy testing or sharing the results of such testing can "opt out" of the study at the time of the initial assent, without having to declare specific reasons. If the pregnancy test is positive, the subject will not be able to participate in the protocol. Please see also section 5.3, Lifestyle Considerations, which details contraceptive requirements.

8.4 Unanticipated Problems

8.4.1 Definition of Unanticipated Problems (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

1. Is unexpected (in terms of nature, specificity, severity, or frequency) given (a) the research procedures described in the protocol-related documents, such as the IRB-approved protocol and informed permission document and (b) the characteristics of the subject population being studied; AND
2. Is related or possibly related to participation in the research (*possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); AND
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, legal, or social harm) than was previously known or recognized.

Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) may be medical or non-medical in nature, and include – but are not limited to – serious, unexpected, and related adverse events and unanticipated adverse device effects

8.4.2 Unanticipated Problem Reporting

UPIRSOs will be reported by the PI to the IRB in accordance with Yale IRB Policy 710, using the appropriate forms found on the website. All study-related events involving risk but NOT meeting the prompt reporting requirements described in IRB Policy 710 will be reported to the IRB in summary form at the time of continuing review. If appropriate, such summary may be a simple brief statement that events have occurred at the expected frequency and level of severity as previously documented.

Events that require prompt reporting include adverse events, if the events are unexpected, related, and serious, and may include subject complaints, protocol deviations, and other untoward events involving risk. Prompt reporting entails the following timeframes:

1. Events that may require a temporary or permanent interruption of study activities to avoid potential harm to subjects will be reported to the IRB immediately (if possible), followed by a written report to the IRB using the UPIRSO Reporting Form (710 FR 4) no more than 5 calendar days after the PI becomes aware of the event
2. Internal events (those occurring at a study site under the jurisdiction of the Yale IRB) will be reported to IRB within 5 calendar days of the PI becoming aware of the event

The following events may represent UPIRSOs that should be promptly reported:

- Adverse device effects that are unanticipated;
- Adverse events or injuries that are serious, unexpected, and related;
- Breaches of confidentiality involving risks;
- DSMB reports, interim analyses, or other oversight committee/monitoring reports altering the risk/benefit profile by identification of increased risks;
- Revisions to safety information, such as Investigational New Drug (IND) Safety Reports, SUSARS and MedWatch Reports, that meet the definition of aUPIRSO;
- New information indicating an unexpected increase in risks or decrease in potential benefits (e.g.literature/scientific reports or other published findings);
- Protocol deviations, violations, or other accidental or unintentional changes to the protocol or procedures involving risks or with the potential to recur;
- Unapproved changes made to the research to eliminate an apparent immediate hazard to a subject;

- Other problem or finding (e.g., loss of study data or forms) that an investigator or research staff member believes could influence the safe conduct of the research.

UPIRSOs will also be reported to the DSMB, OHRP and the NIMH PO within 10 business days of the investigator learning of the event. UPIRSOs that are also AEs would additionally be reported to the FDA according to the schedules described in Table 2.

Suspensions or Terminations and Serious or Continuing Noncompliance

Any suspension or termination of approval of the study by the FDA/DSMB/OHRP/IRB must include a statement of the reason(s) for the action and must be reported promptly to the NIMH PO within 3 business days of receipt. Serious or continuing noncompliance will be Reported to the NIMH PO within 10 business days of IRB determination

According to HHS regulations 45 CFR 46.103(a) and (b)(5), The Yale Human Research protection Program has written procedures to ensure that the following incidents related to regulatory requirements pertaining to research conducted under an OHRP- approved assurance are promptly reported to OHRP: a) Any unanticipated problems involving risks to subjects or others; b) Any serious or continuing noncompliance with this policy or the requirements or determinations of the IRB; and c) Any suspension or termination of IRB approval.

8.4.3 Reporting Unanticipated Problems to Participants

The unanticipated problems that will be reported to participants are determined by the Yale Institutional Review Board (IRB) in accordance with IRB Policy 710.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

Aim 1: To evaluate the safety of treating adolescents with SRI-resistant depression at high suicide risk with a conservative repeat-dosing ketamine paradigm followed by standard of care treatment over 4 months.

> **Hypothesis:** We anticipate no untoward effects on medical outcomes (cardiovascular function and bladder health) or cognitive function (measured via Cogstate).

Aim 2: To evaluate the 48-hour impact of ketamine on suicidal ideation compared to midazolam, and to identify connectome phenotypes predictive of ideation post-treatment.

> **Hypothesis:** Ketamine will reduce suicidal thinking (Columbia Suicide Rating Scale, recent ideation subscale) compared to midazolam. CPM will identify networks predictive of ideation, validated via k-fold or leave-one-out cross-validation within the sample. The network measures obtained at this fixed ketamine dose will inform the design of larger clinical trials.

Aim 3 (exploratory): To describe the trajectory of suicidal thinking, depressive symptoms, and use of mental health resources in both ketamine responders and non-responders over 4 months.

9.2 Sample Size Determination

The original effect size calculation was based on power analysis of a single, randomized-controlled trial of ketamine involving 80 adult subjects using the Scale for Suicidal Ideation. As noted below *(in italics)*, there are additional sources of information that were used in recalculating the anticipated range of possible effect sizes for important primary and secondary outcomes in this trial and the anticipated sample size has been readjusted to 40-66 subjects.

Primary outcome (suicidality, as measured by the C-SSRS recent ideation subscale: to detect an effect size of 0.75[117] of ketamine versus midazolam at this rapid timepoint with 80% power at the two-sided 0.05 significance level, using a two sample t-test, a sample size of 30 subjects per group is required. An additional 3 subjects per group will be enrolled to accommodate a 10% attrition rate, requiring a total sample size of 33 subjects per group (N=66).

Rationale for the Revised Anticipated Effect Size: 0.75-0.85 for the Primary Outcome

- *The power calculation for the original grant proposal was based on a randomized, midazolam-controlled trial of 80 adult subjects with Major depression and suicidal ideation, which demonstrated an effect size of 0.75 at 1 day following initial infusion on the Scale for Suicidal Ideation. [117] (BLUE in Figure 6)*
- *A meta-analysis which included data from 176 individual adult participants from 10 randomized controlled trials of ketamine for depression including adults with treatment-resistant depression and suicidal ideation demonstrated an effect size of 0.85 at 1 day following initial infusion as measured on the MADRS item 10 that measures suicidal ideation.[119] (GRAY in Figure 6)*

Secondary Aims Analyses: Secondary outcome analysis will apply the Benhamini-

Hockberg procedure[118] to control for multiple testing. For an alpha = 0.01 (corrected) for depression outcomes and 80% power, the minimal detectable effect size would be 0.91. Our prior studies with ketamine in depressed adolescents demonstrate an effect size of 0.75 for depression endpoints (MADRS) at rapid timepoints, and thus we are powered to approximately 60% to detect a difference for antidepressant outcomes.

Rationale for the Revised Anticipated Effect Size: 0.78-0.89 for the Depression Symptoms

- *Our original single-dose, midazolam-controlled, crossover trial in adolescents with treatment-resistant depression which provided the pilot data for this initial grant proposal demonstrated an effect size of 0.78 on the MADRS at day 1 for improving depressive symptoms.[120] (ORANGE in Figure 6)*
- *The meta-analysis which included data from 176 individual adult participants from 10 randomized controlled trials of ketamine for depression including adults with treatment-resistant depression and suicidal ideation demonstrated an effect size of 0.89 at day 1 following initial infusion for improving depression symptoms on the MADRS scale. [119] (YELLOW in Figure 6)*

Given that even with our original sample size we were underpowered to detect secondary outcomes if we adjust for multiple hypothesis testing, we instead plan not to adjust for multiple comparisons in secondary analyses (use alpha=0.05) and treat the analyses as exploratory and limit reporting to point estimates and 95% CI without formal hypothesis testing.[121]

Imaging outcomes: The current study proposes to image 66 adolescents, half initially randomized to ketamine and half to midazolam. CPM has successfully built predictive models using only 25 subjects[55], and thus we believe that 33 subjects per group is sufficient to perform the proposed treatment phenotyping. We have estimated an attrition rate of 10% based on previous experience with pediatric ketamine trials, although participant withdrawal generally occurs later in the course of the trial (while the imaging is occurring relatively early on). Even with an additional attrition of 15% for miscellaneous causes (e.g. braces, motion, severe claustrophobia), we still meet the threshold of 25 subjects. *\We anticipate that a sample size between 40-66 will get us the necessary sample size to have the power to complete neuroimaging analysis (N≥25) with currently expected additional exclusion (e.g. due to braces and permanent retainers) or declining participation in the MRI portion of the study (e.g. due to claustrophobia or additional subject burden).*

Figure 6 depicts power versus sample size for the four effect sizes discussed above. Table 3 describes the anticipated power for sample sizes for the 4 possible anticipated effect sizes described above in the range of 30-66 subjects. Based on the above power calculations, we would need to enroll 46-58 subjects to have 80% power to detect a between-group difference in our primary outcome related to suicidal ideation and a total of 42-54 subjects to have 80% power to detect a between-group difference in our secondary exploratory outcomes.

Table 3: Power Calculation for Anticipated Effect Sizes regarding Suicidal Ideation and Depression Symptoms

Total Sample Size	Suicidal Ideation		Depression	
	d=0.75	d=0.85	d=0.78	d=0.89
30	50.9%	61.3%	54.1%	65.3%

32	53.7%	64.3%	57.0%	68.3%
34	56.4%	67.1%	59.7%	71.1%
36	58.9%	69.8%	62.3%	73.7%
38	61.4%	72.2%	64.8%	76.1%
40	63.7%	74.5%	67.1%	78.3%
42	66.0%	76.7%	69.4%	**80.3%**
44	68.1%	78.6%	71.5%	82.2%
46	70.1%	**80.5%**	73.5%	83.9%
48	72.0%	82.2%	75.3%	85.5%
50	73.8%	83.8%	77.1%	86.9%
52	75.6%	85.2%	78.8%	88.2%
54	77.2%	86.5%	**80.3%**	89.4%
56	78.7%	87.8%	81.8%	90.5%
58	**80.1%**	88.9%	83.1%	91.5%
60	81.5%	89.9%	84.4%	92.4%
62	82.8%	90.9%	85.6%	93.2%
64	84.0%	91.7%	86.7%	93.9%
66	85.1%	92.5%	87.7%	94.5%
SAMPLE SIZES NEEDED TO ACHIEVE 80% POWER				
[redacted]	58	46	54	42

Table 3: Power Calculation for Anticipated Effect Sizes regarding Suicidal Ideation and Depression Symptoms. Table 3 depicts the power calculations for the anticipated range of observed effect sizes for suicidal ideation and depression symptoms for possible sample sizes in the current study assuming alpha=0.05 and a 100% completion rate. Sources utilized for the range of anticipated effect sizes for suicidal ideation and depression are outlined above. Shading of the effect sizes in the table correspond to the corresponding color of the power curve for that effect size in Figure 6.

Figure 6: Power Curve for Anticipated Effect Sizes regarding Suicidal Ideation and Depression Symptoms

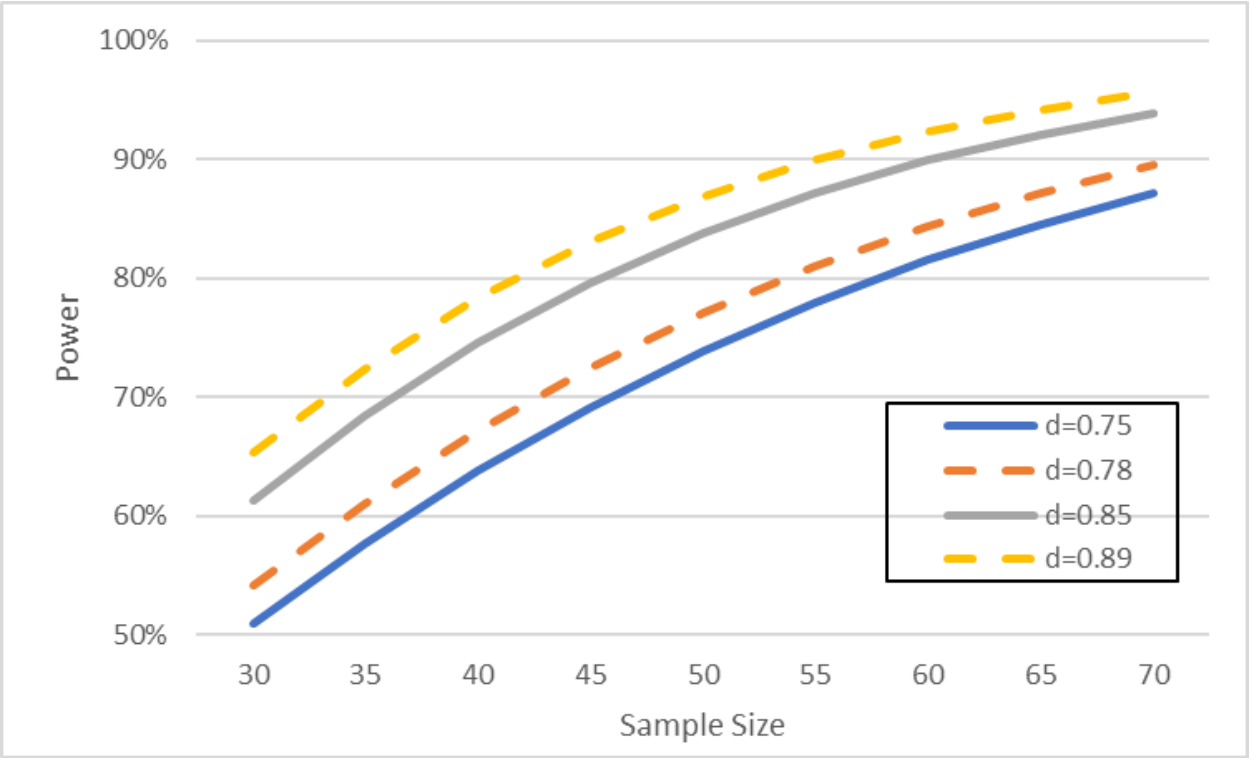

Figure 6: Power Curve for Anticipated Effect Sizes regarding Suicidal Ideation and Depression Symptoms. Figure 6 depicts the power curves for anticipated effect sizes for suicidal ideation (solid lines) and depressive symptoms (dashed lines) over the potential sample size range for this trial if granted a 2-year no cost extension. Sources utilized for effect sizes used for power calculations for suicidal ideation[3-4] and depression[2,4] are described above.

9.3 Populations for Analyses

- Intention-to-Treat (ITT) Analysis Dataset (i.e., all randomized participants)
- Per-Protocol Analysis Dataset: defines a subset of the participants in the full analysis (ITT) set who complied with the protocol sufficiently to ensure that these data would be likely to represent the effects of study intervention according to the underlying scientific model

9.4 Statistical Analyses

9.4.1 General Approach

There are no RCTs testing ketamine's rapid anti-suicidal effects in high-risk youth, and our ability to predict responses to treatment based on biological data is poor. The primary outcome is C-SSRS, recent ideation subscale, at 48-hours post-dose comparing participants who were randomized to ketamine to those who received the active placebo, midazolam. Key secondary outcomes include 48-hour MADRS and Day 11 C-SSRS, MADRS, and CDRS-R. CPM will be used to identify pre-treatment connectome fingerprints that predict treatment response and how circuits change post-treatment.

Data will be examined prior to analysis using descriptive statistics, presented as means ± SD or median (interquartile range) for continuous characteristics, and as frequencies (%) for categorical characteristics. Where appropriate, randomized groups will be compared on baseline continuous characteristics (e.g. age) using two-sample Welch's t-test or Wilcoxon rank sum test, and on categorical characteristics using the chi-square test or Fisher's exact test as appropriate. Continuous outcome measures will be assessed for normality using

histograms and Q-Q plots, and transformations will be applied as necessary. Short-term outcomes will be analyzed by linear mixed models with rating scales as the dependent variables and baseline values, treatment, time (48 hours, Day 11), and treatment-by-time interaction as independent variables (fixed effects). Subjects will be modeled as a random effect to account for the correlated data from same subject. Linear contrasts will be used to estimate treatment group differences and 95% confidence intervals at each timepoint. We will formally test the predictive power of a variety of features associated with outcome in clinical trials in depression and suicidality by including them as covariate(s) separately and together (except for age and age-at-onset) in the analytical models described above.

9.4.2 Analysis of the Primary Efficacy Endpoint(s)

The primary outcome of C-SSRS at 48-hours will be compared between groups using an analysis of covariance (ANCOVA) model, with treatment group (ketamine versus

midazolam) and baseline C-SSRS as the independent variables (covariates). Our primary analyses will be intent-to-treat using all available data.

9.4.3 Analysis of the Secondary Endpoint(s)

Safety outcomes: To examine the safety and tolerability of 4 doses of ketamine in this developmental population we will closely track adverse events and side effects in both the ketamine and midazolam treated groups for the duration of the study. We will use descriptive statistics to characterize the rates of these events for both groups and will use Fisher's exact tests to test for differences between the groups.

Additional clinical outcomes: Short-term outcomes (e.g. MADRS, CDRS-R) will be analyzed by linear mixed models with rating scales as the dependent variables and baseline values, treatment, time (48 hours, Day 11), and treatment-by-time interaction as independent variables (fixed effects). Subjects will be modeled as a random effect to account for the correlated data from same subject. Linear contrasts will be used to estimate treatment group differences and 95% confidence intervals at each timepoint. We will formally test the predictive power of a variety of features associated with outcome in clinical trials in depression and suicidality by including them as covariate(s) separately and together (except for age and age-at-onset) in the analytical models described above. *Comparison of response proportions:* Response is defined as a 50% reduction in C-SSRS (suicidality) or MADRS (depression). The proportion of responders at rapid (48-hour) and short-term (Day 11) timepoints between treatment groups will be performed by the generalized linear mixed models with a random subject effect in which binary response outcome is modeled as a function of covariates including treatment, time, treatment by time interaction and baseline value. Depression status as a mediator of anti-suicidal response: Lastly, as exploratory analysis, to estimate the direct effect and indirect mediation effects of MADRS on the association of treatment with C-SSRS scores, we will fit multilevel mediation models[122] within multilevel structural equation modeling framework using Mplus software. We will also consider an alternative mediation analysis method within the mixed-effect modeling framework described by Bauer[123]. These secondary clinical outcomes shall be considered exploratory and used for hypothesis generating purposes for future studies as our statistical threshold will be set at $p<0.05$ and will not correct for multiple comparisons.

Neuroimaging/ CPM outcomes: FMRI data will be motion corrected using SPM12. Data will be analyzed using BioImage Suite[124,125] and custom scripts in Matlab (Mathworks). Linear and quadratic drift, mean signal from white matter, gray matter, cerebrospinal fluid, and a 24-parameter motion model (6 motion parameters, 6 temporal derivatives, and their squares) will be regressed from the data. Finally, data will be temporally smoothed with a zero mean unit variance Gaussian filter. Data from runs with excessive head motion (*a priori* as > 2 mm translation or > 3 degrees rotation during a single run) will be excluded. Head motion, calculated as mean frame-to-frame displacement, will be measured as a function of state between different task or rest runs to ensure that motion is not a primary driver of condition. Uniform smoothing will be run to remove residual effects of motion [126]. The best performing individual atlas will initially be registered to individual participant space via concatenation of a series of linear and non-linear registrations between the functional images, 2D and 3D anatomical scans as previously described[58]. From this step the individualized atlas will proceed and a custom atlas for each subject at each time point will be produced with information on the correspondence between nodes across (time and subject) atlases maintained. Connectivity matrices are then calculated for the different acquisition conditions and fed into the multidimensional CPM along with subject C-SSRS data.

Multidimensional CPM algorithm: We have extended our original CPM framework by modifying the feature selection step to incorporate information from multiple connectivity matrices (from both task- and resting-state acquisitions). For the new feature selection step, we use canonical correlation analysis (CCA) instead of linear regression. CCA is a multivariate method for inferring information from cross-covariance matrices by finding linear combinations of the data that maximizes the correlation between the data (*i.e.* connectivity matrices from task and rest) and variable of interest (suicidal ideation as measured via the C-SSRS). Latent brain networks from multiple task conditions are used to predict latent behavioral profiles in a single modeling step. Similar to CPM, edges are selected if the CCA produces a significant correlation as measured by the likelihood test (using p-value threshold of 0.01). Once the most predictive edges are selected, the new projections of those edges estimated from the CCA are summed to form a single subject suicidal ideation summary score.

Model Validation: After the CPM model is built from the training data, it can be used to predict scores from connectome data for novel subjects. We will initially test leave-one-out analyses but as the size of the data set grows we will perform k-fold cross-validation (k=10).

Such test data sets allow performance to be evaluated using either the mean squared error (MSE) or the correlation between the predicted and observed behavioral values. Lower MSE and higher correlation indicate a more predictive model. Validation in novel subjects is aimed at demonstrating that the models generalize and are not simply over-fitting a specific set of data.

Connectome Changes: To examine the impact of treatment on the functional connectome identified above we can compare masked connectivity matrices across timepoints. Here the pre-treatment multidimensional CPM identifies edges and nodes that are associated with suicidal ideation. These model edges and nodes can be contrasted with data collected post-treatment (for both placebo and ketamine) to determine the extent to which treatment modifies this pre-treatment functional phenotype. This contrast can be performed with a paired t-test corrected for multiple comparisons and the change distribution can be compared with the change distribution from the placebo group to measure the significance of this change. Such connectivity changes can also be regressed with the pre- and post-treatment suicidal ideation score change to test the hypothesis that the extent of change in the functional connectome is associated with the degree of change in ideation score.

9.4.4 Safety Analyses

Please see section 9.4.3. Safety Outcomes and section 9.4.6 Planned Interim Analyses.

9.4.5 Baseline Descriptive Statistics

Data will be examined prior to analysis using descriptive statistics, presented as means ± SD or median (interquartile range) for continuous characteristics, and as frequencies (%) for categorical characteristics. Where appropriate, randomized groups will be compared on baseline continuous characteristics (e.g. age) using two-sample Welch's t-test or Wilcoxon rank sum test, and on categorical characteristics using the chi-square test or Fisher's exact test as appropriate. Continuous outcome measures will be assessed for normality using histograms and Q-Q plots, and transformations will be applied as necessary. Sex will be

treated as a biological variable in our analyses. We will explicitly test for sex effects in all aspects of this work by building both joint and separate sex-based CPMs and then contrasting the nodes and edges identified in the separate models from the joint model to test for sex effects (paired t-test with correction for multiple comparisons based on the number of edges selected in the models).

9.4.6 Planned Interim Analyses

There is no interim analysis for efficacy outcomes.

Given the importance of monitoring safety outcomes with this experimental treatment, we have developed an interim safety analysis plan and stopping guidelines based on monitoring the rates of suicide events. Suicide events include suicide attempts, interrupted suicide events, and high levels of suicidal ideation that necessitate emergency evaluation or hospitalization. In addition to tables and listings of adverse and serious adverse events, these specific analyses will be presented to the DSMB at all meetings following study initiation.

1) Comparison of rates of suicide events between randomized groups (ketamine and midazolam) during the double-blind phase. A Fisher's exact test will be used and if the p-value crosses the one-tailed 0.025 significance level (indicating a greater likelihood of events in the ketamine group), further enrollment and ketamine treatment will be stopped until the DSMB has reviewed the data and made a recommendation about study discontinuation.

2) Evaluation of the rate of suicide events in all participants who receive ketamine to determine if it is unacceptably higher than expected. We will compare the rate of suicide events in all subjects that receive ketamine (i.e. those initially randomized to ketamine **and** those that cross-over to receive ketamine following the double blind phase) to an acceptable rate of 33.8%/6 months. Using a one-sided 0.025 significance level, if the rate is significantly higher than 33.8%/6 months, further enrollment and ketamine treatment will be stopped, until the DSMB has reviewed the data and made a recommendation about study discontinuation.

In order to maintain the blind and the integrity of the study data, the study team has both a blinded and unblinded statistician. Any DSMB reporting of data by treatment group (i.e. blinded data) will be prepared solely by the unblinded statistician and sent directly to the DSMB liaison. The unblinded statistician will not discuss the results of these analyses with the rest of the study team in a manner which challenges the blind. Any relevant DSMB queries of this data will go directly to the unblinded statistician, and DSMB communications regarding ongoing reviews will proceed as detailed in the NIMH DSMB charter.

9.4.7 Sub-Group Analyses

NA

9.4.8 Tabulation of Individual participant Data

Coded individual participant data will be listed by measure and time point.

9.4.9 Exploratory Analyses

Weekly data on mood and suicidal ideation will be analyzed by mixed-effects regression methods to model the trajectories over the 4-month open phase, regardless of the treatment

assignments. Significance changes of these data from D15 will be tested, with least square mean values estimated at each follow-up time points. These estimates and corresponding 95 CI% will be compared across the 5 response groups. The number of ER, IOP, PHP, or inpatient visits between ketamine responders and non-responders will be compared by the Poisson or negative-binomial regression analysis. Paired t-tests will be used to compare the changes of mood and suicidal ideation in patients who did not have a response to midazolam in the blinded phase and went on to receive an open ketamine.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

Institutional Review Board (IRB) Review

The protocol will be submitted to the IRB for review and approval. Approval of the protocol must be obtained before initiating any research activity. Any change to the protocol or study team will require an approved IRB amendment before implementation. The IRB will determine whether informed permission and assent and HIPAA authorization are required. The IRB will conduct continuing review at intervals appropriate to the degree of risk, but not less than once per year. A study closure report will be submitted to the IRB after all research activities have been completed. Other study events (e.g. data breaches, protocol deviations) will be submitted per Yale University's IRB's policies.

Study Modification

Any study modifications will be completed in writing to the protocol documents, permissions, and assents, and submitted to the Yale IRB for approval, as well as to the relevant regulatory authority prior to implementation. Version control will be used to track all changes, and only the most recent versions of IRB-approved documents will be used. If a modification causes a substantial change to permission or assent documents, participants and parents will be re-consented with the up-to-date documents.

In addition to IRB review and approval, the NIMH DSMB may require review of protocol/study modifications that are substantial, such as modifications to the eligibility criteria or study design. In ambiguous cases, the Sponsor-Investigator will inquire with the DSMB to ascertain if review is warranted.

10.1.1 Informed Consent Process

Informed Permission and Assent Processes

- Only the most recent IRB-approved version of the informed permission and assent documents will be used to consent/assent potential subjects.
- If there are two parents or guardians, both most provide permission and each must sign a separate permission form. Completed parental permission forms and the adolescent assent

document must be obtained from every subject who takes part in a study prior to performing any study-related activities.

- When a potential study subject is identified, the investigator or delegate discusses the study in detail with the participant and their parents. An explanation of the study, its risks and benefits, and what would be required of the subject is discussed. The potential subject and parents or guardians are given a copies of the informed parental permission form and assent documents to read in a quiet environment without distraction, including the option to take the form home to review and discuss with others. Ample time and opportunity are provided for the potential subject, parents, and/or legally authorized representative to ask about the details of the study, to consider other available options, and to decide whether to participate. All potential participants have the opportunity to discuss study participation without their parents or guardians present, to ensure maximum privacy and comfort in discussing any sensitive issues (e.g. pregnancy, drug use) and asking study-related questions, and to ensure that the adolescent is freely choosing to participate without any undue pressure or influence. All questions and concerns to both the adolescent and their parents/guardians are addressed throughout this process by the individual obtaining permission and assent.
- The individual obtaining permission/assent will ensure to the degree possible, and based on his/her judgment, that the potential subject and parents or guardian have comprehended the information provided about the research. A short quiz at the end of the informed consent forms will aid in assessing understanding.
- If a potential subject and parents/guardians decide to participate, the parents/guardians are asked to sign the permission form only after all questions and concerns have been addressed. The adolescent subject is similarly asked to sign the assent document only after all questions and concerns have been addressed
- The permission forms and adolescent assent form must be signed and dated by the parents and participant, along with the individual obtaining consent.
- This study will allow for e-consenting using the Part 11-compliant RedCap system. The RedCap ICF documents will be identical to their HIC-approved paper counterparts. While we anticipate that many or most study discussions and consenting will occur in person, the capacity for e-consenting allows flexibility should a participant want to take some more time to review the study documents at home after an initial discussion. We may also remotely consent potential subjects when subjects need to travel a long distance in order to participate in the trial.
    - The e-consent process is as follows: Any time a permission/assent/consent process is conducted remotely, study staff will be present with the participant and their family via Zoom session. Participants and parents identify an electronic device to access REDCap and open the eConsent as a survey. Time is allotted for any additional review of the research study with the participant, answering questions, and completing the assessment quiz. Research staff review the results of the quiz and review any remaining questions. Participant signature is obtained in RedCap. In the part 11-compliant system, each participant and parent will have a unique login and password to verify their identity, which only they will know. Staff will then review the eConsent form to confirm it is filled out correctly and sign the eConsent as the ‘person obtaining consent’.
    - A compact PDF copy of the signed eConsent will be automatically stored in the File Repository. A copy of the signed eConsent can be provided to the participant and their family either as a paper or electronic document
- All signed paper consents will be uploaded and stored in the same Part 11-compliant RedCap EDC. The paper original signed permission forms and adolescent assents will be kept in a locked filing cabinet in the Child Study Center.

- A copy of the parental permission documents, adolescent assent, and any other documentation about the research (e.g., calendars, instructions) shall be provided to the subject and their parent or guardian.
- Documentation of the permission and assent processes will be entered in the subject's source documents (see section on Documentation Related to the Informed Permission/Assent Process below).

Additional Assent Information: Assent is an adolescent's affirmative agreement to participate in research. Assent will be sought in addition to the permission of a legally authorized representative or surrogate when the individual is sufficiently cognitively capable of understanding the nature of his or her participation in a research study, failure to object will not be construed as assent (45 CFR 46.402(b); 21 CFR 50.3(n)). A child is any individual who at the time of enrollment in a research study has not attained the legal age for consent to treatments or procedures involved in the research under the applicable law of the jurisdiction in which the research will be conducted (45 CFR 46.402(a); 21 CFR 50.3(o)). In the state of Connecticut, the age of majority is 18. Assent will be documented through the adolescent signing an assent form. The assent form will be written in language that is appropriate to the child or adolescent subject's maturity and cognitive level and used as part of the assent process to 1) describe the research study, including the research procedures, risks and benefits, and 2) obtain the child or adolescent'swritten agreement to participate in the study.

Informed Consent Process: If a participant turns 18 while participating in the study, their initial assent and parental permissions are no longer sufficient. Once the participant turns 18, they will now be asked to provide consent for the study, using a separate consent document. The informed consent document is identical to the permission form, with the content addressed to "you" rather than "your child or adolescent". The same principles of informed consent apply, as described above for permission and assent.

Re-permission/re-assent/re-consent: If during the course of the study, the protocol has been modified in such a way that changes are made to the informed permission or assent forms, subjects who have already given their informed permission/provided their assent may be required by the IRB to be re-permissioned using the updated form. If the IRB determines that re-consenting is required or if it is deemed appropriate by the PI but in the absence of an IRB requirement, all subjects currently enrolled in the study must sign the updated informed permission form to acknowledge the changes. The subject may be re-consented at the next site visit unless otherwise stated by the IRB or Sponsor-Investigator.

Immediate hazards or issues of safety, however, should be communicated to the participant and the participant's parent/guardian upon receipt of the new information or as directed by the Sponsor-Investigator. This communication shall be documented in the medical record/research chart by the individual who communicated the information to the participant.

Documentation of the Permission/Assent/Consent Process
The individual obtaining informed permission will document the process via a note or completion of the source document template. The documentation will include information pertinent to the Permission Process (initial consenting and all follow-up consenting through the completion of the study):

- Name and title of person who explained the study

- Date(s) of study explanation (if different than date of document signing)
- Name and title of person who obtained Informed Permission/Assent/Consent (if different than above)
- The actual date Informed Permission/Assent was obtained
- Individual(s) present when Informed Permission/Assent was obtained
- Summary of the consenting process that describes that the study was discussed in detail including the study design, risk and potential benefits for their participation in the study, alternative treatments available, and voluntary nature of participation. In addition, indicate that sufficient time was provided for potential subject and their parents to read/review permission and assent forms and have questions answered to his or her satisfaction and has signed the document and received a copy, and has contact information if questions arise.
- Score of quiz, list of any questions that were missed, and summary of discussion around missed questions

10.1.2 Study Discontinuation and Closure

If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and the DSMB and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.
Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants (please see Section 9.4.4, Safety Analyses)
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

10.1.3 Confidentiality and Privacy

Subject confidentiality is held in strict trust by the research team. Subject medical record review will be limited to the just the elements needed to complete the study. Only authorized HIPAA and GCP trained study team members will be allowed to extract research data from medical records and enter it into RedCap. No direct subject identifiers will be entered into RedCap. All NIH-funded research is covered by a Certificate of Confidentiality, which applies to the current study. Each subject will be assigned a unique study number. A master list linking the unique study number to the human subject will be maintained in a locked drawer in the Child Study Center.

Clinical data, outcomes of diagnostic instruments, and research data will be collected by the principal investigator and other study personnel and stored in a locked file cabinet in a locked office. Data will be entered into a database on a password-protected computer in a locked office, by study personnel. Since this is an investigator-initiated study, the PI and study team will develop Clinical Research Forms (CRFs) for this study. These forms will be labeled with a unique random study code that cannot identify the patient. The key linking the code to the subject's identifiable information will be kept in an electronic excel file which is kept in a password protected file, on a password protected computer on the secure Yale server. A paper copy of this "master file" will be kept in a locked file cabinet as noted above. This master file will be kept separately from any coded data so that the identity of the participant will not be disclosed. The results of the medical and psychiatric evaluations conducted as part of this

research will be available to clinicians caring for the subject unless the participant requests otherwise. The Yale Human Investigation Committee may review records of this research. In the case of published reports of this study, the identities of all participants will be protected. All data obtained from subjects will be coded and stored in locked cabinets/password protected computer in an office that is locked to ensure confidentiality. Information that will breach subject confidentiality will not be shared. Rather, data will only be released upon written consent of the subject and will be available for review by the Yale Human Investigation Committee. The PI will also conduct periodic assessments to ensure that confidentiality provisions established at the onset of the study are maintained throughout the study and during data analysis. Additionally, all staff involved in the handling of subject data are/or will be trained on the requirements of HIPAA Privacy Rule and Human Subject Protection. If the PI should leave Yale, the PI will collaborate with her Department Chair and Faculty Advisor to ensure that proper and continued protection of individually identifiable information and protected health information continues.

10.1.4 Future Use of Stored Specimens and Data

Data will be kept in a locked filing cabinet whose access is only obtainable by study personnel and electronic clinical data will be kept on a password protected server. As an NIMH-funded study, data sharing through the NIMH Data Archive (NDA) is required. NDA is a large database where deidentified study data from many NIMH studies are stored and managed. Any researcher who requests access to the deidentified data of the NDA is bound to adhere to strict data safety practices and to avoid any attempts at deidentification. The intention to submit deidentified subject data to the NDA is included in permission and assent documents, and participants or guardians are able to opt out of this data sharing at any time. Opting out of NDA data sharing does not in any way impact trial eligibility.

With the parental permission and participant's assent, and as approved by the IRB, de-identified biological samples will be stored to examine the pharmacokinetics of ketamine metabolism in pediatric patients. The specimens will be coded in a way that allows linkage to the phenotypic data from each participant, maintaining the blinding of the identity of the participant. During the conduct of the study, the parents or an individual participant can choose to withdraw permission/assent to have biological specimens stored for future research. However, withdrawal of permission/assent with regard to bio sample storage may not be possible after the study is completed.

10.1.5 Key Roles and Study Governance

The Study team is led by the PI, Dr. Michael Bloch, MD, MS. Dr. Bloch will oversee all of the clinical trial operations, including patient screening, enrollment and consent, treatment, and follow up. His clinical team includes an efficacy rater who conducts the blinded ratings (this rater in not present during any of the ketamine or midazolam infusions), a pediatric psychotherapist with experience working with adolescents with CBT, and a clinical coordinator, who schedules patients and assessments, and also assists with the upkeep of regulatory binders and reports.

Dr. Gerard Sanacora, MD, PhD, serves as the Scientific Director of the Interventional Psychiatry Service (IPS, where medication treatments will occur during this study), and will act as a liaison between the study team and IPS to facilitate participant treatment flow.

Dr. Angeli Landeros, MD, is an associate research scientist with extensive experience running pediatric depression clinical trials for rapid antidepressants with Dr. Bloch. She will assist with participant recruitment and trial logistics.

Dr. R. Todd Constable, PhD, serves as the co-Director of the Magnetic Resource Research Center. He will oversee the neuroimaging portion of the study, including leadership on MRI sequence parameters, development of the individualized node parcellation algorithm, data analysis and pre-processing, and connectcome-based predictive modeling algorithms.

Dr. Eugenia Buta, PhD is responsible for the statistical analysis of the clinical data and generating reports for the DSMB.

10.1.6 Safety Oversight

The study will have a NIMH constituted Data Safety Monitoring Board (DSMB) who will periodically review and evaluate the accumulated study data for participant safety, study conduct, progress, and efficacy. The PI will also conduct an internal review every six months to monitor data collection and management of timelines (e.g., implementing data quality assurance procedures; updating, editing, and data checking all files; ensuring that protocol procedures are being followed, as per the site's related SOPs). Dr. Dai and the support services at the Yale Center for Analytic Science are experienced in study design, preparation of study forms, and management of data and preparation of reports related to the progress of each study, and will be responsible for preparing reports for the DSMB.

Safety Monitoring:

Participants are very closely monitored in this study, with twice weekly in-person visits during the 2-week blinded phase, and weekly visits during the 4-month open phase. The first 8 weeks of the open phase will be in-person assessments that coincide with the weekly CBT that the patient is receiving. During the last 8 weeks of the open phase, weekly assessments may be either in person or over the phone if the participant is doing well. There are four monthly in-person full batteries that include mood and anxiety assessments, as well as cognitive testing. Finally, there are medical safety screens built into our protocol, such that an ECG, serum laboratory values (hematologic, hepatic, and renal function) and a urinalysis are obtained at baseline, the end of month 2 of the open phase, and the end of month 4 of the open phase. Any participant who presents with new symptoms or physical complaints will have a medical and laboratory exam prior to the scheduled timepoint.

The multidisciplinary clinical team (efficacy rater, clinical coordinator, psychotherapist, and other participating trial staff) is led by the PI, and meets weekly to coordinate treatment efforts, monitor each patient's progress, and to determine whether further participation puts the patient at risk. The PI may discontinue a patient from study participation if it appears that the study is causing medical or psychiatric safety risks. A patient is also withdrawn at any time if he or she so requests or if a female patient becomes pregnant. At the end of the study or when a patient discontinues the study prematurely, efficacy evaluations are repeated. In addition, a physical exam, vital signs, weight, and laboratory measurements are obtained. Patients are also assessed for adverse experiences with the SAFTEE-GI and SAFTEE-SI.

Please also see section 9.4.4, Safety Monitoring, regarding ongoing safety monitoring at the group level.

10.1.7 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is compliant with currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s). The main features are below.

- Monitoring for this study will be performed by NIMH Clinical Research Education, Support and Training Program (CREST)
  - Monitoring will be conducted throughout the study, and involve targeted data verification of key data variables
  - The site PI will be provided copies of monitoring reports within 10 days of each visit and will be provided to the NIMH DSMB liaison within 30 days of the visit.
  - Details of clinical site monitoring are documented in the CMP. The CMP describes who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.
  - The site will perform internal quality management of study conduct, data collection, documentation and completion. An individualized quality management plan will be developed to describe the site's quality management.

10.1.8 Quality Assurance and Quality Control

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the NIMH, and inspection by local and regulatory authorities, including the FDA.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into Yale REDCap, a 21 CFR Part 11-compliant data capture system provided by Yale University. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

For neuroimaging data, we perform many steps to ensure data quality in our imaging center including daily phantom studies to test temporal stability, SNR, ghosting measures, and contrast levels. The MR techs perform daily stability tests, and scripts automatically upload the data, analyze the above measures, and send an alert if any measures are outside the acceptable range. During subject scanning, we have a real-time motion correction algorithm that provides feedback on subject motion compliance. Subjects can be provided with verbal feedback to try to decrease motion if it is too high or specific runs can be repeated if the motion is too high. Following these steps, a number of other QC steps are taken during analysis as described above (including motion censoring) for those subjects that remained still enough to produce useable data.

Rigor: Scientific rigor for the proposed study is established in the following manner: (a) experimental design follows state-of-the-art study guidelines; (b) safety, efficacy, and neuroimaging data collection is based on well-validated protocols (see below for quality assurance), and the same protocol will be used for all subjects; (c) we consider important biological variables, such as sex, and strictly follow inclusion and exclusion criteria; (d) all results will be compared to well-validated, previously published results; and finally, (e) connectome predictors of suicidal ideation generated via CPM will be based on a 10-fold cross-validated prediction of constructs—from the standpoint of scientific rigor, cross-validation is a more conservative way to infer the presence of a brain-behavior relationship than correlation. All results will be corrected for multiple comparisons using the false discovery rate.

Replication: The ultimate replication is a study wherein a model is built in one population and validated in another. To encourage replication of this work, we (1) will make the clinical trial protocol available, (2) disseminate outcomes as indicated on ClinicalTrials.gov, and (3) will make available all of our neuroimaging data, software, and models. We are hopeful that the clinical results and connectome predictors identified from the current study will be inform the design of a larger trial in suicidal youth, consistent with the aims of the RFA.

Please see also sections 10.1.6 Safety Monitoring and 10.1.7 Clinical Monitoring above

10.1.9 Data Handling and Record Keeping

Private identifiable information will be collected (name, date of birth, age, telephone number, address, medical and psychiatric history, diagnoses, laboratory tests, and psychiatric rating scores) but will be kept confidential and will not be divulged in any publication emanating from this work. The urine toxicology results will not be kept and, as an NIH-funded study, this trial is covered by a Certificate of Confidentiality. Risks to subject confidentiality will be minimized by adopting suitable data storage procedures. All data for each subject will be identified by numerical ID. The master list of subject IDs and names will be stored in a locked file at a separate location. Once data are collected and entered, hard copies of the testing protocols and clinical notes will be stored in a locked filing cabinet. All research data and the clinical information containing PHI are stored separately in locked file cabinets. Permission and assent forms are stored in yet another location. Throughout these procedures, all reasonable precautions are taken to minimize linking any PHI to the research data. All computers are password protected and furnished with firewalls and anti-spy and anti-virus software in accordance with the general guidelines of the Yale Information Technology and HIPAA offices. All data will be stored on a secure server and cloud backup managed by the Yale ITS Office. Reports based on these data will not identify subjects by name. The database will be maintained within the existing data management system, providing a high

degree of security and quality monitoring. Clinical data will be entered directly into RedCap from the source documents.

Access to Source Documents
Source documents include participant data and records that include but are not limited to the following: hospital or medical records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records and records, MAR from infusion procedures, recorded data from automated instruments, laboratory values, subject files, structural and functional MRI images. Data will be collected electronically in the RedCap 21 CFR Part 11-compliant system whenever possible. Any paper source documents will be kept in binders located in rooms that are locked when not in use, and are only accessible to authorized, trained personnel.

Study Records
Study records include but are not limited to regulatory documents, protocols, permissions, assents, consenting checklists, case report forms, and subject medical records.

Retention of Records
Study documents will be retained for a minimum of 2 years after the formal discontinuation of clinical development of the study intervention. These documents will be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable.

Data or Specimen Storage/Security
Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into Yale REDCap, a 21 CFR Part 11-compliant a data capture system provided by Yale University. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

Handling of Missing Data

We will make every reasonable effort to retain all available patients to preserve the integrity of the study data and to minimize possible bias due to the missing data points. We will minimize the loss of data by having clear data collection and performance standards to achieve highest data quality. We will monitor data collection to ensure quality standards are met, including targeted levels of data capture.

We will undertake sensitivity analyses to assess the impact of the missing response data on our conclusions. We will perform single-step (e.g., missing values on the response variable replaced by predicted values from linear or generalized linear models of the longitudinal data over time) or multiple imputation and compare the performance of the imputed data results compared to complete case analyses. It is important to note that the linear mixed model method we propose to use is valid under the assumption that missing data is missing at random (MAR). *Since it is not possible to test the MAR assumption and since for every MNAR model there is an equally well fitting MAR model, we believe that the only suitable approach is to perform sensitivity analyses according to different plausible scenarios for missing data and make sure the conclusions are consistent. If the results are not consistent depending on the assumptions for missing data we will report the conclusions based on each set of assumptions.*

10.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation. All deviations must be addressed in study source documents and reported Yale HRPP per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. All deviations will also be reported in summary format to the DSMB in the triannual reports and to the NIMH program officer in annual progress reports.

10.1.11 Publication and Data Sharing Policy

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

NIH Expectation	Study Action
The applicant will ensure that clinical trial under the award are registered and results information is submitted to ClinicalTrials.gov as outlined in the policy and according to the specific timelines stated in the policy	Michael Bloch, MD, MS (PI) in conjunction with the Yale Center for Clinical Investigation (YCCI), will be responsible for handling ClinicalTrials.gov requirements for this project. We will register the trial prior to enrolling the first patient.

Informed consent forms (ICFs) for the clinical trial will include a specific statement relating to posting of clinical trial information at ClinicalTrials.gov.	Per guidance from Title 21CFR.50.25 (Part 50, Protection of Human Subjects, Subpart B, Informed Consent of Human Subjects), we will include the following statement in the ICF: "A description of this clinical trial will be available on www.clinicaltrials.gov, as required by US Law. This website will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time."
The recipient institution has an internal policy in place to ensure that clinical trials registration and results reporting occur in compliance with policy requirements.	Once a record is established, we will confirm accuracy of record content, resolve problems and maintain records including content update and modifications. We will also be responsible for aggregate results reporting and adverse event reporting at the conclusion of the project. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 5 years after the completion of the primary endpoint by contacting the PI.
Under the NIH Public Access Policy, the public deserves access to the published results of NIH funded research.	The PI will ensure that all peer-reviewed manuscripts relating to this work will be digitally archived with PubMed Central upon acceptance for publication.

10.1.12 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership in conjunction with the appropriate conflict of interest review committee has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

All investigators will follow the applicable conflict of interest policies. Yale University maintains an up-to-date, written, enforced policy on financial conflicts of interest that complies with the requirements of Title 42 C.F.R. Part 50, Subpart F. As part of Yale's standard process, investigators are required to disclose, in writing, significant financial interests that relate to their research and other Yale responsibilities. In accordance with Yale's COI policy, Yale's Conflict of Interest Committee (or its designee) determines whether significant financial interests (SFIs) present financial conflicts of interest, and by what means such conflicts should be avoided or managed.

Dr. Michael Bloch (the Principal Investigator of this award) and Drs. Angeli Landeros, Todd Constable, Feng Dai, and Gerard Sanacora have submitted PHS-compliant conflict of interest disclosures to Yale's Conflict of Interest Office. Yale's COI Policy, posted at https://your.yale.edu/policies-procedures/other/yale-university-policy-conflict-interest, describes the specific processes and procedures that are in place to identity, address and report financial conflicts of interest.

10.2 Additional Considerations

Funding from 1R01MH125203 (PI Bloch)

10.3 Abbreviations

Abbreviation	Explanation
CADSS	Clinician-Administered Dissociative States Scale
CBT	Cognitive Behavioral Therapy
CDRS-R	Children's Depression Rating Scale- Revised
CPM	Connective-based Predictive Modeling
C-SSRS	Columbia-Suicide Severity Rating Scale
DBP	Diastolic Blood Pressure
DHNK	Dehydronorketamine
ECG	Electrocardiogram
GCP	Good Clinical Practice
HCP	Human Connectome Project
HNK	Hydroxynorketamine
HR	Heart rate
HSP	Human Subjects Protection
ICH	International Conference for Harmonisation
IDS	Investigational Drug Service
MADRS	Montgomery Asberg Depression Rating Scale
MAR	Medication Administration record
MDD	Major Depressive Disorder
MRI	Magnetic Resonance Imaging
NMDA	N-methyl-D-aspartate receptor
NK	Norketamine
PHI	Protected Health Information
SBP	Systolic Blood Pressure
SOP	Standard Operating Procedures

TORDIA	Treatment of Resistant Depression in Adolescents Trial
TRD	Treatment Resistant Depression
RDoC	Research Domain Criteria
RFA	Request for Applications
SSRI	Selective Serotonin Reuptake Inhibitor

10.4 Protocol Amendment History

Revision #	Version Date
0	09/04/2020
V1.2	09/14/2020
V1.3	09/15/2020
V1.4	10/27/2020
V2.0	03/05/21
V2.1	04/12/21
V2.2	02AUG2021
V2.3	01SEPT2021
V2.4	19OCT2021
V2.5	15NOV21
V3.0	18FEB22
V 4.4	30JAN2023
V 4.5	11SEPT2023
V 4.6	15APR2024
V 4.7	05SEPT2024

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