SEVERE ADOLESCENT DEPRESSION – KETAMINE INTERMEDIATE DURATION STUDY (SAD – KIDS)

Protocol Number: 2000023857

National Clinical Trial (NCT) Identified Number: NCT03889756

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

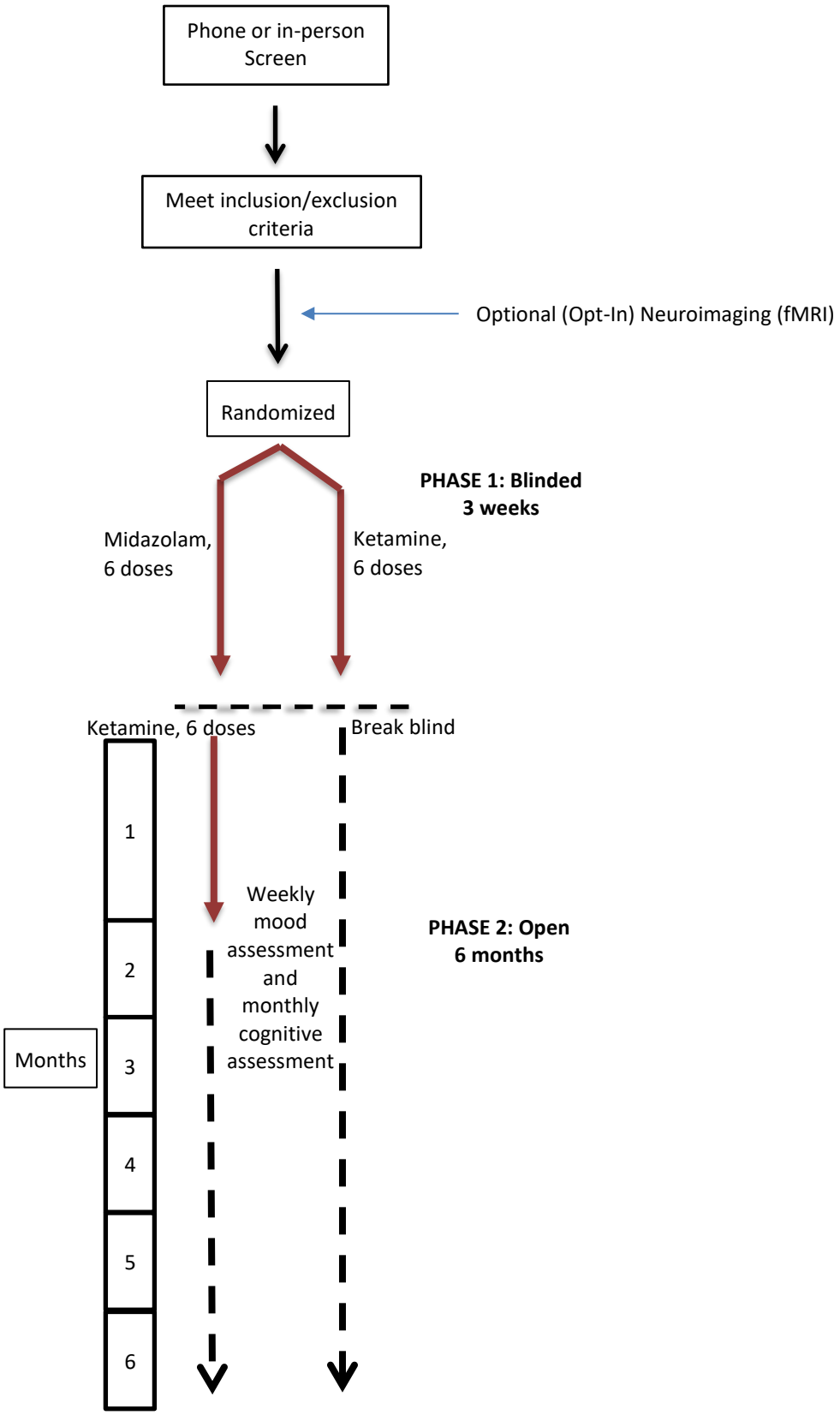
1.1 SYNOPSIS

Title:	Ketamine for severe adolescent depression: intermediate-term safety and efficacy
Study Description:	The purpose of this study is to evaluate the intermediate-term efficacy and tolerability of a multiple-dosing ketamine infusion paradigm for the treatment of medication-refractory major depressive disorder (MDD) in a pediatric population (13 to 17 year-olds).
Objectives:	Primary Objective: (1) To evaluate the efficacy and tolerability of a multiple-dosing ketamine infusion paradigm (2 infusions per week for 3 weeks) compared to midazolam in adolescents with treatment resistant depression (TRD). Secondary Objectives: (2) To evaluate the durability of ketamine's antidepressant response and the maintenance requirements over six months. (3) To evaluate the safety of the multiple-dosing ketamine paradigm by assessing cognitive function over six months via Cogstate battery. Exploratory Objective: To identify pre-treatment functional connectome signatures predictive of post-treatment efficacy

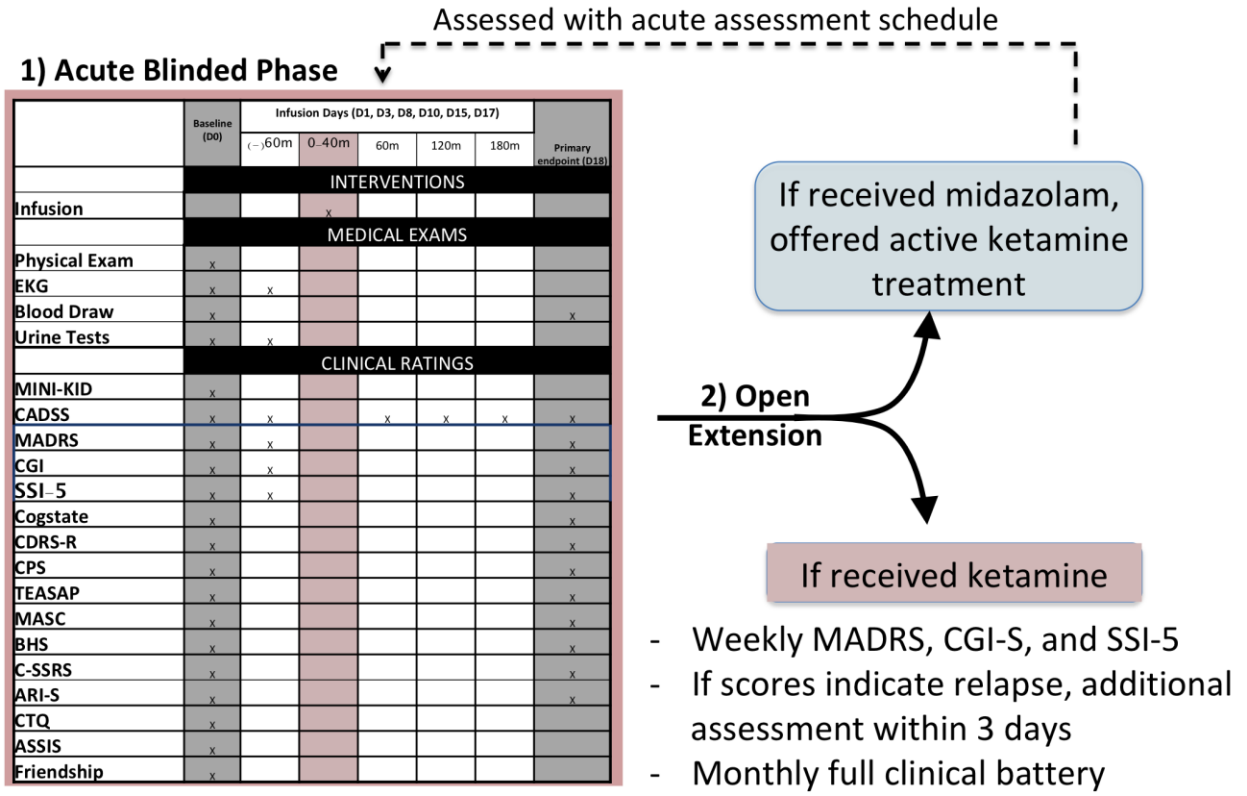
Endpoints:	Primary Endpoint: Improved Children's Depression Rating Scale-Revised (CDRS-R) score at Day 18. Secondary Endpoints: Time to relapse in ketamine responders, maintenance requirements over six months, neurocognitive measures, and analysis of ketamine metabolites.
Study Population:	Up to 24 adolescents ages 13-17 years with medication-refractory major depressive disorder (MDD) will be recruited for this study through the Yale Child Study Center. Average weights of subjects are anticipated to be 45-54kg for females 13-17 years old and 45-65kg for males 13-17 years old, and we have implemented a weight limit of 80kg.
Phase:	II/III
Description of Sites/Facilities Enrolling Participants:	The Yale Child Study Center (YCSC) will be the only site enrolling participants.
Description of Study Intervention:	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 are not to exceed a maximum total dose of 40mg/day of Ketamine and 3.6mg/day of Midazolam) (doses of both medications will not exceed a maximum total dose corresponding to a weight of 80kgs).
Study Duration:	60 months.
Participant Duration:	6 months.

1.2 SCHEMA

See next page.


Phone or in-person Screen
Meet inclusion/exclusion criteria
Optional (Opt-In) Neuroimaging (fMRI)
Randomized
PHASE 1: Blinded
3 weeks
Midazolam, 6 doses
Ketamine, 6 doses
Ketamine, 6 doses
Break blind
Months
1
2
3
4
5
6
Weekly mood assessment and monthly cognitive assessment
PHASE 2: Open
6 months

1.3 SCHEDULE OF ACTIVITIES (SOA)



	Baseline (D0)	Infusion Days (D1, D3, D8, D10, D15, D17)					Primary endpoint (D18)
		(-)60m	0-40m	60m	120m	180m	
	INTERVENTIONS						
Infusion			x				
	MEDICAL EXAMS						
Physical Exam	x						
EKG	x	x					
Blood Draw	x						x
Urine Tests	x	x					
	CLINICAL RATINGS						
MINI-KID	x						
CADSS	x	x		x	x	x	x
MADRS	x	x					x
CGI	x	x					x
SSI-5	x	x					x
Cogstate	x						x
CDRS-R	x						x
CPS	x						x
TEASAP	x						x
MASC	x						x
BHS	x						x
C-SSRS	x						x
ARI-S	x						x
CTQ	x						
ASSIS	x						
Friendship	x						

Figure 4: Summary of procedures and definition of clinical rating scales: MINI-KID: Mini International Neuropsychiatric Interview for Children & Adolescents; CADSS: Clinician-Administered Dissociative State Scale; MADRS: Montgomery-Asberg Depression Rating; CGI: Clinician Global Impressions; SSI-5: Beck Suicidal Scale; Cogstate: a neurocognitive battery tailored to adolescents; CDRS-R: Children's Depression Rating Scale, Revised; CPS: Pleasure Scale for Children; TEASAP: Treatment-Emergent Activation and Suicidality Assessment; MASC: Multidimensional Anxiety Scale for Children; BHS: Beck Hopelessness Scale; C-SSRS: Columbia-Suicide Severity Rating Scale; ARI-S: Affective Reactivity Index Self-report; CTQ: Childhood Trauma Questionnaire ; ASSIS: Social Networks Interview; Friendship: Friendship Questionnaire.

2 INTRODUCTION

2.1 STUDY RATIONALE

MDD is a significant pediatric health problem with a point prevalence of up to 8% and a lifetime prevalence approaching 25% by the end of adolescence[1-4]. Adolescent MDD is associated with significant comorbidity including poor social and scholastic functioning, early pregnancy, and increased risk of physical illness and substance abuse[5-7]. It is also linked with significant mortality, with an increased risk for suicide (the 3rd leading cause of death in 15-24 year-olds)[1,8]. Treatment can be difficult, with 40% of patients failing to achieve remission from selective serotonin reuptake inhibitors (SSRI)[9]. Of that SSRI-resistant population, nearly half remain depressed despite switching medications and adding

psychotherapy[10,11]. Of those that do improve, 1 in 4 relapse within a year[12]. While modest therapeutic efficacy is problematic, pediatric SSRI studies highlight the dangers of assuming that adolescents respond identically to medications as adults, as SSRIs are linked to small but significant increases in suicidality in children and adolescents, but not adults[13]. These data highlight the need for novel therapies targeting distinct neurochemical systems that are thoughtfully considered in a developmental context.

Treatment in psychiatry remains in the era of trial-and-error prescribing and reliable biomarkers that predict treatment response have remained elusive. A novel computational approach to neuroimaging data, connectome predictive modeling (CPM), has been successfully applied to predict psychiatric symptoms[14-17] and treatment responses[18,19] in adult populations. As an exploratory counterpart to the primary clinical trial, we will offer participants a single pre-treatment fMRI session on an opt-in basis, to explore the feasibility of utilizing CPM to predict antidepressant responses to ketamine.

2.2 BACKGROUND

Ketamine

The glutamate system is implicated in the pathophysiology of depression, and ketamine, an NMDA receptor antagonist and glutamatergic modulator, has been demonstrated in multiple controlled clinical trials to have rapidly acting antidepressant and anti-suicidal effects[20-25] (also see preliminary data section). In adult studies, ketamine has a robust average effect size of >1.2 and a number needed to treat (NNT) of only 2.3 in the medication-refractory depressed population[26]. While ketamine improves a range of depressive symptoms in adults, it notably reduces anhedonia[27], a symptom associated with poor therapeutic response in adolescent MDD[28]. Ketamine also specifically reduces suicidality in adults[29], a dimension of adolescent MDD that is resistant to, or perhaps even increased, by SSRIs[13,30]. Similarly, several controlled studies in adults have suggested ketamine may be effective in reducing obsessive compulsive disorder (OCD) and post-traumatic stress disorder (PTSD) symptoms as well as comorbid anxiety symptoms in subjects with primary MDD. While the glutamate system continues to mature in adolescence[31], preclinical data suggests that ketamine reverses depressive phenotypes in adolescent rats[32]. Due to its safety and success in treating adult MDD, ketamine is beginning to be considered for use in severe, treatment-refractory affective disorders in adolescence[33]. Outside of meager case report level data, however, clinicians have no published prospective evidence available to guide them regarding ketamine use in adolescent treatment-resistant depression (TRD). Thus, carefully monitored prospective studies regarding ketamine's safety and efficacy are needed to provide responsible, evidence-based care to this population in need.

PRELIMINARY DATA:

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 has a wide therapeutic window and has been used safely in Pediatrics for over 40 years for sedation prior to medical procedures and dentistry[34,35]. 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 there are no prospective studies of ketamine in pediatric psychiatric populations, the recently published retrospective report of ketamine use in pediatric bipolar depression did not describe any serious safety problems or adverse events associated with its use[33]. Yale University has been safely using ketamine in research studies with adult psychiatric patients for over 20 years[20].

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)[36].

Adult Major Depressive Disorder

A placebo-controlled study completed here at Yale first demonstrated the surprising finding that a single dose of ketamine (0.5 mg/kg, intravenously) had rapid antidepressant effects in depressed patients[21]. In these subjects, a ketamine infusion produced mild psychotomimetic symptoms and euphoria that dissipated within 120 minutes, while the antidepressant effects of ketamine infusion emerged over the first 180 minutes and persisted over 72 hours[21]. Fifty percent of depressed patients receiving ketamine were treatment responders at Day 3 compared to 12.5% in the placebo infusion group[21]. Another recent double-blind study performed at NIMH confirmed the rapid antidepressant effects of ketamine[22]. In this second study, 72% of ketamine infused patients responded to treatment compared to no treatment responders in the placebo group[22]. Since these initial studies at Yale, multiple controlled clinical studies have demonstrated that ketamine yields rapid antidepressant effect in unipolar and bipolar depression[21,22,37-39] (Figure 1) with an average effect size of 1.2 and a number needed to treat (NNT) of only 2.3[26]. Ketamine's anti-depressant effects peak 1-3 days following a single infusion. Ketamine's antidepressant effect is observed long after ketamine has been metabolized and excreted by the body and after ketamine's sedative and dissociative effects have dissipated. How to extend ketamine's rapid, potent, but short-lasting effects has become an intense area of study in adult psychiatry, and is a critical issue for moving this treatment into clinical use. In adults, repeated dosing schedules show robust antidepressant effects at the end of the series[40], and an extension of efficacy compared to a single infusion. Trials have suggested that there is no superiority of three-times-a-week paradigms over twice-a-week-infusions[40]. While a single, acute infusion has an action that peaks at one day, and subsides by seven days (Fig. 1), the twice-a-week infusion paradigm results in more sustained antidepressant effects[41]. Our clinical experience suggests that effects can often be maintained by more infrequent maintenance infusions (~once per month). There is a considerable range of antidepressant

duration, with some individuals achieving remission for >3 months following the acute series, and others appear to require maintenance sooner[41]. The individual factors predicting duration are largely unknown.

Pediatric Major Depressive Disorder

Our research group is the first to conduct a randomized, controlled trial of ketamine in adolescent TRD. We have been running a four-week crossover trial in 13-17 year-old adolescents with TRD (defined here as having failed at least one 8-week trial of a standard antidepressant at therapeutic dosing, although most of our subjects have failed multiple agents). This study was designed to assess the short-term efficacy and safety of a single ketamine infusion (0.5mg/kg over 40 minutes) compared to an active control, midazolam (0.045mg/kg over 40 minutes). Infusion 1 and 2 are separated by two weeks, and drug treatments (ketamine or midazolam) are randomized by the Yale Investigational Drug service. Adolescents with a history of mania, psychosis, or substance use disorders are excluded. A sample size of 18 subjects is powered to >80% to detect a difference of ketamine from midazolam (α=0.05). While we have not yet broken the blind in this study, we can report on the clinical outcomes of the initial 12 trial participants (Box 1). Our approximately 60% response rate is similar to that reported in the adult TRD literature with ketamine[42]. Five of our subjects also completed a parallel pilot neuroimaging study in which they received structural and functional MRI imaging at baseline, at 24 hours following infusion 1, and 24 hours following infusion 2, which will be analyzed at the completion of the trial. Blood samples for ketamine metabolites and the potential response biomarker, D-serine, were collected for all participants. Overall, 4 of the 11 subjects who received an infusion elected to continue to receive ketamine treatment after the trial. Reasons for individuals who responded in the trial but elected not to receive additional ketamine clinically thereafter included expense, geography, or desire to pursue alternative treatments. Taken together these preliminary data suggest that a single ketamine infusion is well-tolerated medically and psychiatrically in adolescents with TRD, and may have a similar response rate to that reported in the adult literature. Also similar to adults, ketamine's effects have been ephemeral, resulting in the need for additional maintenance infusions in subjects who responded in the trial. As described above, repeated dosing paradigms are being actively investigated in adults as a way of extending ketamine's durability of response. We have some experience with repeated dosing clinically in adolescents, described recently in a case report[43]. Here we treated a 16-year-old adolescent boy with TRD and several serious suicide attempts with the multi-infusion ketamine paradigm described in adults [44]. He had exhausted conventional medication options and opted for ketamine treatment in lieu of electroconvulsive therapy. He tolerated the infusions well and had a significant reduction in his depressive symptoms (evidenced by decreased scores on the CDRS-R and MADRS), improved hopelessness (BHS), and resolution of his suicidal ideation (SSI) (Fig. 2). We note a transient increase in depressive symptoms around day 30, coinciding with an insurance denial for this patient for his preferred discharged plan. These symptoms resolved on their own (i.e. prior to a maintenance infusion a month later) and reinforce that while ketamine appears effective for the treatment of severe depressive symptoms, it does not numb the recipient to natural reactions to life's ups and downs. Taken together, we are nearing completion of the first randomized controlled trial of ketamine in adolescents with TRD, with promising results of effectiveness, albeit short-lived. Adult data suggests that repeated infusion paradigms extend the antidepressant efficacy of ketamine, and we have shown that this is safely tolerated at the case report level. While ketamine has been used in Pediatrics as an anesthetic for over

fifty years with an impressive safety record for acute use, a critical question is whether ketamine is safe with repeated use. Cohort studies of adults who chronically abuse ketamine (at higher doses, often with additional substances or contaminants) suggest that bladder dysfunction and cognitive difficulties may be possible long-term side-effects of ketamine use[45,46]. Despite the lack of longer-term evidence, ketamine is increasingly being used in an off-label, unregulated way by physicians, including Child Psychiatrists[47]. While some of these community ketamine centers may amount to unscrupulous practices, others are likely driven by a genuine desperation for treatment for those patients who have failed to respond to first and second-line treatments. Regardless, now is a critical time to understand the long-term efficacy and safety of ketamine as a treatment for depression.

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[48] indicates the importance of this modality. Despite the excitement, we lack clinically oriented tools that can take advantage of this connectivity information. The Constable Lab has developed connectome-based predictive modeling (CPM) as a data-driven approach to relate brain functional organization to behavior [15,16,49-52]. 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[53]. 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[54]. Finally, modeling methods that are designed to generalize across multiple behaviors and diagnostic groups are needed[55]. 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 depressive symptoms. 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 depression axis, leading to better predictive models that generalize across heterogeneous groups of patients, as we have shown in autism[17], ADHD[16], and cocaine use disorder[18].

Specific Aims and Hypotheses:

Aim 1: To evaluate the efficacy and tolerability of a multiple-dosing ketamine infusion paradigm (2 infusions per week for 3 weeks) compared to midazolam in adolescents with treatment resistant depression (TRD).

Hypothesis 1: Repeated ketamine will be tolerated well medically and psychiatrically. We expect a significant reduction in CDRS-R in those treated with ketamine at the end of the dosing paradigm (Day 18).

Aim 2: To evaluate the durability of ketamine's antidepressant response and the maintenance requirements over six months.
Hypothesis 2: The repeated dosing paradigm will result in a prolonged antidepressant response (measured via CDRS-R), as is seen in adults and in our adolescent case report.

Aim 3: To evaluate the safety of the multiple-dosing ketamine paradigm on cognitive function over six months via Cogstate battery.
Hypothesis 3: Cogstate extensive battery will show no adverse effects on attention, working memory, or executive functioning over the 6 months following ketamine treatment.

Exploratory Aim 4: To evaluate the ability of pre-treatment functional connectome signatures derived from resting and task-based fMRI to predict post-treatment antidepressant responses to ketamine.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Ketamine administration:

Ketamine is a medication approved by the Food and Drug Administration to be used as an anesthetic in both children and adults. It is a dissociative anesthetic that has been used in humans since the late 1960's. Despite extensive medical experience there is no clear evidence of long-term toxicity associated with ketamine administration. However, the acute behavioral side effects of this medication warrant particular attention. Krystal et al, administered ketamine to healthy subjects at the West Haven VAMC[20]. The study showed that ketamine produces dose-related effects at sub-anesthetic effects in healthy subjects. At 0.1 mg/kg administered over 40 minutes, the low dose ketamine in the VA protocol, produced little more than a tingling in the extremities and a "little buzzing in the head". At 0.5 mg/kg, the VA study's high ketamine dose, and the dose proposed in this protocol, transiently elicited both the positive and negative symptoms of schizophrenia, dissociative symptoms, attentional impairments, a preferential impairment in delayed over immediate recall, increased perseverative errors on the Wisconsin card sort test, and decreased verbal fluency. In the sub-anesthetic dose utilized in this study, perceptual changes dissipate within 10-20 minutes following the termination of ketamine infusion. Ketamine also increased prolactin and cortisol, while blunting a test day decline in plasma HVA. Ketamine increased systolic and diastolic blood pressure by approximately 10-15 mm Hg, without a clear effect on pulse. Ketamine did not produce gross disorientation, as evidenced by complete absence of an effect on the MMSE and the successful completion of all categories on the Wisconsin card sort test. There have been no medical complications of ketamine administration to date. Out of the 18 adult subjects administered ketamine 0.5 mg/kg over 40 minutes, 8 complained of blurred vision and two subjects vomited. Both the individuals who vomited were noted to have horizontal nystagmus. In this study, the absolute dose of ketamine is not to exceed 40mg, corresponding to a weight of 80kg. There have not been adverse psychological reactions in any of the healthy adult subjects. In the Yale Pediatric Sedation Team's experience, there have not been serious after-effects reported in follow-up phone calls

after sedation with ketamine for medical purposes, although one family reported that their child had "weird dreams" the night following the procedure. There is no doubt that ketamine has clear and, in some cases, dramatic effects upon cognitive function. Some people find these effects pleasant or interesting and others find these effects frightening. In prior studies, all subjects have been thoroughly prepared for possible ketamine response prior to testing and debriefed at the end of each test day. As a result, only two subjects terminated their participation in either study prematurely. No subject has had adverse or lingering responses to ketamine following a test day. Also, none of the subjects experienced "flashbacks" to their ketamine experiences following a test day. These findings are very consistent with the earlier work of Domino and his colleagues. Thus, ketamine appears to be safe and, despite the intensity of its short-term behavioral effects, well tolerated. The extent to which the effects of ketamine are perceived as unpleasant is context dependent and can be reduced by preparing individuals in advance for the possible responses to ketamine. The response to ketamine appears to be reduced by a number of medications, including benzodiazepines and antipsychotic agents.

Since 1989, Yale researchers have administered ketamine to over 140 healthy subjects and over the last 25 years have been at the forefront of testing ketamine in psychiatric disorders like depression and PTSD. Adverse effects in response to ketamine infusion have been **mild and transient**, with no evidence of any clinically significant adverse side effects. We have reported 8 adverse events associated with ketamine administration to the Institutional Review Boards of Yale and/or the West Haven VA since October of 2000. None was considered serious and all resolved shortly after discontinuation of the ketamine infusion or within two weeks of the ketamine test day. Adverse events included nausea and vomiting, sedation, hypotension, insomnia and nightmares, headaches, visual and somatosensory perceptual alterations, strong paranoid feelings, and anxiety. The pediatric participants in our outpatient study have tolerated ketamine very well.

None of the patients or healthy subjects studied to date has had any long-term adverse consequences as a result of ketamine administration. This impression is supported by follow-up data up to 2 years on 132 healthy subjects participating in ketamine studies at Yale University and at Washington University at St. Louis (unpublished data). We examined follow-up assessments collected in a sub-sample of 132 healthy subjects who returned for subsequent testing over a duration of 1 week to 2 years. These subjects completed a similar battery of assessments when they reappeared as they completed in their earlier testing. In this analysis, no significant changes occurred in any measure between their initial and follow-up assessment.

In a previous study where ketamine was administered to depressed subjects there were adverse effects, though none were severe, and none persisted beyond 110 minutes. Conversely, there was a significant transient improvement of depressive symptoms.

Additional risks of ketamine must be considered in the pediatric population, particularly in light of earlier studies with antidepressants in which pediatric patients experienced side effects/risks that adults did not. While ketamine specifically reduces suicidality in adults[29,56], antidepressant medications have been associated with increased suicidal ideation in pediatric but not adult populations[13]. While we have not noted any increase in suicidality from ketamine treatment in our outpatient pediatric study, we will

minimize the risks of increased suicidality in this study by ensuring that subjects will (1) undergo regular psychiatric assessments of suicidality, (2) have direct access to a child psychiatrist throughout the study, and (3) will be psychiatrically hospitalized if they present as an imminent danger to self or others.

As ketamine can be abused outside of its medical purpose, the risk of any potential addictive properties must also be considered. Ketamine has been used in the pediatric population for years, and the rates of ketamine addiction in the United States remain very low[57]. The ketamine infusion protocol in this trial is given slowly at a low dose (smaller than what is used in anesthesia) to minimize psychotomimetic effects (the high) associated with it. Adult studies have not reported problems with addiction following this ketamine protocol[58]. The potential risk of abuse will be mitigated by excluding subjects with a history of substance use disorder. Additionally, subjects and their parents will be warned about abuse potential in all consent/assent documents.

Midazolam administration:

Midazolam, the active control in this study, is a medication that is approved by the Food and Drug Administration as a sedative for both children and adults. It is a benzodiazepine with a short half-life that is used commonly in pediatric sedation and dentistry [59-64] and it is frequently administered rapidly at higher doses than proposed in the current study (we propose 0.045mg/kg over 40 minutes versus sedation dosing of 0.05 – 0.1mg/kg over 2 minutes[64,65]). In this study, the total midazolam dose is not to exceed 3.6mg, corresponding to a weight of 80kg. The most common side effects related to midazolam infusions are hiccups, nausea, vomiting, coughing, headache, and drowsiness. In doses higher than those involved in the proposed study, serious cardiorespiratory adverse events have occurred after administration of midazolam, including respiratory depression, airway obstruction, oxygen desaturation, apnea, respiratory arrest and/or cardiac arrest. In younger children, these higher doses have been associated with a risk for paradoxical disinhibition or agitation. Midazolam, like ketamine, has a short half-life, and important adverse events related either drug are not observed after 30 minutes following drug administration[33]. Our research group has experience using midazolam in pediatric ketamine studies (our outpatient crossover trial and ongoing Janssen esketamine trial, which uses a higher dose of midazolam than proposed here) and has not encountered any adverse events. While the risks of serious adverse cardiorespiratory events are low given the dose chosen for this study, airway equipment and qualified physician personnel will be readily available. The reversal medication, flumazenil, will also be available, which can rapidly abort the effects of midazolam should any untoward effects occur. All benzodiazepines have the potential for physical and psychological dependence after prolonged use. The low number of exposures (six infusions) and low dose proposed in this study would not be expected to result in any physiologic dependence. Benzodiazepines as a class are also at risk to be abused for recreational use outside of medical purposes. The addictive potential of midazolam will be mitigated by using a low dose and a slow infusion paradigm. Midazolam is used very frequently in the pediatric setting and is not thought to be associated with precipitating subsequent benzodiazepine abuse. The risks will further be mitigated by screening out adolescent subjects with concurrent

substance use disorders. Subjects will also be warned about the abuse potential in all consent/assent documents.

Blood drawing/intravenous placement:

Bruising or thrombosis can occur with placement of the intravenous line. A total of 85cc will be drawn over 3 weeks (30cc at baseline, 40cc on first infusion day (Day 1), 15cc on Day 18), which equates to 1.5cc/kg – 2.2cc/kg (based on average weights of 13-17yo adolescents). The risks of blood draws include brief pain at the time of needle insertion, bruising, swelling at needle site and rarely, fainting or infection.

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 complete them.

Clinical Deterioration:

There is a risk that a participant may experience an increase of 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 18). 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 CDRS-R and MADRS, as well as ratings and frequent contact with clinic personnel. The following are criteria for evaluation and possible pharmacological and/or non-pharmacological treatments: (1) an increase of 25% in CDRS-R score at any time over the course of treatment and (2) new-onset of suicidal ideation or an increase in passive 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. Participants who have completed the trial *will only be discharged with appropriate referrals to an appropriate level of care given their severity.*

MRI (for those who opt-in to neuroimaging):

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 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.

These risks will be mitigated by the following plans: 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 consent 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.

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. The potential benefits to

society of these investigations are considerable. Depression continues 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.

Midazolam arm:

While the active control 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 will have scheduled contact with Child Psychiatry twice weekly during the blinded phase, when they may be receiving 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 at Day 18, will be offered active ketamine treatment.

Brain imaging (to those who opt-in): No direct benefits to the subject are to be expected.

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
20 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, ketamine also may alleviate symptoms of depression and anxiety in adolescents.
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 (see Preliminary Data).

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[66]. 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[66]. 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[66]. 7) Urinary: in people who recreationally abuse ketamine, bladder inflammation and cystitis have been reported, which appear to be both dose and frequency dependent[67]. 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[68]. 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.

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 control 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.

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 0.05 to 0.1mg/kg (a maximum of 0.6mg/kg total) 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 than 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 single infusion 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 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Aim 1: To evaluate the efficacy and tolerability of a multiple-dosing ketamine infusion paradigm (2 infusions per week for 3 weeks) compared to midazolam in adolescents with treatment resistant depression (TRD).	Aim 1: The primary outcome will be Children's Depression Rating Scale - Revised (CDRS-R) score at Day 18.	Aim 1: Based on adult literature, ketamine effects on mood reach peak 24 hours after the infusion and we would like to determine if this is the same in the pediatric population.
Secondary		
Aim 2: To evaluate the durability of ketamine's antidepressant response and the maintenance requirements over six months. Aim 3: To evaluate the safety of the multiple-dosing ketamine paradigm	Aim 2: Secondary outcomes include time to relapse in ketamine responders, maintenance requirements over six months, and neurocognitive measures. Aim 3: Percent change from baseline of individual neurocognitive test scores	Aim 2: Response in pediatric patients. Aim 3: There are currently no studies in pediatric patients evaluating the neurocognitive impact

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
on cognitive function over six months via Cogstate battery.		of repeated ketamine dosing.
Exploratory		
Aim 4: To evaluate the ability of pre-treatment functional connectome signatures derived from resting and task-based fMRI to predict post-treatment antidepressant responses to ketamine.	Aim 4: Pre-treatment functional connectome signatures correlated with post-treatment CDRS-R score	Aim 4: Prior successful applications of this approach to predicting psychiatric symptoms[16,17] and treatment responses[18]

4 STUDY DESIGN

4.1 OVERALL DESIGN

Specific Aims and Hypotheses:

Aim 1: To evaluate the efficacy and tolerability of a multiple-dosing ketamine infusion paradigm (2 infusions per week for 3 weeks) compared to midazolam in adolescents with treatment resistant depression (TRD).

Hypothesis 1: Repeated ketamine will be tolerated well medically and psychiatrically. We expect a significant reduction in CDRS-R in those treated with ketamine at the end of the dosing paradigm (Day 18)

Aim 2: To evaluate the durability of ketamine's antidepressant response and the maintenance requirements over six months (the total number of ketamine infusions will not exceed 8 doses over the lifetime of the patient).

Hypothesis 2: The repeated dosing paradigm will result in a prolonged antidepressant response (measured via MADRS), as is seen in adults and in our adolescent case report.

Aim 3: To evaluate the safety of the multiple-dosing ketamine paradigm on cognitive function over six months via Cogstate battery.

Hypothesis 3: Cogstate extensive battery will show no adverse effects on attention, working memory, or executive functioning over the 6 months following ketamine treatment.

Exploratory Aim 4: To evaluate the ability of pre-treatment functional connectome signatures derived from resting and task-based fMRI to predict post-treatment antidepressant responses to ketamine.

Overview:

We propose a two-phase study (Fig 3). **Phase 1 is a double-blind, midazolam-controlled parallel design trial** to evaluate the safety and efficacy of a multiple-dose paradigm to treat adolescent TRD (six infusions over three weeks, each 0.5mg/kg over 40 minutes) compared to midazolam (each 0.045mg/kg over 40 minutes). Investigators will be blind to the treatment assignment during this phase, and physicians absent from the infusion paradigm will perform blinded clinical efficacy ratings. **Phase 2 is an open extension**, in which the blind will be broken and midazolam-treated subjects who remain symptomatic will be offered ketamine. Those who respond to ketamine from either group will be followed carefully and offered up to two more symptom-triggered maintenance infusions over 6 months (up to 2 more infusions for a maximum of 8 total). All subjects will be followed closely over 6 months while receiving standard of care as per AACAP guidelines (42). Those who opt-in to the predictive neuroimaging portion of the study will have an additional one-time visit to the Magnetic Resonance Resource Center (MRRC) following enrollment but prior to randomization to complete a 1.5 fMRI session including rest and task-based activities.

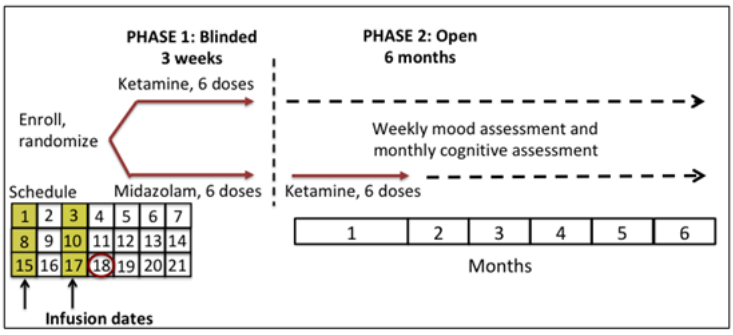


Fig 3: Approach. In phase 1, subjects are randomized to ketamine or midazolam (6 infusions over 3 weeks, days highlighted in yellow). At the end of phase 1, the blind is broken, and midazolam-treated subjects are offered open ketamine treatment in phase 2. All subjects receive standard of care treatment (dashed black lines), and are assessed weekly for mood and monthly via Cogstate battery.

Screening/Baseline (Visit 1):

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. 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 enroll, the patient and his/her parent/guardian will be asked to sign the assent and parental permission forms, respectively. At this same visit, the additional neuroimaging opt-in study will be discussed as well. 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. If necessary, a release of information (ROI) will be obtained to access prior medical records and to allow for communication with the health care provider(s). 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)[69]. Additionally, the participant and his/her parent/guardian will complete clinical ratings related to the participant's 1) MDD symptoms and 2) symptoms of other commonly comorbid psychiatric conditions. A medical assessment including vital signs, physical exam, baseline serum labs, urine drug screen, and urine pregnancy test will be completed prior to enrollment. The clinical assessment will take approximately 2 hours.

Assessments and Ratings (Figure 4 provides a detailed assessment and procedures schedule).

a. *Medical Assessments:* Vital signs, physical exam, and clinical laboratory tests (i.e. CBC with differential, complete metabolic panel (CMP) (including electrolytes, LFTs, BUN, creatinine and glucose), TFTs, and routine urinalysis) will be completed. A total of 30 cc (2 US tablespoons) of blood will be drawn via venipuncture at this visit. In addition, an EKG will be performed and read in order to rule out any cardiac abnormalities.

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 consent 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 consent, 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 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 consent, without having to declare specific reasons. If subjects choose not to enroll, then any previously collected drug test results will be destroyed.

Drug use information **(in the adolescent ≥ 13 years of age)** 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.

b. *Psychiatric Assessments:* Ratings will be conducted by trained research staff. A detailed description of the most common assessments included but not limited to, is listed below. The timing of the clinical assessments is depicted in Table 1 (located at the end of this Research Plan).

All Subjects:

(1) Children's Depression Rating Scale, Revised (CDRS-R): a standardized rating scale that assesses depression severity in children and adolescents[70]
(2) Montgomery-Asberg Depression Rating Scale (MADRS): a standardized rating scale that assesses depression severity in children and adolescents[71]
(3) Columbia-Pleasure Scale for Children (PSC): a standardized rating scale to assess anhedonia[72]
(4) Treatment-Emergent Activation and Suicidality Assessment Profile (TEASAP): a rating scale designed to detect increased behavioral activation and suicidality[73]
(5) Clinical Global Impressions (CGI): a widely used instrument used to assess overall severity of illness and symptom improvement on 1-7 point scales[74]
(6) Multidimensional Anxiety Scale for Children (MASC): a multidimensional assessment of anxiety in children and adolescents [75]
(7) Clinician-Administered Dissociative States Scale (CADSS): self and interviewer administered items that evaluate dissociative symptoms[76]
(8) Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID): structured clinical interview evaluating psychiatric disorders[69]
(9) Columbia-Suicide Severity Rating Scale (C-SSRS) [77]
(10) Beck Hopelessness Scale (BHS): instrument to assess hopelessness[78]
(11) Childhood Trauma Questionnaire (CTQ): a self-report screening measure for maltreatment histories in children[79]
(12) Social Networks Interview (ASSIS): a brief measure of social supports[80]
(13) Friendship Questionnaire
(14) Affective Reactivity Index Self-report (ARI-S)[81]
(15) Scale for Suicide Ideation- 5 (SSI-5): This scale measures the first 5 items on the SSI (19 items). SSI-5 has been shown to be more sensitive to ketamine treatment than the 19-item [82]
(16) Cogstate: a neurocognitive battery tailored to adolescents

Visit 1b: Optional (Opt-In) Neuroimaging session:

Participants will have a separate scheduled session at the MRRC. Female participants will have received a serum pregnancy test as part of the baseline visit (described above under "Medical

Assessments"). A short number of additional rating scales and cognitive testing will be performed including : Edinburgh Handedness Inventory and the Wechsler Abbreviated Scale of Intelligence. Following completion of the scales, the participants will proceed to the MRI suite. 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.

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, the 1 perception/movie watching run, and 1-resting-state run will be acquired over 6 minutes (total of 30 minutes of connectivity data) using multiband EPI (voxel size 2mm3)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.

Tasks performed during the experiment sessions will include:

1. Card guessing task[83]: 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 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[84,85]: 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[86]: 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[87], or by responding to questions that require them to interpret social "hints" in vignettes[88].
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 1: Double-Blind Intervention (Visits 2 – 8):

Subjects will receive 6 infusions of either ketamine (0.5mg/kg IV) or midazolam (0.045mg/kg IV) over a three-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.

Infusions (Day 1, 3, 8, 10, 15, & 17, Visits 2 - 7):

Subjects who, in the opinion of the principal investigator, 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 and potential biomarkers, Day 1 only) and one iv on infusion days without bloodwork (Day 3, 8, 10, 15, 17). 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 hour for 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 6 infusions, scheduled on study day 1, 3, 8, 10, 12, 15, and 17 (corresponding to visits 2 – 7). All participants will return on study day 18 (Visit 8) for extensive mood and cognitive measures, as well as follow-up blood collection for potential biomarkers of response. The CDRS-R on Study Day 18 will serve as the primary outcome measure for this portion of the study.

Ketamine has been used safely in pediatrics for over 40 years for conscious sedation and dentistry[35], however it does have significant risks. The most common side effects when ketamine is infused at the current rate and dose in adult studies are (1) increased blood pressure, respiratory rate, pulse, (2) pain/rash at the injection site, (3) temporary psychiatric symptoms including, but not limited to, disorientation, anxiety, dysphoria, flashbacks, hallucinations, and psychotic-like symptoms (which occur less frequently in younger patients[35]). These reactions are typically self-limited and occur in ~12% of adults given higher doses of ketamine than proposed in this study[89]. The most serious side effects are (1) increased intraocular pressure, (2) allergic reaction, (3) laryngospasm, (4) elevated blood pressure resulting in stroke, heart attack, or death, and (5) substance abuse, all of which are rare[34,35]. These risks will be mitigated by providing comprehensive monitoring in IPS during and two hours following the infusion, supervised by trained physicians with access to airway equipment. Ketamine has been studied in over 10,000 psychiatric patients in more than 100 separate studies. Our institution has had experience using ketamine safely in research studies involving adult psychiatric patients for the last 20 years, and our current research team has significant experience using ketamine in pediatric psychiatric populations. This dose of ketamine is considered subanesthetic in both adults and children[34], and thus we expect minimal cardiorespiratory and neurologic side effects. Case report level data suggests a favorable side effect profile for intranasal ketamine for adolescents with refractory disorders[33]. However, if side effects do not dissipate we will additionally offer patients hospitalization at Yale Psychiatric Hospital, Adolescent Unit for psychiatric side effects or YNHH for medical side effects.

Midazolam, the active control in this study, similarly has a robust history of use in pediatric sedation and dentistry[59-64,90]. Also similar to ketamine, the dose proposed in the current study (0.045mg/kg over 40 minutes) is lower and slower than doses used in most pediatric sedation procedures (0.05 – 0.1mg/kg over 2 minutes)[64,65]. The most common side effects related to midazolam infusions are hiccups, nausea, vomiting, coughing, headache, and drowsiness. In doses higher than those involved in the proposed study, serious cardiorespiratory adverse events have occurred after administration of midazolam, including respiratory depression, airway obstruction, oxygen desaturation, apnea, respiratory arrest and/or cardiac arrest. In younger children, these higher doses have been associated with a risk for paradoxical disinhibition. Midazolam, like ketamine, has a short half-life, and important adverse events related either drug are not observed after 30 minutes following drug administration[34]. Our research team also has significant experience using midazolam in pediatric psychiatric populations from our first ketamine study. While the risks of serious adverse cardiorespiratory events are low given the dose chosen for this study, airway equipment and qualified physician personnel will be readily available.

Midazolam is the most appropriate control for ketamine in the proposed study, as the psychotomimetic effects of ketamine make it extremely difficult to blind when compared to saline[26]. Specifically, the typically mild dizziness, nausea and dissociative effects on ketamine functionally un-blind subjects and investigators. As such, more recent ketamine studies have used midazolam as a control. Using an appropriate active control in this efficacy trial is critical to establishing genuine clinical efficacy for ketamine (and not just treatment expectancy or subject performance bias).

Phase 2: Open Phase Intervention (Visits 9 – 39):

Month 1:

Consistent with our ethical obligation to treat suffering children with active interventions, we will break the blind following the acute phase, and offer those subjects who received midazolam open active treatment according to the protocol described above. That is, they will receive 6 infusions of open ketamine over 3 weeks (Study Days 22, 24, 29, 31, 36, and 38, corresponding to Study Visits 9 – 14), followed by an in-person assessment on Day 39 (Visit 15). Blood draws will occur on Study Day 22 for ketamine metabolites and biomarkers (Visit 9) and for biomarkers on Day 39 (Visit 15).

The subjects who received ketamine in the blinded phase will be evaluated for **response** (defined as >50% decrease from baseline MADRS at any point between D1 and D18). **Remission** is defined as a MADRS < 9.

(1) Non-responders will not be offered additional ketamine, but will receive standard of care depression treatment[91], weekly telephone mood assessment (Days 25, 32, 39 corresponding to Visits 9, 10, 11) and monthly in-depth mood and cognitive assessments (Day 46, Visit 12), see Figure 4.

(2) Responders will also be followed weekly via MADRS, CGI-S, and SSI-5 to assess for relapse (Days 25, 32, 39, 46 corresponding to Visits 9, 10, 11, 12). **Relapse** is defined as ≥50% of baseline MADRS *AND* [MADRS >20 *OR* SSI-5 ≥ 6] *AND* a >10 point absolute increase from MADRS at end of ketamine treatment phase *AND* a >15 point absolute increase from lowest MADRS score during the ketamine treatment phase on 2 consecutive assessments. Any subject with a weekly assessment indicative of relapse will be seen again within 3 days for a second assessment. Time to relapse will be tracked and **up to two symptom-triggered maintenance** infusions will be offered if relapse criteria is met and there is a consensus between the physician, study subject, and their guardian. Relapse periods can, however, be variable, and we will limit maintenance infusions to no more than 1 every 2 weeks (up to 2 more infusions for a maximum of 8 total). Surveillance labs (same as admission labs) will be obtained prior to any maintenance infusion OR within 1 month of the end of the study including a urine toxicology test to track substance abuse, as suggested by FDA. A biomarker blood draw (10cc) may be taken prior to the maintenance infusion.

Months 2-6:

At this point in the trial, all subjects will have received active treatment (half receiving ketamine in the blinded phase, and half receiving ketamine in the open phase after having been assigned to midazolam in the blinded phase, assuming that there are no subjects whose symptoms remit after midazolam treatment). All responders will be followed to track time to relapse, and will be offered up to two symptom-triggered maintenance infusions as described above (up to 2 more infusions for a maximum of 8 total). All non-responders will also be followed during this period while receiving standard of care. The general assessment schedule will be weekly phone follow-up and monthly in-person follow up, however any assessments indicative of relapse will trigger an unscheduled, in-person visit.

Telephone:

Each subject will schedule follow-up telephone calls with a member of the research staff to monitor his/her MDD symptoms and any Adverse Events associated with potential abuse. During these telephone calls, participants will complete a series of clinical ratings (see Table 1) conducted by a trained research staff member. These telephone calls will take approximately 1 hour.

Telephone visit schedule for those who initially received ketamine: Study Days 25, 32, 39, 53, 60, 67, 81, 88, 94, 108, 115, 122, 136, 143, 150, 164, 171, and 178, which correspond to scheduled study visits 9-11, 13-15, 17-19, 21-23, 25-27, and 29-31.

Telephone visit schedule for those initially received midazolam, then received open ketamine: Study Days 46, 53, 60, 74, 81, 88, 101, 108, 115, 129, 136, 143, 157, 164, 171, 185, 192, and 199, which correspond to scheduled study visits 16-18, 20-22, 24-26, 28-30, 32-34, and 36-38.

In-person:

Subjects will return to the Yale Child Study Center for follow-up in person visits to monitor their symptoms of depression and physical health, and any Adverse Events associated with potential abuse (these will be systematically collected in an AE log by study personnel). During these visits, the participant will complete a series of clinical ratings in addition to the Cogstate neurocognitive battery (see Table 1), to be conducted by a trained research staff member. All of the outpatient study follow-up visits will take approximately 1-2 hours.

In-person visit schedule for those who initially received ketamine: Study days 46, 74, 101, 129, 157, and 185, which correspond to scheduled study visits 12, 16, 20, 24, 28, and 32.In-person visit schedule for those initially received midazolam, then received open ketamine: Study Days 67, 94, 122, 150, 178, and 206, which correspond to scheduled study visits 19, 23, 27, 31, 35, and 39.

Figure 4: Overall Study Assessment Schedule

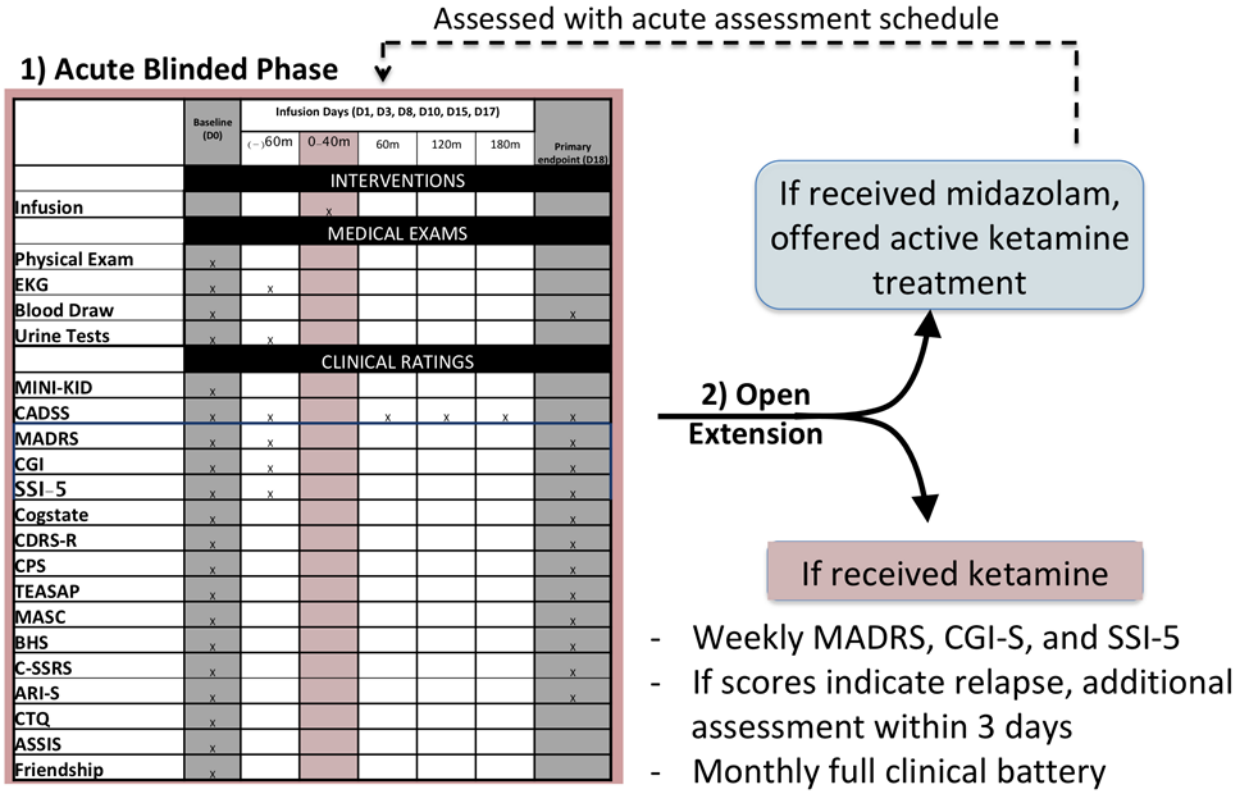


Figure 4: Summary of procedures and definition of clinical rating scales: MINI-KID: Mini International Neuropsychiatric Interview for Children & Adolescents; CADSS: Clinician-Administered Dissociative State Scale; MADRS: Montgomery-Asberg Depression Rating; CGI: Clinician Global Impressions; SSI-5: Beck Suicidal Scale; Cogstate: a neurocognitive battery tailored to adolescents; CDRS-R: Children's Depression Rating Scale, Revised; CPS: Pleasure Scale for Children; TEASAP: Treatment-Emergent Activation and Suicidality Assessment; MASC: Multidimensional Anxiety Scale for Children; BHS: Beck Hopelessness Scale; C-SSRS: Columbia-Suicide Severity Rating Scale; ARI-S: Affective Reactivity Index Self-report; CTQ: Childhood Trauma Questionnaire ; ASSIS: Social Networks Interview; Friendship: Friendship Questionnaire.

Discharge from the Study:

The determination of risk of harm to self or others will be made by a credentialed clinician (MD, PhD, MSW, PA, APRN); this assessment will be determined by a clinical assessment performed at the time of discharge from the study. The decision to discharge a subject from any study visit will be made by a credentialed clinician.

In the event that a subject is judged to remain significantly depressed, anxious, and/or is at an 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. Participants who have completed the trial will only be discharged with appropriate referrals to an appropriate level of care given their severity.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Midazolam was chosen an active control in this study to enhance the blinding of ketamine, whose mild psychotomimetic effects make saline placebos minimally useful. Here we have chosen a parallel design trial to compared repeated dosing of ketamine to midazolam. To balance the rigor of a parallel design trial with an ethical obligation to offer active treatment to sick children, we have chosen to break the blind after the acute phase to offer active treat to children who received placebo and continue to experience severe depressive symptoms. We will gain a significant amount of information about durability of response and maintenance requirements from the 6-month open phase of this trial.

4.3 JUSTIFICATION FOR DOSE

Pediatric Major Depressive Disorder

Our research group is the first to conduct a randomized, controlled trial of ketamine in adolescent TRD. We have been running a four-week crossover trial in 13-17 year-old adolescents with TRD (defined here as having failed at least one 8-week trial of a standard antidepressant at therapeutic dosing, although most of our subjects have failed multiple agents). This study was designed to assess the short-term efficacy and safety of a single ketamine infusion (0.5mg/kg over 40 minutes) compared to an active control, midazolam (0.045mg/kg over 40 minutes). Infusion 1 and 2 are separated by two weeks, and drug treatments (ketamine or midazolam) are randomized by the Yale Investigational Drug service. Adolescents with a history of mania, psychosis, or substance use disorders are excluded. A sample size of 18

Box 1: Preliminary Results of Adolescent MDD Ketamine Crossover Trial

12 subjects consented

- 10 completed the 4-week trial
- 1 received Infusion 1, improved, and discontinued to receive ketamine in the community
- 1 experienced panic in the first seconds of Infusion 1 and discontinued

10 subjects completed

- 6 of 10 responded to one (but not

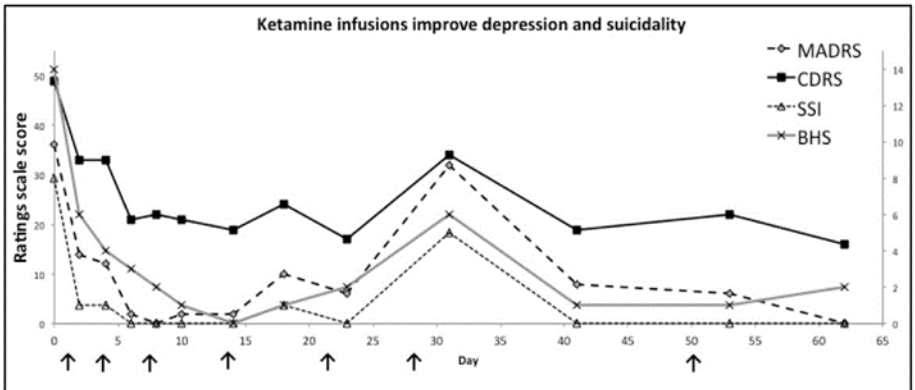


Figure 2: Ketamine improves depression and suicidality in an adolescent with severe treatment-refractory MDD with suicidal ideation. MADRS/CDRS scores are shown on the left Y axis, and SSI/BHS scores on the right. Days are shown on the X axis with arrows denoting the dates of ketamine infusions. Adapted from[7]

subjects is powered to >80% to detect a difference of ketamine from midazolam (α=0.05). While we have not yet broken the blind in this study, we can report on the clinical outcomes of the initial 12 trial participants (Box 1). Our approximately 60% response rate is similar to that reported in the adult TRD literature with ketamine[42]. Five of our subjects also completed a parallel pilot neuroimaging study in which they received structural and functional MRI imaging at baseline, at 24 hours following infusion 1, and 24 hours following infusion 2, which will be analyzed at the completion of the trial. Blood samples for ketamine metabolites and the potential response biomarker, D-serine, were collected for all participants. Overall, 4 of the 11 subjects who received an infusion elected to continue to receive ketamine treatment after the trial. Reasons for individuals who responded in the trial but elected not to receive additional ketamine clinically thereafter included expense, geography, or desire to pursue alternative treatments. Taken together these preliminary data suggest that a single ketamine infusion is well-tolerated medically and psychiatrically in adolescents with TRD, and may have a similar response rate to that reported in the adult literature. Also similar to adults, ketamine's effects have been ephemeral, resulting in the need for additional maintenance infusions in subjects who responded in the trial. As described above, repeated dosing paradigms are being actively investigated in adults as a way of extending ketamine's durability of response. We have some experience with repeated dosing clinically in adolescents, described recently in a case report[43]. Here we treated a 16 year-old adolescent boy with TRD and several serious suicide attempts with the multi-infusion ketamine paradigm described in adults[44]. He had exhausted conventional mediation options and opted for ketamine treatment in lieu of electroconvulsive therapy. He tolerated the infusions well and had a significant reduction in his depressive symptoms (evidenced by decreased scores on the CDRS-R and MADRS), improved hopelessness (BHS), and resolution of his suicidal ideation (SSI) (Fig. 2). We note a transient increase in depressive symptoms around day 30, coinciding with an insurance denial for this patient for his preferred discharged plan. These symptoms resolved on their own (i.e. prior to a maintenance infusion a month later), and reinforce that while ketamine appears effective for the treatment of severe depressive symptoms, it does not numb the recipient to natural reactions to life's ups and downs. Taken together, we are nearing completion of the first randomized controlled trial of ketamine in adolescents with TRD, with promising results of effectiveness, albeit short-lived. Adult data suggests that repeated infusion paradigms extend the antidepressant efficacy of ketamine, and we have shown that this is safety tolerated at the case report level. While ketamine has been used in Pediatrics as an anesthetic for over fifty years with an impressive safety record for acute use, a critical question is whether ketamine is safe with repeated use. Cohort studies of adults who chronically abuse ketamine (at higher doses, often with additional substances or contaminants) suggest that bladder dysfunction and cognitive difficulties may be possible long-term side-effects of ketamine use[45,46]. Despite the lack of longer-term evidence, ketamine is increasingly being used in an off-label, unregulated way by physicians, including Child Psychiatrists[47]. While some of these community ketamine centers may amount to unscrupulous practices, others are likely driven by a genuine desperation for treatment for those patients who have failed to respond to first and second-line treatments. Regardless, **now is a critical time to understand the long-term efficacy and safety of ketamine as a treatment for depression**.

Ketamine has been shown to cause neuronal vacuolations in sexually mature rats starting at a dose of 40 mg/kg, with a no observable adverse effect level (NOAEL) of 20 mg/kg[92]. Currently, we do not have enough data in the public domain to determine if ketamine-induced neuronal vacuoles progress to

irreversible degeneration and necrosis at higher doses or lower, repeated doses. Utilizing a conservative approach and considering the potential benefit for patients enrolled in clinical trials, we have agreed to limit ketamine dosing to 40 mg/day, which provides at least a 5-fold margin of safety based on body surface area calculations.
The long-term effects of ketamine are unknown and the FDA's current policy for ketamine usage restricts use to no more than eight doses in the lifetime of the patient for the treatment of psychiatric disorders. This limitation is based on the absence of nonclinical data demonstrating safety.

For the purposes of this protocol, dosing of ketamine will not exceed 40 mg/day, dosing of midazolam will not exceed 3.6mg/day, and the total number of ketamine infusions will not exceed eight doses over the lifetime of the participants. We have implemented a weight limit of 80kg for participants in the trial.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit and the last scheduled procedure shown in the Schema, Section 1.2, and Schedule of Activities (SoA), Section 1.3.

The end of the study is defined as completion of the last visit or procedure shown in the Schema and SoA in the trial globally.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

1) Male or female ages 13-17 years.
2) Meet DSM-5 criteria for Major Depressive Disorder by structured interview (MINI-KID+).
3) Children's Depression Rating Scale, Revised CDRS-R score ≥40 at screening.
4) Failure to achieve remission with at least 2 antidepressant trials (e.g. SSRI, SNRI or TCA), meaning at least 6 weeks at therapeutic dosing, including at least 4 weeks of stable dosing.
5) Stable psychiatric medications and doses for the month prior to enrollment. Subjects may continue to engage in any ongoing psychotherapy.
6) Medically and neurologically healthy based on physical examination and medical history.
7) Parents able to provide written informed consent and adolescents must additionally provide assent.

5.2 EXCLUSION CRITERIA

1) History of psychotic disorder, manic episode, autism spectrum disorder diagnosed by MINI-KID.
2) History of substance dependence diagnosis by MINI-KID (excluding tobacco) or positive urine toxicology.
3) Intellectual disability (IQ<70) per medical history.
4) Pregnancy (urine pregnancy tests on the day of infusions for menstruating girls) or lactation.
5) Inability to provide written informed consent according to the Yale Human Investigation Committee (HIC) guidelines in English.
6) Prior participation in Ketamine studies and participants that have used Ketamine for recreational purposes.
7) Pre-existing cardiovascular disease or untreated or unstable hypertension.
8) Currently taking Benzodiazepines or other medications that may cause respiratory depression.
9) Body weight greater than 80kgs.

For participation in the opt-in fMRI scans only:

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:

- Take proper precaution to not become pregnant during the study.
- Not engage in demanding work for the first 3 days after the ketamine infusion.
- Not drive or operate heavy machinery 24 hours after an infusion.
- Attend all scheduled appointments.
- Refrain from using any illegal substances.
- Maintain stable psychiatric medications and dosing 4 weeks prior to and during the study.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Adolescents ages 13-17 years with medication-refractory MDD will be recruited for this study. As many as 24 adolescents will be recruited through the Yale Child Study Center and Yale Psychiatric Hospital. The YCSC serves a large population of children with MDD and has forged relationships with

local schools for referrals of children and adolescents with MDD to the clinics. We will additionally employ letters to child mental health clinicians, educational lectures on adolescent MDD at mental health centers, and outreach to adolescent MDD researchers in the Boston-New York City area.

Averages weights of subjects are anticipated to be 45-54kg for females 13-17 years old and 45-65kg for males 13-17 years old*, and we have implemented a weight limit of 80kg*.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Ketamine has been used safely in pediatrics for over 40 years for conscious sedation and dentistry[35], however it does have significant risks. The most common side effects when ketamine is infused at the current rate and dose in adult studies are (1) increased blood pressure, respiratory rate, pulse, (2) pain/rash at the injection site, (3) temporary psychiatric symptoms including, but not limited to, disorientation, anxiety, dysphoria, flashbacks, hallucinations, and psychotic-like symptoms (which occur less frequently in younger patients[35]). These reactions are typically self-limited and occur in ~12% of adults given higher doses of ketamine than proposed in this study[89]. The most serious side effects are (1) increased intraocular pressure, (2) allergic reaction, (3) laryngospasm, (4) elevated blood pressure resulting in stroke, heart attack, or death, and (5) substance abuse, all of which are rare[34,35]. These risks will be mitigated by providing comprehensive monitoring in IPS during and two hours following the infusion, supervised by trained physicians with access to airway equipment. Ketamine has been studied in over 10,000 psychiatric patients in more than 100 separate studies. Our institution has had experience using ketamine safely in research studies involving adult psychiatric patients for the last 20 years, and our current research team has significant experience using ketamine in pediatric psychiatric populations. This dose of ketamine is considered subanesthetic in both adults and children[34], and thus we expect minimal cardiorespiratory and neurologic side effects. Case report level data suggests a favorable side effect profile for intranasal ketamine for adolescents with refractory disorders[33]. However, if side effects do not dissipate we will additionally offer patients hospitalization at Yale Psychiatric Hospital, Adolescent Unit for psychiatric side effects or YNHH for medical side effects.

Midazolam, the active control in this study, similarly has a robust history of use in pediatric sedation and dentistry[59-64,90]. Also similar to ketamine, the dose proposed in the current study (0.045mg/kg over 40 minutes) is lower and slower than doses used in most pediatric sedation procedures (0.05 – 0.1mg/kg over 2 minutes)[64,65]. The most common side effects related to midazolam infusions are hiccups, nausea, vomiting, coughing, headache, and drowsiness. In doses higher than those involved in the proposed study, serious cardiorespiratory adverse events have occurred after administration of midazolam, including respiratory depression, airway obstruction, oxygen desaturation, apnea, respiratory arrest and/or cardiac arrest. In younger children, these higher doses have been associated with a risk for paradoxical disinhibition. Midazolam, like ketamine, has a short half-life, and

important adverse events related either drug are not observed after 30 minutes following drug administration[34]. Our research team also has significant experience using midazolam in pediatric psychiatric populations from our first ketamine study. While the risks of serious adverse cardiorespiratory events are low given the dose chosen for this study, airway equipment and qualified physician personnel will be readily available.

Midazolam is the most appropriate control for ketamine in the proposed study, as the psychotomimetic effects of ketamine make it extremely difficult to blind when compared to saline[26]. Specifically, the typically mild dizziness, nausea and dissociative effects on ketamine functionally un-blind subjects and investigators. As such, more recent ketamine studies have used midazolam as a control. Using an appropriate active control in this efficacy trial is critical to establishing genuine clinical efficacy for ketamine (and not just treatment expectancy or subject performance bias).

6.1.2 DOSING AND ADMINISTRATION

Infusions (Day 1, 3, 8, 10, 15, & 17, Visits 2 - 7): Subjects who, in the opinion of the principal investigator, 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 and potential biomarkers, Day 1 only) and one IV on infusion days without bloodwork (Day 3, 8, 10, 15, 17). 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 hour for 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 6 infusions, scheduled on study day 1, 3, 8, 10, 12, 15, and 17 (corresponding to visits 2 – 7). All participants will return on study day 18 (Visit 8) for extensive mood and cognitive measures, as well as follow-up blood collection for potential biomarkers of response. The CDRS-R on Study Day 18 will serve as the primary outcome measure for this portion of the study.

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 used by the Yale New Haven Hospital Investigative Drug Service.

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 used by the Yale New Haven Hospital Investigative Drug Service.

Investigational drug accountability and management (purchase, storage, preparation, dispensing, and disposition) will be conducted by Yale-New Haven Health Investigational Drug Service Pharmacy.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Ketamine hydrochloride injection USP 50 mg/ml, 10 ml vials (NDC: 00143-9508-10) will be used.

Midazolam hydrochloride injection USP 1 mg/ml, 5 ml vials (NDC: 00641-6059-10) will be used.

The formulation, appearance, packaging, and labeling of Ketamine and Midazolam can be found in the package inserts included in this application.

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 and Midazolam will be prepared by Yale New Haven Health Investigational Drug Service. Preparation will comply with USP 797 requirements.

Required dose of Ketamine and Midazolam will be diluted with 0.9% Sodium Chloride USP to a total volume of 50 ml. Prepared infusion will be labeled in a blinded fashion to maintain the study blind. Prepared infusion will have 24 hours stability at Room Temperature.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Yale-New Haven Health Investigational Drug Service Pharmacy will generate a randomization table and randomize participants in 1:1 fashion. Investigational Drug Service Pharmacy staff will be the only un-blinded participants in the study. Investigational drug and active control preparation, dispensing and labeling will be in a blinded fashion. Investigational Drug Service Pharmacy will ensure that blinded study team does not have access to the randomization table during the study.

During the trial Investigational Drug Service Pharmacy will break the participant study blind, upon request from the principal or sub-investigator to ensure patient safety, and such will be documented in study records.

Midazolam is the most appropriate control for ketamine in the proposed study, as the psychotomimetic effects of ketamine make it extremely difficult to blind when compared to saline[26]. Specifically, the typically mild dizziness, nausea and dissociative effects on ketamine functionally un-blind subjects and investigators. As such, more recent ketamine studies have used midazolam as a control. Using an appropriate active control in this efficacy trial is critical to establishing genuine clinical efficacy for ketamine (and not just treatment expectancy or subject performance bias). There will be separate safety and efficacy raters to insure that outcomes are truly blinded.

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. Rating scales will be conducted by a member of the study team who will enter all performed procedures on case report forms and in the electronic data capture system.

6.5 CONCOMITANT THERAPY

Stable psychiatric medications and doses for the month prior to enrollment. Subjects may continue to engage in any ongoing psychotherapy. The maximum period that a patient may receive placebo (midazolam) is 18 days (6 infusions of active control over 18 days). During this time the participant would not be receiving any additional treatments [they may remain on any current therapy (daily medications, psychotherapy, etc.)].

6.5.1 RESCUE MEDICINE

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 Dr. Dwyer. A study doctor 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, the site of infusion. In the unlikely event that any psychiatric care more intensive than regular clinic visits is required as a direct result of participation in this study, study personnel will provide emergent care and stabilization. If longer-term psychiatric care is required, beyond what is normally provided by a research clinic, then study personnel will provide referrals and otherwise endeavor to assist participants in arranging such care.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from ketamine or midazolam treatment during the acute blinded phase, or discontinuation of ketamine treatment during the open phase does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The data to be collected at the time of study intervention discontinuation will include the following:

- We will continue to perform monthly clinical batteries and neurocognitive testing, consistent with the aim of characterizing clinical and cognition outcomes following exposure to ketamine. We will continue to check in for weekly mood assessments as tolerated.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request. Discontinuation from the opt-in neuroimaging portion does not impact eligibility for the primary clinical trial.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- 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 which 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
- Experiences claustrophobia in the fMRI scanner (for the opt-in neuroimaging only)

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 consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, 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 4 weeks a letter will be sent to their address and they will be informed of their discontinuation from the study.

The following actions must 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 reschedule the missed visit as soon as possible and 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.
- 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 should 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.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Screening/Baseline (Visit 1):

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. 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 enroll, the patient and his/her parent/guardian will be asked to sign the assent and parental permission forms, respectively. At this same visit, the additional neuroimaging opt-in study will be discussed as well. 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)[69]. Additionally, the participant and his/her parent/guardian will complete clinical ratings related to the participant's 1) MDD symptoms and 2) symptoms of other commonly comorbid psychiatric conditions. A medical assessment including vital signs, physical exam, baseline serum labs, urine drug screen, and urine pregnancy test will be completed prior to enrollment. The clinical assessment will take approximately 2 hours.

Assessments and Ratings (Figure 4 provides a detailed assessment and procedures schedule).

a. *Medical Assessments:* Vital signs, physical exam, and clinical laboratory tests (i.e. CBC with differential, complete metabolic panel (CMP) (including electrolytes, LFTs, BUN, creatinine and glucose), TFTs, and routine urinalysis) will be completed. A total of 30 cc (2 tablespoons) of blood will be drawn via venipuncture at this visit. In addition, an EKG will be performed and read in order to rule out any cardiac abnormalities.

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 consent 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 consent, 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 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 consent, without having to declare specific reasons. If subjects choose not to enroll, then any previously collected drug test results will be destroyed.

Drug use information **(in the adolescent ≥ 13 years of age)** 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.

b. *Psychiatric Assessments:* Ratings will be conducted by trained research staff. A detailed description of the most common assessments included but not limited to, is listed below. The timing of the clinical assessments is depicted in Table 1 (located at the end of this Research Plan).

All Subjects:

(1) Children's Depression Rating Scale, Revised (CDRS-R): a standardized rating scale that assesses depression severity in children and adolescents[70]
(2) Montgomery-Asberg Depression Rating Scale (MADRS): a standardized rating scale that assesses depression severity in children and adolescents[71]
(3) Columbia-Pleasure Scale for Children (PSC): a standardized rating scale to assess anhedonia[72]
(4) Treatment-Emergent Activation and Suicidality Assessment Profile (TEASAP): a rating scale designed to detect increased behavioral activation and suicidality[73]
(5) Clinical Global Impressions (CGI): a widely used instrument used to assess overall severity of illness and symptom improvement on 1-7 point scales[74]
(6) Multidimensional Anxiety Scale for Children (MASC): a multidimensional assessment of anxiety in children and adolescents [75]
(7) Clinician-Administered Dissociative States Scale (CADSS): self and interviewer administered items that evaluate dissociative symptoms[76]
(8) Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID): structured clinical interview evaluating psychiatric disorders[69]
(9) Columbia-Suicide Severity Rating Scale (C-SSRS) [77]
(10) Beck Hopelessness Scale (BHS): instrument to assess hopelessness[78]
(11) Childhood Trauma Questionnaire (CTQ): a self-report screening measure for maltreatment histories in children[79]
(12) Social Networks Interview (ASSIS): a brief measure of social supports[80]
(13) Friendship Questionnaire
(14) Affective Reactivity Index Self-report (ARI-S)[81]
(15) Scale for Suicide Ideation- 5 (SSI-5): This scale measures the first 5 items on the SSI (19 items). SSI-5 has been shown to be more sensitive to ketamine treatment than the 19-item [82]
(16) Cogstate: a neurocognitive battery tailored to adolescents

Visit 1b: Optional (Opt-In) Neuroimaging session: There are no efficacy assessments scheduled during this visit.

Phase 1: Double-Blind Intervention (Visits 2 – 8):

Subjects will receive 6 infusions of either ketamine (0.5mg/kg IV) or midazolam (0.045mg/kg IV) over a three-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.

Infusions (Day 1, 3, 8, 10, 15, & 17, Visits 2 - 7):

Subjects who, in the opinion of the principal investigator, 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 and potential biomarkers, Day 1 only) and one iv on infusion days without bloodwork (Day 3, 8, 10, 15, 17). 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 hour for 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 6 infusions, scheduled on study day 1, 3, 8, 10, 12, 15, and 17 (corresponding to visits 2 – 7). All participants will return on study day 18 (Visit 8) for extensive mood and cognitive measures, as well as follow-up blood collection for potential biomarkers of response. The CDRS-R on Study Day 18 will serve as the primary outcome measure for this portion of the study.

Ketamine has been used safely in pediatrics for over 40 years for conscious sedation and dentistry[35], however it does have significant risks. The most common side effects when ketamine is infused at the current rate and dose in adult studies are (1) increased blood pressure, respiratory rate, pulse, (2) pain/rash at the injection site, (3) temporary psychiatric symptoms including, but not limited to, disorientation, anxiety, dysphoria, flashbacks, hallucinations, and psychotic-like symptoms (which occur less frequently in younger patients[35]). These reactions are typically self-limited and occur in ~12% of adults given higher doses of ketamine than proposed in this study[89]. The most serious side effects are (1) increased intraocular pressure, (2) allergic reaction, (3) laryngospasm, (4) elevated blood pressure resulting in stroke, heart attack, or death, and (5) substance abuse, all of which are rare[34,35]. These risks will be mitigated by providing comprehensive monitoring in IPS during and two hours following the infusion, supervised by trained physicians with access to airway equipment. Ketamine has been studied in over 10,000 psychiatric patients in more than 100 separate studies. Our institution has had experience using ketamine safely in research studies involving adult psychiatric patients for the last 20 years, and our current research team has significant experience using ketamine in pediatric psychiatric

populations. This dose of ketamine is considered subanesthetic in both adults and children[34], and thus we expect minimal cardiorespiratory and neurologic side effects. Case report level data suggests a favorable side effect profile for intranasal ketamine for adolescents with refractory disorders[33]. However, if side effects do not dissipate we will additionally offer patients hospitalization at Yale Psychiatric Hospital, Adolescent Unit for psychiatric side effects or YNHH for medical side effects.

Midazolam, the active control in this study, similarly has a robust history of use in pediatric sedation and dentistry[59-64,90]. Also similar to ketamine, the dose proposed in the current study (0.045mg/kg over 40 minutes) is lower and slower than doses used in most pediatric sedation procedures (0.05 – 0.1mg/kg over 2 minutes)[64,65]. The most common side effects related to midazolam infusions are hiccups, nausea, vomiting, coughing, headache, and drowsiness. In doses higher than those involved in the proposed study, serious cardiorespiratory adverse events have occurred after administration of midazolam, including respiratory depression, airway obstruction, oxygen desaturation, apnea, respiratory arrest and/or cardiac arrest. In younger children, these higher doses have been associated with a risk for paradoxical disinhibition. Midazolam, like ketamine, has a short half-life, and important adverse events related either drug are not observed after 30 minutes following drug administration[34]. Our research team also has significant experience using midazolam in pediatric psychiatric populations from our first ketamine study. While the risks of serious adverse cardiorespiratory events are low given the dose chosen for this study, airway equipment and qualified physician personnel will be readily available.

Midazolam is the most appropriate control for ketamine in the proposed study, as the psychotomimetic effects of ketamine make it extremely difficult to blind when compared to saline[26]. Specifically, the typically mild dizziness, nausea and dissociative effects on ketamine functionally un-blind subjects and investigators. As such, more recent ketamine studies have used midazolam as a control. Using an appropriate active control in this efficacy trial is critical to establishing genuine clinical efficacy for ketamine (and not just treatment expectancy or subject performance bias).

Phase 2: Open Phase Intervention (Visits 9 – 39):

Month 1: Consistent with our ethical obligation to treat suffering children with active interventions, we will break the blind following the acute phase, and offer those subjects who received midazolam open active treatment according to the protocol described above. That is, they will receive 6 infusions of open ketamine over 3 weeks (Study Days 22, 24, 29, 31, 36, and 38, corresponding to Study Visits 9 – 14), followed by an in-person assessment on Day 39 (Visit 15). Blood draws will occur on Study Day 22 for ketamine metabolites and biomarkers (Visit 9) and for biomarkers on Day 39 (Visit 15).

The subjects who received ketamine in the blinded phase will be evaluated for **response** (defined as >50% decrease from baseline MADRS at any point between D1 and D18). **Remission** is defined as a MADRS < 9.

(1) Non-responders will not be offered additional ketamine, but will receive standard of care depression treatment (42), weekly telephone mood assessment (Days 25, 32, 39 corresponding to Visits 9, 10, 11) and monthly in-depth mood and cognitive assessments (Day 46, Visit 12), see Figure 4.

(2) Responders will also be followed weekly via MADRS, CGI-S, and SSI-5 to assess for relapse (Days 25, 32, 39, 46 corresponding to Visits 9, 10, 11, 12). **Relapse** is defined as ≥50% of baseline MADRS *AND* [MADRS >20 *OR* SSI-5 ≥ 6 *AND* a >10 point absolute increase from MADRS at end of ketamine treatment phase *AND* a >15 point absolute increase from lowest MADRS score during the ketamine treatment phase] on 2 consecutive assessments. Any subject with a weekly assessment indicative of relapse will be seen again within 3 days for a second assessment. Time to relapse will be tracked and **up to two symptom-triggered maintenance** infusions will be offered if relapse criteria is met and there is a consensus between the physician, study subject, and their guardian. Relapse periods can, however, be variable, and we will limit maintenance infusions to no more than 1 every 2 weeks (up to 2 more infusions for a maximum of 8 total). Surveillance labs (same as admission labs) will be obtained prior to any maintenance infusion OR within 1 month of the end of the study including a urine toxicology to track substance abuse, as suggested by FDA. A biomarker blood draw (10cc) may be taken prior to the maintenance infusion.

Months 2-6: At this point in the trial, all subjects will have received active treatment (half receiving ketamine in the blinded phase, and half receiving ketamine in the open phase after having been assigned to midazolam in the blinded phase, assuming that there are no subjects whose symptoms remit after midazolam treatment). All responders will be followed to track time to relapse, and will be offered up to two symptom-triggered maintenance infusions as described above (up to 2 more infusions for a maximum of 8 total). All non-responders will also be followed during this period while receiving standard of care. The general assessment schedule will be weekly phone follow-up and monthly in-person follow up, however any assessments indicative of relapse will trigger an unscheduled, in-person visit.

Telephone: Each subject will schedule follow-up telephone calls with a member of the research staff to monitor his/her MDD symptoms and any Adverse Events associated with potential abuse. During these telephone calls, participants will complete a series of clinical ratings (see Table 1) conducted by a trained research staff member. These telephone calls will take approximately 1 hour.

Telephone visit schedule for those who initially received ketamine: Study Days 25, 32, 39, 53, 60, 67, 81, 88, 94, 108, 115, 122, 136, 143, 150, 164, 171, and178, which correspond to scheduled study visits 9-11, 13-15, 17-19, 21-23, 25-27, and 29-31.

Telephone visit schedule for those initially received midazolam, then received open ketamine: Study Days 46, 53, 60, 74, 81, 88, 101, 108, 115, 129, 136, 143, 157, 164, 171, 185, 192, and 199, which correspond to scheduled study visits 16-18, 20-22, 24-26, 28-30, 32-34, and 36-38.

In-person: Subjects will return to the Yale Child Study Center for follow-up in person visits to monitor their symptoms of depression, physical health, and any Adverse Events associated with potential abuse

(these will be systematically collected in an AE log by study personnel). During these visits, the participant will complete a series of clinical ratings in addition to the Cogstate neurocognitive battery (see Table 1), to be conducted by a trained research staff member. All of the outpatient study follow-up visits will take approximately 1-2 hours.

In-person visit schedule for those who initially received ketamine: Study days 46, 74, 101, 129, 157, and 185, which correspond to scheduled study visits 12, 16, 20, 24, 28, and 32.In-person visit schedule for those initially received midazolam, then received open ketamine: Study Days 67, 94, 122, 150, 178, and 206, which correspond to scheduled study visits 19, 23, 27, 31, 35, and 39.

8.2 SAFETY AND OTHER ASSESSMENTS

Screening/Baseline (Visit 1):

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. 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 enroll, the patient and his/her parent/guardian will be asked to sign the assent and parental permission forms, respectively. At this same visit, the additional neuroimaging opt-in study will be discussed as well. 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)[69]. Additionally, the participant and his/her parent/guardian will complete clinical ratings related to the participant's 1) MDD symptoms and 2) symptoms of other commonly comorbid psychiatric conditions. A medical assessment including vital signs, physical exam, baseline serum labs, urine drug screen, and urine pregnancy test will be completed prior to enrollment. The clinical assessment will take approximately 2 hours.

Assessments and Ratings (Figure 4 provides a detailed assessment and procedures schedule).

a. *Medical Assessments:* Vital signs, physical exam, and clinical laboratory tests (i.e. CBC with differential, complete metabolic panel (CMP) (including electrolytes, LFTs, BUN, creatinine and glucose), TFTs, and routine urinalysis) will be completed. A total of 30 cc (2 tablespoons) of blood will be drawn via venipuncture at this visit. In addition, an EKG will be performed and read in order to rule out any cardiac abnormalities.

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 consent 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 consent, 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 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 consent, without having to declare specific reasons. If subjects choose not to enroll, then any previously collected drug test results will be destroyed.

Drug use information **(in the adolescent ≥ 13 years of age)** 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.

b. *Psychiatric Assessments:* Ratings will be conducted by trained research staff. A detailed description of the most common assessments included but not limited to, is listed below. The timing of the clinical assessments is depicted in Table 1 (located at the end of this Research Plan).

All Subjects:

(1) Children’s Depression Rating Scale, Revised (CDRS-R): a standardized rating scale that assesses depression severity in children and adolescents[70]
(2) Montgomery-Asberg Depression Rating Scale (MADRS): a standardized rating scale that assesses depression severity in children and adolescents[71]
(3) Columbia-Pleasure Scale for Children (PSC): a standardized rating scale to assess anhedonia[72]
(4) Treatment-Emergent Activation and Suicidality Assessment Profile (TEASAP): a rating scale designed to detect increased behavioral activation and suicidality[73]
(5) Clinical Global Impressions (CGI): a widely used instrument used to assess overall severity of illness and symptom improvement on 1-7 point scales[74]
(6) Multidimensional Anxiety Scale for Children (MASC): a multidimensional assessment of anxiety in children and adolescents [75]
(7) Clinician-Administered Dissociative States Scale (CADSS): self and interviewer administered items that evaluate dissociative symptoms[76]
(8) Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID): structured clinical interview evaluating psychiatric disorders[69]
(9) Columbia-Suicide Severity Rating Scale (C-SSRS) [77]

(10) Beck Hopelessness Scale (BHS): instrument to assess hopelessness[78]

(11) Childhood Trauma Questionnaire (CTQ): a self-report screening measure for maltreatment histories in children[79]

(12) Social Networks Interview (ASSIS): a brief measure of social supports[80]

(13) Friendship Questionnaire

(14) Affective Reactivity Index Self-report (ARI-S)[81]

(15) Scale for Suicide Ideation- 5 (SSI-5): This scale measures the first 5 items on the SSI (19 items). SSI-5 has been shown to be more sensitive to ketamine treatment than the 19-item [82]

(16) Cogstate: a neurocognitive battery tailored to adolescents

Visit 1b: Optional (Opt-In) Neuroimaging session (Other Assessments):

Participants will have a separate scheduled session at the MRRC. Female participants will have received a serum pregnancy test as part of the baseline visit (described above under "Medical Assessments"). A short number of additional rating scales and cognitive testing will be performed including : Edinburgh Handedness Inventory and the Wechsler Abbreviated Scale of Intelligence. Following completion of the scales, the participants will proceed to the MRI suite. 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.

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, the 1 perception/movie watching run, and 1-resting-state run will be acquired over 6 minutes (total of 30 minutes of connectivity data) using multiband EPI (voxel size 2mm3)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.

Tasks performed during the experiment sessions will include:

7. Card guessing task[83]: 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 or greater than five. The card will then be flipped over and subjects will receive reward feedback based on their response.
8. N-back task[84,85]: 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).
9. Response inhibition task/Stop-Signal (e.g.,[86]): Subjects will see items on the screen and either respond (go trial) or not respond (no-go trial), depending on the presented item.
10. 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[87], or by responding to questions that require them to interpret social "hints" in vignettes[88].
11. 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.
12. Resting state run: Subjects will be asked to stay still with eyes open during the resting runs. There is no task involved.

Phase 1: Double-Blind Intervention (Visits 2 – 8): Subjects will receive 6 infusions of either ketamine (0.5mg/kg IV) or midazolam (0.045mg/kg IV) over a three-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.

Infusions (Day 1, 3, 8, 10, 15, & 17, Visits 2 - 7): Subjects who, in the opinion of the principal investigator, 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 and potential biomarkers, Day 1 only) and one IV on infusion days without bloodwork (Day 3, 8, 10, 15, 17). 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 hour for 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 6 infusions, scheduled on study day 1, 3, 8, 10, 12, 15, and 17 (corresponding to visits 2 – 7). All participants will return on study day 18 (Visit 8) for extensive mood and cognitive measures, as well as follow-up blood collection for potential biomarkers of response. The CDRS-R on Study Day 18 will serve as the primary outcome measure for this portion of the study.

Ketamine has been used safely in pediatrics for over 40 years for conscious sedation and dentistry[35], however it does have significant risks. The most common side effects when ketamine is infused at the current rate and dose in adult studies are (1) increased blood pressure, respiratory rate, pulse, (2) pain/rash at the injection site, (3) temporary psychiatric symptoms including, but not limited to, disorientation, anxiety, dysphoria, flashbacks, hallucinations, and psychotic-like symptoms (which occur less frequently in younger patients[35]). These reactions are typically self-limited and occur in ~12% of adults given higher doses of ketamine than proposed in this study[89]. The most serious side effects are (1) increased intraocular pressure, (2) allergic reaction, (3) laryngospasm, (4) elevated blood pressure resulting in stroke, heart attack, or death, and (5) substance abuse, all of which are rare[34,35]. These risks will be mitigated by providing comprehensive monitoring in IPS during and two hours following the infusion, supervised by trained physicians with access to airway equipment. Ketamine has been studied in over 10,000 psychiatric patients in more than 100 separate studies. Our institution has had experience using ketamine safely in research studies involving adult psychiatric patients for the last 20 years, and our current research team has significant experience using ketamine in pediatric psychiatric populations. This dose of ketamine is considered subanesthetic in both adults and children[34], and thus we expect minimal cardiorespiratory and neurologic side effects. Case report level data suggests a favorable side effect profile for intranasal ketamine for adolescents with refractory disorders[33]. However, if side effects do not dissipate we will additionally offer patients hospitalization at Yale Psychiatric Hospital, Adolescent Unit for psychiatric side effects or YNHH for medical side effects.

Midazolam, the active control in this study, similarly has a robust history of use in pediatric sedation and dentistry[59-64,90]. Also similar to ketamine, the dose proposed in the current study (0.045mg/kg over 40 minutes) is lower and slower than doses used in most pediatric sedation procedures (0.05 – 0.1mg/kg over 2 minutes)[64,65]. The most common side effects related to midazolam infusions are hiccups, nausea, vomiting, coughing, headache, and drowsiness. In doses higher than those involved in the proposed study, serious cardiorespiratory adverse events have occurred after administration of midazolam, including respiratory depression, airway obstruction, oxygen desaturation, apnea, respiratory arrest and/or cardiac arrest. In younger children, these higher doses have been associated with a risk for paradoxical disinhibition. Midazolam, like ketamine, has a short half-life, and important adverse events related either drug are not observed after 30 minutes following drug administration[34]. Our research team also has significant experience using midazolam in pediatric psychiatric populations from our first ketamine study. While the risks of serious adverse

cardiorespiratory events are low given the dose chosen for this study, airway equipment and qualified physician personnel will be readily available.

Midazolam is the most appropriate control for ketamine in the proposed study, as the psychotomimetic effects of ketamine make it extremely difficult to blind when compared to saline[26]. Specifically, the typically mild dizziness, nausea and dissociative effects on ketamine functionally un-blind subjects and investigators. As such, more recent ketamine studies have used midazolam as a control. Using an appropriate active control in this efficacy trial is critical to establishing genuine clinical efficacy for ketamine (and not just treatment expectancy or subject performance bias).

Phase 2: Open Phase Intervention (Visits 9 – 39):

Month 1: Consistent with our ethical obligation to treat suffering children with active interventions, we will break the blind following the acute phase, and offer those subjects who received midazolam open active treatment according to the protocol described above. That is, they will receive 6 infusions of open ketamine over 3 weeks (Study Days 22, 24, 29, 31, 36, and 38, corresponding to Study Visits 9 – 14), followed by an in-person assessment on Day 39 (Visit 15). Blood draws will occur on Study Day 22 for ketamine metabolites and biomarkers (Visit 9) and for biomarkers on Day 39 (Visit 15).

The subjects who received ketamine in the blinded phase will be evaluated for **response** (defined as >50% decrease from baseline MADRS at any point between D1 and D18). **Remission** is defined as a MADRS < 9.

(1) Non-responders will not be offered additional ketamine, but will receive standard of care depression treatment (42), weekly telephone mood assessment (Days 25, 32, 39 corresponding to Visits 9, 10, 11) and monthly in-depth mood and cognitive assessments (Day 46, Visit 12), see Figure 4.

(2) Responders will also be followed weekly via MADRS, CGI-S, and SSI-5 to assess for relapse (Days 25, 32, 39, 46 corresponding to Visits 9, 10, 11, 12). **Relapse** is defined as ≥50% of baseline MADRS *AND* [MADRS >20 *OR* CGI-S ≥ 5 *OR* SSI-5 ≥ 6] *AND* a >10 point absolute increase from MADRS at end of ketamine treatment phase *AND* a >15 point absolute increase from lowest MADRS score during the ketamine treatment phase on 2 consecutive assessments. Any subject with a weekly assessment indicative of relapse will be seen again within 3 days for a second assessment. Time to relapse will be tracked and **up to two symptom-triggered maintenance** infusions will be offered if relapse criteria is met and there is a consensus between the physician, study subject, and their guardian. Relapse periods can, however, be variable, and we will limit maintenance infusions to no more than 1 every 2 weeks (up to 2 more infusions for a maximum of 8 total). Surveillance labs (same as admission labs) will be obtained prior to any maintenance infusion OR within 1 month of the end of the study including a urine toxicology to track substance abuse, as suggested by FDA. A biomarker blood draw (10cc) may be taken prior to the maintenance infusion.

Months 2-6: At this point in the trial, all subjects will have received active treatment (half receiving ketamine in the blinded phase, and half receiving ketamine in the open phase after having been assigned to midazolam in the blinded phase, assuming that there are no subjects whose symptoms remit after midazolam treatment). All responders will be followed to track time to relapse, and will be offered up to two symptom-triggered maintenance infusions as described above so that the total number of ketamine infusions does not exceed 8 doses over the lifetime of the patient. All non-responders will also be followed during this period while receiving standard of care. The general assessment schedule will be weekly phone follow-up and monthly in-person follow up, however any assessments indicative of relapse will trigger an unscheduled, in-person visit.

Telephone: Each subject will schedule follow-up telephone calls with a member of the research staff to monitor his/her MDD symptoms and any Adverse Events associated with potential abuse. During these telephone calls, participants will complete a series of clinical ratings (see Table 1) conducted by a trained research staff member. These telephone calls will take approximately 1 hour.

Telephone visit schedule for those who initially received ketamine: Study Days 25, 32, 39, 53, 60, 67, 81, 88, 94, 108, 115, 122, 136, 143, 150, 164, 171, and178, which correspond to scheduled study visits 9-11, 13-15, 17-19, 21-23, 25-27, and 29-31.

Telephone visit schedule for those initially received midazolam, then received open ketamine: Study Days 46, 53, 60, 74, 81, 88, 101, 108, 115, 129, 136, 143, 157, 164, 171, 185, 192, and 199, which correspond to scheduled study visits 16-18, 20-22, 24-26, 28-30, 32-34, and 36-38.

In-person: Subjects will return to the Yale Child Study Center for follow-up in person visits to monitor their symptoms of depression, physical health and any Adverse Events associated with potential abuse (these will be systematically collected in an AE log by study personnel). During these visits, the participant will complete a series of clinical ratings in addition to the Cogstate neurocognitive battery (see Table 1), to be conducted by a trained research staff member. All of the outpatient study follow-up visits will take approximately 1-2 hours.

In-person visit schedule for those who initially received ketamine: Study days 46, 74, 101, 129, 157, and 185, which correspond to scheduled study visits 12, 16, 20, 24, 28, and 32. In-person visit schedule for those initially received midazolam, then received open ketamine: Study Days 67, 94, 122, 150, 178, and 206, which correspond to scheduled study visits 19, 23, 27, 31, 35, and 39.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event 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 adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, 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, or a congenital anomaly/birth defect. Important medical events 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

8.3.3.1 SEVERITY OF EVENT

For adverse events (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 with functioning.
- **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".

In addition to grading the adverse event, the PI will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it results in any of the following outcomes:

1. Death;
2. A life-threatening experience in-patient hospitalization or prolongation of existing hospitalization;
3. A persistent or significant disability or incapacity;
4. A congenital anomaly or birth defect; OR
5. Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the Yale IRB is necessary.

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) will have their relationship to study intervention assessed by the principal investigator (Dr. Michael Bloch), who will examine and evaluate the participant based on temporal relationship and his 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 (de-challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory re-challenge 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 (de-challenge). Re-challenge information is not required to fulfill this definition.
- **Possibly 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).
- **Unrelated** – 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.>

8.3.3.3 EXPECTEDNESS

The principal investigator (Dr. Michael Bloch) will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

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 (CRF). 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 informed consent 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 investigator will inquire 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

All adverse events not meeting the *prompt* reporting requirements described in the Yale Institutional Review Board (IRB) Policy 710 (Appendix 1) will be reported to the IRB in summary form at the time of continuing review.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

The principal investigator will report the following types of events to the Yale Institutional Review Board (IRB):

Any incident, experience or outcome that meets ALL 3 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 consent 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*. ***Please note*** that adverse events are reportable to the IRB as UPIRSOs **only** if they meet all 3 criteria listed above. We will additionally report serious adverse events 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.

These UPIRSOs/SAEs will be reported to the IRB in accordance with IRB Policy 710 (Appendix 1), using the appropriate forms found on the website. All related events involving risk but not meeting the *prompt* reporting requirements described in IRB Policy 710 should 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**.** In lieu of a summary of external events, a current DSMB report can be submitted for research studies that are subject to oversight by a DSMB (or other monitoring entity that is monitoring the study on behalf of an industry sponsor).

8.3.7 REPORTING EVENTS TO PARTICIPANTS

The AEs and SAEs that will be reported to participants are determined by the Yale Institutional Review Board (IRB) in accordance with IRB Policy 710 (Appendix 1).

8.3.8 EVENTS OF SPECIAL INTEREST

Not applicable.

8.3.9 REPORTING OF PREGNANCY

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 consent 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 consent, without having to declare specific reasons. If the pregnancy test is positive, the subject will not be able to participate in the protocol.

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 consent 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.

8.4.2 UNANTICIPATED PROBLEM REPORTING

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. Please note that adverse events are reportable to the Yale Institutional Review Board (IRB) as UPIRSOs only if they meet all 3 criteria listed above. We will additionally report serious adverse events 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.

These UPIRSOs/SAEs will be reported to the IRB in accordance with IRB Policy 710 (Appendix 1), using the appropriate forms found on the website. All related events involving risk but not meeting the prompt reporting requirements described in IRB Policy 710 should 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. In lieu of a summary of external events, a current DSMB report can be submitted for research studies that are subject to oversight by a DSMB (or other monitoring entity that is monitoring the study on behalf of an industry sponsor).>

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 (Appendix 1).

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

- Primary Efficacy Endpoint(s):

Hypothesis 1: Repeated ketamine will be tolerated well medically and psychiatrically. We expect a significant reduction in MADRS/CDRS-R in those treated with ketamine at the end of the dosing paradigm (Day 18)

- Secondary Efficacy Endpoint(s):

Hypothesis 2: The repeated dosing paradigm will result in a prolonged antidepressant response (measured via MADRS/CDRSD-R), as is seen in adults and in our adolescent case report.

Hypothesis 3: Cogstate extensive battery will show no adverse effects on attention, working memory, or executive functioning over the 6 months following ketamine treatment.

Exploratory Aim 4: To identify functional connectome phenotypes predictive of depression scores (Day 18 CDRS-R) post-ketamine or midazolam treatment.

9.2 SAMPLE SIZE DETERMINATION

Controlled studies of ketamine (both saline and midazolam controls) in adult MDD report effect sizes of 1.5[22,93]. If of similar efficacy in adolescents, a sample size of 24 (n=12 per group) would have

power greater than 0.8 to detect a difference from midazolam (α=0.05) for an effect size≥1.0 for the acute, blinded phase. A previous double-blind, midazolam-controlled trial examining the benefits of twice-weekly ketamine treatments in adults with treatment-refractory depression demonstrated an effect size=1.1 after 2 weeks of treatment[40].

The primary outcome will be change in CDRS-R score from baseline to Day 18. This outcome will be analyzed using a mixed-effect model with repeated measures, with baseline CDRS-R score as a covariate, treatment and time-by-treatment interaction as fixed effects, and subject as a random effect. Scores of the other rating scales and time course will be assessed as secondary measures in a similar manner. Treatment response will be defined as 40% reduction in CDRS-R score on Day 18 relative to baseline CDRS-R. A Fischer exact test will be used to compare the proportion of treatment responders in each group.

The open arm of this study is an exploratory aim, designed to examine ketamine's antidepressant durability in adolescents after a repeated dosing paradigm, and to examine symptom triggered maintenance requirements. Important descriptive information to be gathered from this phase includes (1) average time to relapse after ketamine treatment; (2) number of subjects who remain in sustained remission during the 6 month follow-up period and (3) average frequency of needed of single ketamine maintenance treatments. The open arm will also follow longitudinal neurocognitive measures via Cogstate as a safety measure following repeated ketamine. While the arm ultimately has an increased number of ketamine-treated subjects (24 ketamine treated subjects assuming no significant midazolam-responders) it is still likely underpowered to detect a difference on Cogstate measures. This phase will provide preliminary data for a subsequent study, and will help estimate the number of subjects needed to power that study.

The opt-in neuroimaging portion of the study is exploratory, to examine the feasibility of incorporating pre-treatment neuroimaging into a pediatric treatment clinical trial. That said, CPM has successfully build predictive models using only 25 subjects[15], so it is possible that the imaging portion could provide more than feasibility data.

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

The primary outcome will be change in CDRS-R score from baseline to Day 18. This outcome will be analyzed using a mixed-effect model with repeated measures, with baseline CDRS-R score as a covariate, treatment and time-by-treatment interaction as fixed effects, and subject as a random effect. Scores of the other rating scales and time course will be assessed as secondary measures in a similar manner. Treatment response will be defined as 40% reduction in CDRS-R score on Day 18 relative to baseline CDRS-R. A Fischer exact test will be used to compare the proportion of treatment responders in each group.

The open arm of this study is an exploratory aim, designed to examine ketamine's antidepressant durability in adolescents after a repeated dosing paradigm, and to examine symptom triggered maintenance requirements. Important descriptive information to be gathered from this phase includes (1) average time to relapse after ketamine treatment; (2) number of subjects who remain in sustained remission during the 6 month follow-up period and (3) average frequency of needed of single ketamine maintenance treatments. The open arm will also follow longitudinal neurocognitive measures via Cogstate as a safety measure following repeated ketamine. While the arm ultimately has an increased number of ketamine-treated subjects (24 ketamine treated subjects assuming no significant midazolam-responders) it is still likely underpowered to detect a difference on Cogstate measures. This phase will provide preliminary data for a subsequent study, and will help estimate the number of subjects needed to power that study.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The primary outcome will be change in CDRS-R score from baseline to Day 18. This outcome will be analyzed using a mixed-effect model with repeated measures, with baseline CDRS-R score as a covariate, treatment and time-by-treatment interaction as fixed effects, and subject as a random effect. Scores of the other rating scales and time course will be assessed as secondary measures in a similar manner. Treatment response will be defined as 40% reduction in CDRS-R score on Day 18 relative to baseline CDRS-R. A Fischer exact test will be used to compare the proportion of treatment responders in each group.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

The primary outcome will be change in CDRS-R score from baseline to Day 18. This outcome will be analyzed using a mixed-effect model with repeated measures, with baseline CDRS-R score as a covariate, treatment and time-by-treatment interaction as fixed effects, and subject as a random effect. Scores of the other rating scales and time course will be assessed as secondary measures in a similar manner. Treatment response will be defined as 40% reduction in CDRS-R score on Day 18 relative to baseline CDRS-R. A Fischer exact test will be used to compare the proportion of treatment responders in each group.

9.4.4 SAFETY ANALYSES

The open arm of this study is an exploratory aim, designed to examine ketamine's antidepressant durability in adolescents after a repeated dosing paradigm, and to examine symptom triggered maintenance requirements. Important descriptive information to be gathered from this phase includes (1) average time to relapse after ketamine treatment; (2) number of subjects who remain in sustained remission during the 6 month follow-up period and (3) average frequency of needed of single ketamine maintenance treatments. The open arm will also follow longitudinal neurocognitive measures via Cogstate as a safety measure following repeated ketamine. While the arm ultimately has an increased number of ketamine-treated subjects (24 ketamine treated subjects assuming no significant midazolam-responders) it is still likely underpowered to detect a difference on Cogstate measures. This phase will provide preliminary data for a subsequent study, and will help estimate the number of subjects needed to power that study.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Not applicable.

9.4.6 PLANNED INTERIM ANALYSES

Not applicable.

9.4.7 SUB-GROUP ANALYSES

Not applicable.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Data will not be listed individually.

9.4.9 EXPLORATORY ANALYSES

The open arm of this study is an exploratory aim, designed to examine ketamine's antidepressant durability in adolescents after a repeated dosing paradigm, and to examine symptom triggered maintenance requirements. Important descriptive information to be gathered from this phase includes (1) average time to relapse after ketamine treatment; (2) number of subjects who remain in sustained remission during the 6 month follow-up period and (3) average frequency of needed of single ketamine maintenance treatments. The open arm will also follow longitudinal neurocognitive measures via Cogstate as a safety measure following repeated ketamine. While the arm ultimately has an increased number of ketamine-treated subjects (24 ketamine treated subjects assuming no significant midazolam-

responders) it is still likely underpowered to detect a difference on Cogstate measures. This phase will provide preliminary data for a subsequent study, and will help estimate the number of subjects needed to power that study.

Neuroimaging/ CPM outcomes: FMRI data will be motion corrected using SPM12. Data will be analyzed using BioImage Suite[94,95] 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 [96]. 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[53]. 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 CDRS-R 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 (depressive symptoms as measured via the CDRS-R). 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 depression 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.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol:

1. ADOLESCENT ASSENT FOR PARTICIPATION IN A RESEARCH PROJECT YALE UNIVERSITY SCHOOL OF MEDICINE—YALE NEW HAVEN HOSPITAL
2. COMPOUND AUTHORIZATION AND PARENTAL PERMISSION FOR PARTICIPATION IN A RESEARCH PROJECT YALE UNIVERSITY SCHOOL OF MEDICINE—YALE NEW HAVEN HOSPITAL 310 FR. 3a (2014-3)
3. ADOLESCENT ASSENT FOR PARTICIPATION IN A RESEARCH PROJECT YALE UNIVERSITY SCHOOL OF MEDICINE—YALE NEW HAVEN HOSPITAL—NEUROIMAGING OPT-IN
4. COMPOUND AUTHORIZATION AND PARENTAL PERMISSION FOR PARTICIPATION IN A RESEARCH PROJECT YALE UNIVERSITY SCHOOL OF MEDICINE—YALE NEW HAVEN HOSPITAL—NEUROIMAGING OPT-IN

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed assent/consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Assent/consent forms will be Institutional Review Board (IRB)-approved and the participant and his/her parents/guardians will be asked to read and review the documents. The investigator will explain the research study to the participant and his/her parents/guardians and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's and his/her parents'/guardians' comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Both the main study and the neuroimaging opt-in study will be discussed together in the same visit. Participants and their parents/guardians will have the opportunity to carefully review the written assent/consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant and his/her parents/guardians will sign the informed

assent/consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed assent/consent document will be given to the participants for their records. The informed assent/consent process will be conducted and documented in the source documents (including the date), and the forms signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor 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
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

10.1.3 CONFIDENTIALITY AND PRIVACY

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. 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. 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 his 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 collected for this study will be analyzed and physically stored at the Yale Child Study Center in a locked filing cabinet in a locked office that is only accessible to study personnel and electronic clinical data will be kept on a password protected server or on password protected and encrypted computers. After the study is completed, the de-identified, archived data will be stored at the above site.

With the participant's approval and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be stored at the Clinical Neuroscience Research Unit (CNRU). These samples could be used to research 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, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to bio sample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the CNRU.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	Sub-Investigator
Michael Bloch, MD, MS, Associate Professor in the Child Study Center, Associate Director, Albert J. Solnit Integrated Training Program, Associate Director of the Tic and OCD Program	Jennifer Dwyer, MD, PhD Assistant Professor, Yale Child Study Center and Department of Radiology of Biomedical Imaging
Yale University	Yale University
230 S Frontage Rd, New Haven, 06519	230 S Frontage Rd, New Haven, 06519
203.745.9921	203.433.2788
Michael.Bloch@yale.edu	Jennifer.Dwyer@yale.edu

10.1.6 SAFETY OVERSIGHT

Data safety monitoring will be conducted by the principal investigator.

10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

- The principal investigator will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified frequency, which must be conducted at a minimum of every 6 months (including when reapproval of the protocol is sought). During the review process, the principal investigator will evaluate whether the study should continue unchanged, require modification/amendment, or close to enrollment. Either the principal investigator or the IRB have the authority to stop or suspend the study, or require modifications.
- Yale may conduct internal audits.

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.

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 research coordinator 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 sponsor, and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

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.

10.1.9.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harminosation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations.

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.

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:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. 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 Dr. Michael Bloch.

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 design and conduct of this trial.

10.2 ADDITIONAL CONSIDERATIONS

Not applicable.

10.3 ABBREVIATIONS

AE	Adverse Event
CFR	Code of Federal Regulations
CMP	Complete Metabolic Panel
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event

SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
1.1	13 APR 2019	Changes requested by FDA and Yale IRB to clarify procedures and risks associated with both drugs used in the protocol.	Update the IND protocol with the revisions and recommendations provided by the FDA. Update the protocol with the revisions and recommendations of the Yale IRB to enhance protocol clarity.
1.2	20 DEC 2019	Add weight limit to the inclusion/exclusion criteria. Clarify response and relapse criteria. Clarify how AE's would be assessed and recorded by study personnel.	Add clarity and add safety for participants.
2	25 SEPT 2020	Review of scales and bibliography. Clarify when post-treatment and monitoring labs are to be performed; as well as when and how participants would be discharged from the trial.	Add clarity to phase 2 monitoring and discharge.
3	31 MAR 2021	Addition of neuroimaging opt-in procedures	Addition of neuroimaging opt-in procedures
3.1	20 APR 2021	Addition of neuroimaging sequence detail	Additional detail requested by MRRC committee

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Appendix 1

Appendix 2