The BIO-K Study: A Single-Arm, Open-Label, Biomarker Development Clinical Trial of Ketamine for Non-Psychotic Unipolar Major Depression and Bipolar I or II Depression

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The BIO-K Study: A Single-Arm, Open-Label, Biomarker Development Clinical Trial of Ketamine for Non-Psychotic Unipolar Major Depression and Bipolar I or II Depression.

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A. BRIEF SUMMARY

This multi-site (Johns Hopkins Hospital, Mayo Clinic (Rochester, MN), Pine Rest Christian Mental Health Services, University of Michigan, and the University of Pennsylvania), open-label, single-arm clinical trial is designed to develop preliminary biomarkers of ketamine response in 100 patients with treatment-resistant non-psychotic unipolar major depression (MDD) or bipolar I or II depression (BPD). Ketamine will be administered intravenously (IV) at a subanesthetic dose of 0.5 mg/kg delivered over 40-minutes or 100-minutes of infusion time. Enrolled subjects will receive a total of three acute phase infusions, delivered at least every other day, within a time window of up to 11 days including the first infusion day. The primary outcome will be remission, as defined by a Montgomery-Åsberg Depression Scale (MÅDRS) score ≤9. The secondary outcome measures include reduction of suicidality, as defined by a 50% reduction in the Beck Scale for Suicidal Ideation (BSS). Exploratory biomarkers for ketamine treatment outcome include baseline to endpoint change in WBC stimulated mTOR, AKT, and GSK3 signaling. Additional sample for the ascertainment of other biomarkers will also be taken.

B. BACKGROUND

B.1. Limitations of conventional pharmacotherapy for Treatment Resistant Depression (TRD)

Pharmacotherapy with conventional antidepressants and mood stabilizing medications is ineffective for many patients with MDD and BPD. Up to one-third of patients with MDD do not respond to conventional antidepressants and fewer than a third of patients experience durable symptomatic remission even after serial therapeutic antidepressant trials [1]. Depression is also the predominant mood state in patients with BPD over the course of illness—yet, few pharmacological treatments have been shown to be effective for treating depressive episodes associated with BPD in adults [2-4]. Not surprisingly, treatment resistance occurs frequently for patients with BPD depression despite intensive pharmacotherapy [5]. Moreover, response to vigorous pharmacological treatment is incomplete or absent for many patients with BPD depressive episodes [6], thus contributing to high rates of recurring and persisting depressive symptoms, psychiatric and general medical comorbidity, and suicide in BPD patients [7-10]. For patients with MDD or BPD who eventually respond to pharmacotherapy with antidepressants and/or mood stabilizing medications, the onset of therapeutic benefit is usually delayed for several weeks [11].

B.2. Glutamate signaling as a pharmacological target for rapid antidepressive responses

The need for more effective treatments with a faster onset of therapeutic effect has been long-recognized, as has the need for discovering novel mechanisms of depressive illness and antidepressive treatment response that go beyond biogenic amine transporter and receptor-ology based mechanisms [12]. Converging evidence from post-mortem and *in vivo* neuroimaging studies shows glutamate abnormalities in the pathophysiology of mood disorders [13-27]. These discoveries demonstrated strong evidence of disrupted glutamate signaling through N-methyl-D-aspartate (NMDA) receptors in depressive mood states, and prompted investigations of the antidepressive activity for compounds that interact with NMDA receptors.

B.3. Rapid antidepressive effects of ketamine, a glutamatergic NMDA-receptor antagonist

Multiple controlled trials have now demonstrated the short-term effectiveness of single or serial administration of low-dose IV ketamine, a potent non-competitive NMDA antagonist [28], for treating the symptoms of depression in patients with MDD and BPD who failed to respond to

multiple trials of pharmacotherapy and, in some cases, electroconvulsive therapy [29]. Those who benefitted from ketamine experienced rapid (within hours) onset of clinical antidepressive response [30]. Positive benefit from ketamine persisted for 3-14 days on average following single infusions [30-38], and for 18-19 days following serial infusions administered over 12-14 days [39,40]. The duration of antidepressive response demonstrated in these clinical studies is well in excess of what would be predicted by ketamine's short elimination half-life of 2-3 hours [41,42].

B.4. Biomarkers for antidepressive effects of ketamine: clinical significance

The therapeutic potential of ketamine (i.e., rapid symptom relief and response in treatment-resistant patients) has stimulated considerable interest in the psychiatric community [43], and the clinical use of ketamine infusion for the treatment of depression is now an intense focus of research worldwide. However, this further progress is challenged by the absence of reliable and valid predictors of antidepressive response to ketamine. The rates of positive antidepressive response to I.V. ketamine are highly variable (25%-85%) after single infusions.[7] When the results of controlled and uncontrolled studies of single and multiple IV infusions of ketamine for treatment-resistant depression are considered, there is an even more striking degree of variability in the cumulative rates of positive antidepressive response during short-term treating, ranging from 7%-90% [44-46]. Currently, there is no evidence-based approach to determining which patients with treatment-resistant MDD or BPD are more likely to respond to IV ketamine infusion therapy. Reliable biomarkers of ketamine response would allow clinicians to risk-stratify patients who are eligible for ketamine treatment according to their likelihood of responding to ketamine, a treatment that is not without potential serious risks, including psychomimetic effects, changes in hemodynamic measures, etc.

B.5. Ketamine antidepressive effects and mammalian target of rapamycin (mTOR) signaling: potential for biomarker development

B.5.a. mTOR signaling, cellular energetics, and potential importance for depressive symptoms.

mTOR signaling plays a significant role in modulating cell energy homeostasis and survival [47]. Accumulating evidence shows that rapid antidepressant effects of ketamine are directly mediated by this intracellular pathway, activation of which promotes synapse formation and efficiency [48]. This rapid potentiation of synaptic plasticity is thought to be the overarching mechanism through which antidepressant actions are conferred to behavioral (preclinical) or mood (clinical) related changes [49].

B.5.b. mTOR signaling changes with ketamine may be crucial to its antidepressive effect, and subsequent biomarker development.

The few biomarker investigations in this area have failed to reliably distinguish ketamine responders and nonresponders, in part because of their focus on simple pharmacokinetic or metabolomic measures for predicting ketamine antidepressive response [50-54]. This approach is not aligned with emerging data showing that antidepressive effects of ketamine are critically mediated through glutamatergic neurotransmission and subsequent mTOR-dependent cell signaling cascades, which rapidly upregulate functional and structural synaptic plasticity in mood-regulating neural pathways [55,56]. These structural and functional changes at the molecular and neural network level appear to be critical to an individual's potential for ketamine treatment response [55,57] It follows that biomarker development efforts will require multimodal, integrated measures across neuroimaging and molecular domains.

Specific to this biomarker development project, mTOR signaling target engagement has been shown to directly correlate with antidepressant treatment response to ketamine [58]. Project

investigators have demonstrated a strong positive correlation between phosphorylation of mTOR, Akt and GSK3 and the antidepressant actions of ketamine (**Fig 1**). A trend was also observed for TrkB. Specifically, a significant and strong positive correlation was observed between latency to first immobility (a preclinical measure of antidepressant efficacy) and the level of protein phosphorylation (expressed as a ratio relative to loading control β-actin) in antidepressant-resistant animals that received ketamine. No significant correlations were observed between latency to first immobility and these biomarkers for control animals (both treatment-resistant & treatment-responsive), with the exception of pTrkB. We also observed a moderate negative correlation between latency to immobility and pmTOR in treatment-resistant animals that did not receive ketamine, suggesting that ketamine was correcting a functional deficit in this intracellular pathway that is critically implicated in rapid antidepressant response in TRD [59].

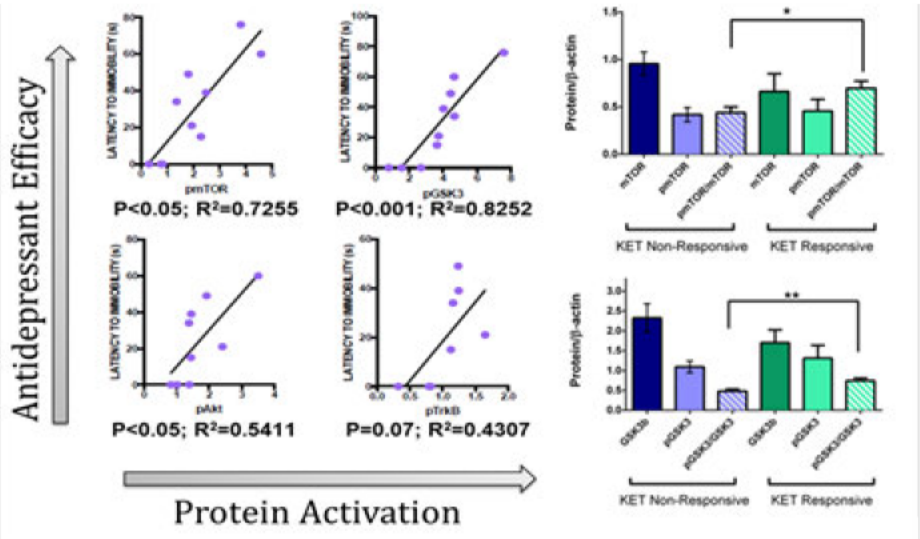


Fig. 1 **LEFT**: Strong, significant positive correlation between levels of phosphorylated Akt, mTOR and GSK3 in the infralimbic cortex and the antidepressant actions of ketamine (latency to immobility) in our preclinical model of treatment resistant depression to ketamine. A positive correlative trend was also observed for TrkB. **RIGHT:** Levels of phosphorylated mTOR and GSK3 were significantly increased in white blood cells of ketamine responders relative to non-responders. Protein levels are expressed as a ratio relative to loading control β-actin. * p<0.05; ** p< 0.01

B.5.c. Peripheral WBC assay for mTOR, GSK3, and AKT signaling—correlation with ketamine-associated preclinical antidepressive effects.

Project investigators recently developed an assay for quantification of mTOR, GSK3, and AKT in peripheral white blood cells *ex vivo*, and demonstrated that these serve as valid proxy biomarkers for the brain (see §D.2.a). Specifically, investigators showed that phosphorylation of these proteins in WBCs directly correlated with the phosphorylation status of the same proteins in prefrontal cortex in a rodent model of TRD. Crucial to this application, investigators also demonstrated that these analogous changes in phosphorylation status of mTOR, GSK3, and AKT in both brain and peripheral WBCs were correlated with antidepressive response to ketamine.

B.6. Overarching goal of the current project

We will conduct a multi-site, open-label, single-arm clinical trial to develop preliminary biomarkers of ketamine response in 100 patients with treatment-resistant non-psychotic MDD or BPD. *The*

overarching goal of this project is to develop multiple biomarkers of ketamine's antidepressive effects in adults with treatment-resistant depression using a time-efficient approach. To accomplish this goal, the investigative sites will be comprised of members of the National Network of Depression Centers (NNDC), several of which have active and successful translational research and biomarker development programs.

While the overarching goal of this collaboration is to develop multiple biomarkers, the primary initial aims of this project will focus on developing a WBC marker of impaired mTOR-dependent cellular energy regulation for predicting ketamine-induced antidepressive response (see §B.5.c). A panel of investigators across multiple sites with experience in the development of biomarkers for severe affective disorders and with the treatment of depression will identify additional specific blood-based biomarkers for predicting antidepressive response to ketamine in adults with treatment-resistant unipolar or bipolar major depression (Samples Subcommittee, see §C.2.b). These additional biomarkers will be tested using blood samples from enrollees in this project.

B.7. Hypotheses/Specific Aims

We will enroll 100 adults with treatment-resistant unipolar or bipolar major depression (TRD) across 8 clinical sites and provide three IV ketamine infusions (0.5 mg/kg, infused over at 40-minutes for first infusion and subsequent infusions at either 40-minutes or 100-minutes) and measure their depressive symptom responses. Biomarkers will be developed using blood samples from study subjects, taken prior to (predictive biomarkers) and following ketamine treatment (change biomarkers). We will begin by studying the predictive value of mTOR target engagement by ketamine using a WBC assay for antidepressive response to ketamine (**Aim 1**); however, samples will be used to develop multiple blood-based biomarkers for ketamine antidepressive effects (**Aim 2**). We will also examine the effect of combining multiple blood-based biomarkers for predicting antidepressive response to ketamine in adults with TRD (**Aim 3**).

Specific to Mayo Clinic-Rochester, a subset of 10 subjects with treatment-resistant unipolar major depression will be enrolled in support of Mayo Clinic IRB # 17-011373, "Central versus Peripheral Glutamate Biomarkers for Treatment Response during a Single Infusion of Intravenous Ketamine for Treatment-Resistant Depression". Subjects will be provided one IV ketamine infusion (0.5 mg/kg, infused over 40 minutes) and will have measured their depressive symptom responses. In this subset of subjects, adults (aged 18-65 years) with treatment-resistant depression will be included. For this subset of subjects, blood draw schedule will defer to those IRB-approved for IRB # 17-011373. Blood samples will be drawn by CRTU staff from the subject before the first ketamine infusion (81ml), during the infusion (1ml at 20 minutes), at infusion stop time (81ml at 40 minutes), and twice after infusion stop time (1ml at 1 hour after the infusion and 1ml at 24 hours after the infusion) for a total of 165ml at Acute Infusion #1. A subject may be eligible to enroll in both trials through trial-specific informed consent forms. If a subject enrolls in both trials, collection of blood samples at Acute Infusion #1 will serve purposes of both trials.

Aim 1: Determine the effectiveness of a WBC marker of impaired cellular energy regulation (see §B5.c.) as a biomarker for predicting antidepressive response to ketamine in adults with TRD.
Hypothesis 1a: Baseline WBC markers of impaired cellular energy regulation will be associated with measures of clinical response to ketamine (predictive biomarker);
Hypothesis 1b: Changes in WBC markers of impaired cellular energy regulation will be associated with clinical response to ketamine (change biomarker).

Aim 2: Examine the effectiveness of other blood-based biomarkers with strong potential for predicting antidepressive response to ketamine in adults with TRD.

For this exploratory aim, we have formed a panel of investigators across multiple sites with experience in the development of biomarkers for severe affective disorders and with the treatment of TRD using IV ketamine (see §B.6., para. 2). These additional biomarkers will be measured using blood samples collected from study participants, and will be correlated with IV ketamine-associated treatment response in order to provide preliminary validation.

Aim 3: Examine the effectiveness of combining blood-based biomarkers for predicting antidepressive response to ketamine in adults with TRD.
A similar approach will be taken to develop additional biomarkers that are of interest to project collaborators.

C. CLINICAL SITES & ORGANIZATIONAL STRUCTURE

C.1. Background

This multi-site biomarker development clinical trial is sponsored by the National Network of Depression Centers (NNDC) and will occur at 6 participating academic centers (clinical sites): Johns Hopkins Hospital, The Mayo Clinic, University of California at San Francisco, University of Michigan, Pine Rest Christian Mental Health Services, and the University of Pennsylvania. The activation of each recruiting site will be on a rolling basis, meaning pre-initiation documentation must be provided to the sponsor before the protocol is implemented at each site. All clinical sites are members of the NNDC, a non-profit consortium of 25 leading clinical and academic Member Centers in the U.S. – including more than 600 interdisciplinary faculty, staff, and trainees – that seeks to integrate innovative research, clinical translation, education, and public policy to better diagnose and treat individuals with depression or bipolar illness and related mood disorders.

Preliminary biomarker development efforts for ketamine antidepressive effects could be carried out at single clinical sites. However, our experience has shown that clinical antidepressive response biomarker studies can be very challenging to conduct in terms of recruitment. Additionally, the variable positive treatment response rates, the multifactorial nature of antidepressive treatment response, and the anticipated heterogeneity of enrolled subjects require that a reasonably large sample be recruited for even preliminary development of robust treatment response biomarkers. As such, the enrollment target of 100 persons with treatment-resistant depression is ambitious but necessary, and the 7-site collaboration for this project is intended to accomplish just that.

C.2. Cross-site scientific and administrative coordination

C.2.a. Global administrative coordination across study sites.

To coordinate efforts across sites, the chief administrative responsibilities for this project will be carried out at two sites (**Fig. 1**). Protocol development and execution, as well as biospecimen storage (all samples) will occur at Mayo Clinic (Rochester, MN). Biomarker analysis (for primary initial aim) will occur at Mayo Clinic (Rochester, MN), and will be further supported at the University of Michigan. Subject enrollment, conductance of the clinical study per this protocol, and data collection will occur at 3 clinical sites:, Johns Hopkins Hospital, Mayo Clinic (Rochester, MN), and University of Michigan. For this protocol version, the sponsor NNDC has discontinued recruitment at site Pine Rest Christian Mental Health Services and reassigned it biomarker development responsibility; the sponsor NNDC has discontinued recruitment at site University of Cincinnati. A site lead investigator at each site will have responsibility over all site-specific aspects of clinical trial conduct, including the collection of biological specimens. However, a centralized database for clinical and biomarker data will be maintained at the University of Michigan. A steering committee composed of the lead investigators from each clinical site, selected sub-investigators, and administrative officers, biostatistics consultants, and The Bio-K Data Safety

Monitoring Board will have project-level oversight of all aspects of study conduct, data analysis, and data presentation and dissemination.

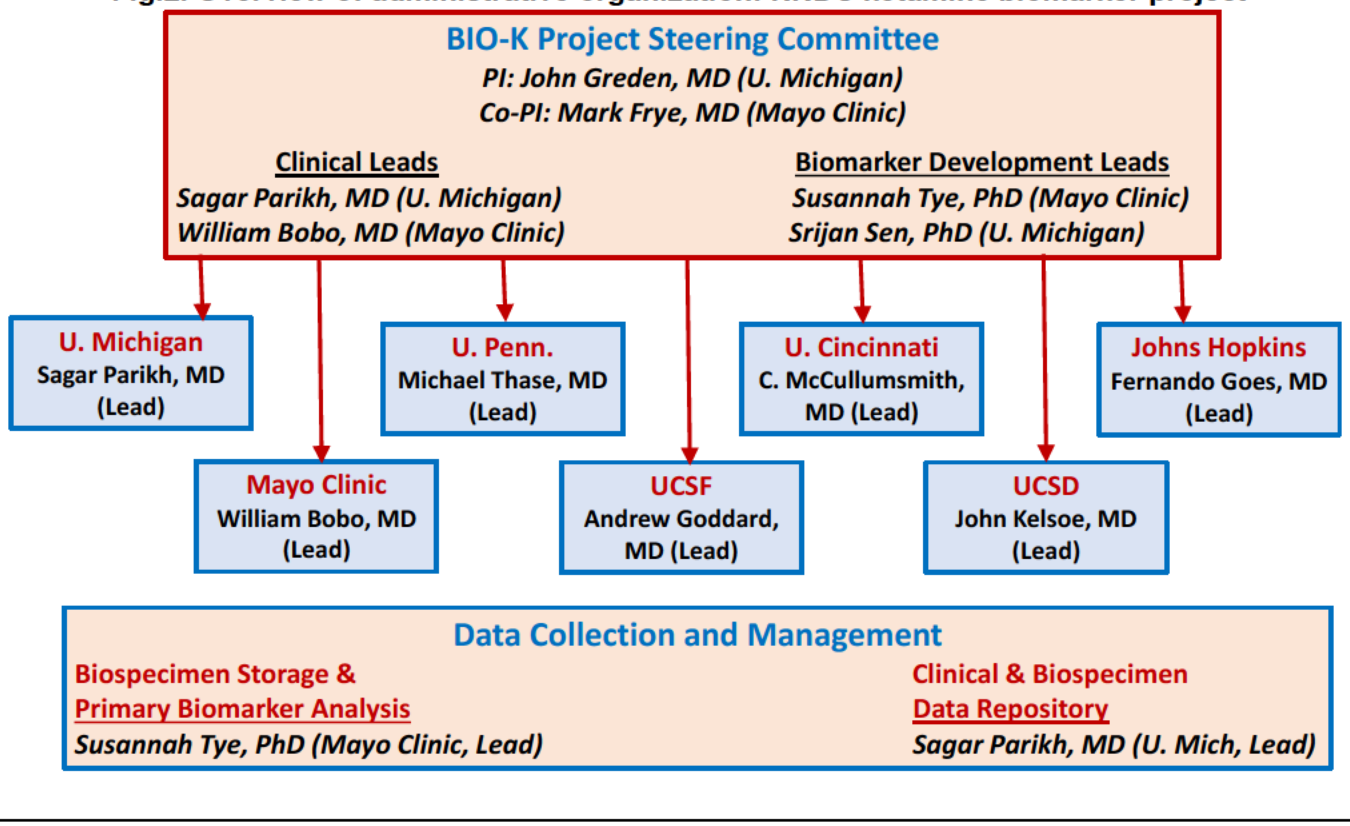
C.2.b. NNDC biomarker development project Samples Sub-Committee.

As noted earlier, a panel of investigators across multiple sites with experience in the development of biomarkers for severe affective disorders and with the treatment of depression using IV ketamine will identify additional specific blood-based biomarkers for predicting antidepressive response to ketamine in adults with treatment-resistant unipolar or bipolar major depression (**Fig. 2**).

C.2.c. Measures Subcommittee.

An additional panel of investigators will finalize the clinical measures for inclusion in the clinical protocol, and determine based on periodic literature review what additional measures may lend themselves to biomarker development (**Fig. 2**).

Fig.2. Overview of administrative organization: NNDC ketamine biomarker project



D. PRELIMINARY STUDIES & PRIOR RELATED RESEARCH

The two main features of this project are the **core clinical protocol** (administration of ketamine, collection of clinical outcomes data) and **biomarker sampling and analysis**. This section of the protocol devoted to PRELIMINARY STUDIES will be divided into two corresponding sections. Section D.1 will describe prior research that supports the effectiveness and safety of the core

clinical protocol for this study. Section D.2 will summarize prior research supporting the WBC marker of impaired cellular energy regulation as a biomarker for predicting antidepressive response to ketamine in adults with TRD.

D.1. Core Clinical Protocol

The feasibility of serial acute (Studies 1 and 2) and continuation phase (Study 2) ketamine infusions, 0.5 mg/kg over 100 minutes was demonstrated in two clinical trials at Mayo Clinic:

Serial Ketamine Infusion Study 1 – twice-weekly acute phase infusions.
Clinical researchers from this NNDC investigative team conducted a pilot study of ketamine infusions administered twice weekly (0.5 mg/kg administered over 100 minutes) to 10 patients with treatment-resistant Major Depression or Bipolar Disorder depression until either remission was achieved or four infusions were given [60]. Patients were followed at weekly intervals for four weeks after completion of the infusions. All subjects received ongoing treatment with oral antidepressive medications. This group demonstrated a rapid reduction in symptoms with a 50% remission rate (50% sustained 1 month after last infusion) and minimal side effects. Cumulative remission rates after 1, 2, and 4 infusions were 10%, 40%, and 50%, respectively. A secondary analysis identified that concurrent benzodiazepines, known to impact NMDA receptor occupancy and functioning, were associated with reduced ketamine response [61]. None of the subjects experienced significant changes in hemodynamic parameters. There were no significant changes from baseline in manic symptoms (as measured by the Young Mania Rating Scale [62]) or psychotic or psychomimetic symptoms (as measured by the Brief Psychiatric Rating Scale total and positive symptom subscale scores [63]). One patient experienced a transient visual hallucination.

Serial Ketamine Infusion Study 2 – thrice-weekly acute phase infusions + 4 weekly continuation phase infusions (acute-phase remitters only).
The same has also safely infused ketamine thrice weekly (0.5 mg/kg administered over 100 min) to 12 inpatients with treatment-resistant Bipolar Disorder depression (n=3) or Major Depression (n=9) who were at high risk for suicide (Patient Health Questionnaire-9 [PHQ-9] item 9 score of 1 or higher, or Suicide Status Form-2 [SSF-2] score >3+ on the item assessing 'overall risk of suicide') [64]. Subjects received pharmacotherapy with antidepressants and/or mood stabilizing medications at the discretion of their primary inpatient clinical teams. None were allowed electroconvulsive therapy or concurrent benzodiazepines on any infusion day. The study included an initial acute phase (thrice-weekly infusions of ketamine, 0.5 mg/kg administered over 100 minutes). Those who remitted during the acute phase entered into a continuation phase, and received 4 weekly IV ketamine infusions (0.5 mg/kg administered over 100 minutes). Five of 12 patients (42%) remitted and 7 of 12 (58%) responded to ketamine treatment during the acute phase. Four of the five remitters did so after the first acute-phase infusion, whereas one additional patient did so after the third acute phase infusion. All 5 acute-phase remitters retained their remission status during the continuation phase.

D.2. Biomarkers

D.2.a. White blood cell (WBC) biomarker for ketamine response

Researchers at one of the NNDC investigative sites, and others, have demonstrated that the antidepressant-like effects of ketamine in animal models of depression are mediated by mammalian target of rapamycin (mTOR) signaling pathways [65,66]. Specifically, investigators demonstrated a strong and highly significant positive correlation between phosphorylation of mTOR and the antidepressant actions of ketamine in an animal model of depression (**Fig. 1**). The same group recently developed an assay for quantification of mTOR in peripheral white blood

cells, ex vivo, and demonstrated that these serve as valid proxy biomarkers for the brain. Specifically, they showed that phosphorylation of these proteins in WBCs directly correlated with the phosphorylation status of the same proteins in prefrontal cortex in our rodent model of treatment-resistant depression, and that these analogous changes in phosphorylation status of mTOR in both brain and peripheral WBCs directly correlated with antidepressive response to ketamine.

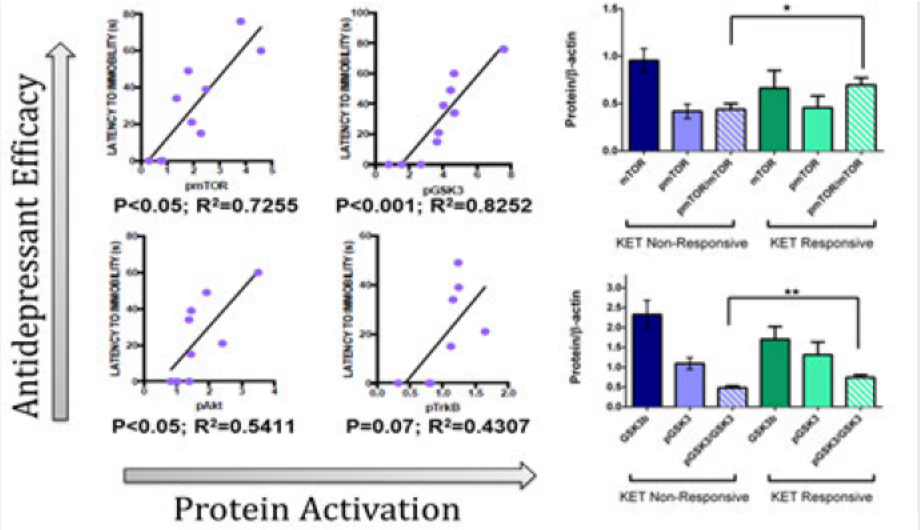


Fig. 1 **LEFT**: Strong, significant positive correlation between levels of phosphorylated Akt, mTOR and GSK3 in the infralimbic cortex and the antidepressant actions of ketamine (latency to immobility) in our preclinical model of treatment resistant depression to ketamine. A positive correlative trend was also observed for TrkB. **RIGHT:** Levels of phosphorylated mTOR and GSK3 were significantly increased in white blood cells of ketamine responders relative to non-responders. Protein levels are expressed as a ratio relative to loading control β-actin. * $p<0.05$; ** $p< 0.01$

D.2.b. Additional biomarkers

As noted earlier, a panel of investigators across multiple sites with experience in the development of biomarkers for severe affective disorders and with the treatment of depression using IV ketamine will identify additional specific blood-based biomarkers for predicting antidepressive response to ketamine in adults with treatment-resistant unipolar or bipolar major depression. The development of these additional biomarkers will be facilitated using the same clinical data and biological samples obtained as part of the proposed core clinical protocol. Therefore, multiple biomarkers will be developed from a single protocol. These are discussed further below.

E. STUDY CONDUCT

E.1. Study Overview

As noted above, the two main components of this study are the core clinical protocol (administration of ketamine, collection of clinical outcomes data) and biomarker sampling and analysis. In broad terms, the core clinical protocol will consist of a multi-site, single-arm, open label clinical trial of 100 adult patients (enrolled at 4 clinical sites) with treatment-resistant MDD or BP given IV infusions of ketamine at subanesthetic doses (0.5 mg/kg, infused over 40 minutes or 100 minutes) (**Fig. 2**). The core clinical protocol will consist of a mandatory acute phase (all clinical sites) and an optional continuation phase (each site may choose to participate or not). During the mandatory **acute phase**, a total of 3 ketamine infusions will be given at least every other day (minimum 1 day between infusions, and a maximum of 4 days between infusions), during a time window of up to 11 days. Biological samples for biomarker development will be obtained before and after each acute phase infusion. Patients who achieve remission of depressive symptoms and suicidal ideation during the acute phase will be eligible for the optional **continuation phase**. For sites that choose to offer continuation phase treatment, patients who respond to or remit (defined below) during the acute phase will receive 4 additional, once weekly continuation phase IV ketamine infusions. Biological specimens will not be collected during the optional continuation phase. At Mayo Clinic-Rochester, these specimens will be collected by the Center for Clinical and Translational Science Clinical Research Unit (CRU), either by mobile staff (inpatients) or at the CRU site (outpatients) where staff will collaborate to prepare infusion logistics, establish venipuncture site, monitor infusion and vital signs during and after infusion (60 minutes).

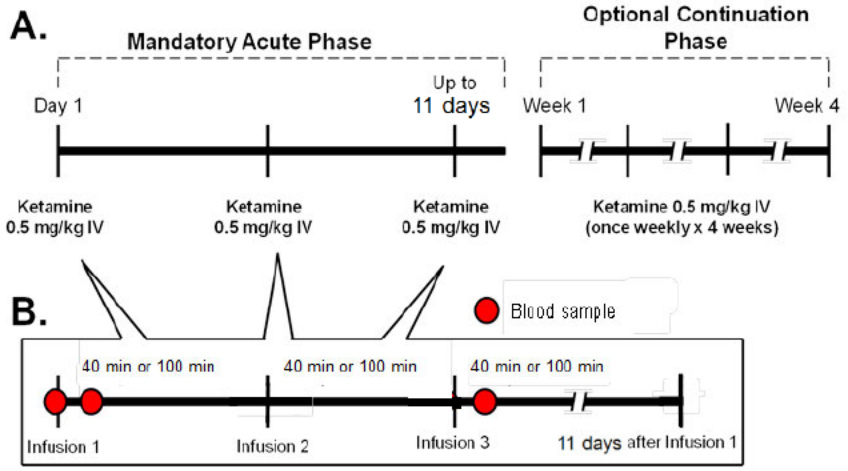


Figure 2 **Study Overview.** The core clinical protocol consists of a mandatory acute phase and an optional continuation phase (Panel A). Up to 11 days are allowed including the first infusion day (Day 1) to administer 3 IV ketamine infusions (0.5 mg/kg, 100 minutes) during the acute phase. Acute phase remitters at specific sites may receive continuation phase infusions. Biological sample collection schedule is summarized in Panel B.

Depressive symptoms, response, remission, suicidality, and global clinical state will be assessed at baseline prior to each ketamine infusion, at the end of infusion (40 or 100 minutes), at the end of post-ketamine infusion observation (60 minutes), and 24 hours after each infusion during the acute and continuation phases, using standardized rating scales. Safety measures will include standardized questionnaires and an open-ended side effect questionnaire. Concerns that arise about worsening suicidality or other psychiatric emergencies will prompt further evaluation by a board certified study psychiatrist, who will determine the need for additional assessment and follow-up.

E.2 Protocol, Protocol Amendments and Pre-Initiation Documentation

Neither the sponsor nor the investigators will modify this protocol without a formal amendment by the sponsor. All protocol amendments must be issued by the sponsor, and signed and dated by the investigator and approved by site IRB/IEC. Protocol amendments must not be implemented without prior IRB/IEC approval, except when necessary to eliminate immediate hazards to the subjects, in which case the amendment must be promptly submitted to the IRB/IEC and relevant competent authority.

Documentation of amendment approval by the investigator and IRB/IEC must be provided to the sponsor. During the course of the study, a situation may arise where a departure from the protocol is necessary and unavoidable. In such an instance, the investigator or appropriate study-site personnel will contact the sponsor. Except in emergency situations, this contact should be made before implementing any departure from the protocol. In all cases, contact with the sponsor must be made as soon as possible to discuss the situation and agree on an appropriate course of action. The data recorded in the CRF and source documents will reflect any departure from the protocol, and the source documents will describe this departure and the circumstances requiring it.

Pre-initiation documentation must be provided to the sponsor before the protocol is implemented at each site. Each site will be provided with a separate document identifying all pre-initiation documents required to be provided to the sponsor, and another pre-enrollment list of documents required by the sponsor before enrollment of first subject.

E.3. Inclusion Criteria

For inclusion in this study, the following are required:

- Ability to provide informed consent;
- Current psychiatric inpatient (voluntary only) or outpatient treatment;
- Male or female;
- Age 18-65 years;
- Meets DSM-5 diagnostic criteria for major depressive disorder, bipolar I disorder, or bipolar II disorder;
- PHQ-9 total score $\geq$ 15 at screening and at baseline (just prior to first acute phase ketamine infusion);
- Treatment-resistant depression, as defined by failure of at least two previous antidepressant or mood stabilizing treatments within the current depressive episode. Failed antidepressant or mood stabilizing treatments can include pharmacotherapy for depression at an adequate dose for at least 8 weeks, or an acute series of at least 6 administrations of electroconvulsive therapy (ECT);
- Ability to pass a comprehension assessment test related to effects of ketamine and trial objectives and criteria.

E.4. Exclusion Criteria

Based on ketamine's known difficulties with induction of perceptual/psychomimetic symptoms, exclusion criteria for this study are as follows:

- Patients with a BMI >40.
- Diagnosis of schizophrenia, schizoaffective disorder, or active psychotic symptoms;
- Ongoing prescription of $\geq$ 4 mg lorazepam equivalents (total) daily, or morning dosing of any benzodiazepine at the time of assessment;
- Currently undergoing ECT, transcranial magnetic stimulation, vagal nerve stimulation, or deep brain stimulation as either an acute or maintenance treatment of depression;
- Any active or unstable medical condition judged by the study psychiatrist as conferring too great a level of medical risk to allow inclusion in the study;

- A cannabis use disorder by DSM-5 criteria in partial remission of less than 3 months will be an exclusion from enrollment. Cannabis use disorders in partial remission of at least 3 months will defer this exclusion.
- Any patient-reported abuse or dependence of cannabis within the prior 3 months from date of screening will be an exclusion.
- Any positive urine toxicology screen as part of the screening visit for illicit, non-prescribed, drug use will be an exclusion.;
- Any current abuse or dependence of alcohol or drugs (excluding nicotine,caffeine, and cannabis). Note: Persons will be allowed to enroll in this study if their drug or alcohol abuse/dependence is in complete (not partial) and sustained (> 1 year) remission;
- Use of any MAOI is prohibited two weeks prior to administration of study drug; if patients are on an MAOI when enrolled, study drug will not be administered until two weeks off MAOI.
- CYP3A4 inducers carbamazepine and modafinil are prohibited two weeks prior to administration of study drug and at least 24 hours after last dose of study drug.
- Current use of Naltrexone.
- History of traumatic brain injury that resulted in loss of consciousness;
- Developmental delay, mental retardation, or intellectual disorder;
- Clinical or self-reported diagnosis of delirium, encephalopathy, or related clinical diagnosis within the prior 12 months;
- Cognitive disorder (mild and major categories per DSM-5);
- Current or prior clinical treatment by ketamine for depression or prior or current participation in another study of ketamine for depression within the prior 6 months.;
- History of either poor antidepressive response to or poor tolerability of ketamine (any route of administration) when previously administered for treating symptoms of depression;
- History of hypothyroidism unless taking a stable dose of thyroid medication and asymptomatic for 6 months;
- Significant unstable medical condition
- Hepatic insufficiency (2.5 X ULN for AST or ALT) within 1 year of consent, past liver transplant recipient, and/or clinical diagnosis of cirrhosis of the liver;
- A diagnosis of Complex Regional Pain Syndrome (CRPS);
- Males active in attempting to conceive a child.
- Pregnancy, or nursing;
- Prisoners;
- Involuntary psychiatric hospitalization.

E.5. Subject Recruitment and Screening

E.5.a. Overview

Subjects will be patients recruited from either outpatient clinics or adult inpatient psychiatric units, and provide voluntary informed consent to participate in the study. Each study site will retain decisional authority as to whether recruitment at their site will occur in outpatient setting, inpatient settings, or both. Patients will be identified for potential inclusion in the study by study coordinators or other study personnel designated by the site lead investigator. Eligible patients will be approached by study staff and the elements of the study will be explained. If the patient meets all inclusion and no exclusion criteria, a site investigator (not providing care for the patient), will further describe the protocol to patient, administer the comprehension assessment test (detailed below), and obtain informed consent. Patients will have the opportunity to have an IRB patient advocate or a significant other present during the consent procedure if desired. One hundred patients will be recruited in the study.

Each site will complete a screening log and subject identification log to facilitate the identification of each subject enrolled and progressed through the study. The subject identification and enrollment log will be treated as confidential, and kept in the master file at each site. All essential documents related to any subject will identify subjects by their assigned identification code and date of birth. For subjects consented and then withdrawn and not having received the first infusion of ketamine, thus no subject identification code assigned, correspondence identifying the subject will include date seen and date of birth. The screening log, made available to all sites as a separate document, reports on all subjects seen by a site investigator to determine eligibility of inclusion in the study.

E.5.b. Comprehension assessment test

A 8-question true or false quiz (comprehension assessment) based on aspects of the study will be administered to each patient, after the co-investigator explains the study, to ensure the patient understands the key concepts, potential side effects of ketamine, and goals of the study. Any incorrect answer will be reviewed by the participant and investigator with an initial by the patient with the answer that has been corrected. For any participant who answers 3 or more questions incorrectly, the study will be postponed with the patient being re-consented within the next 3 days, but no sooner than the following day. If there are still 3 or more questions answered incorrectly, the subject will not be enrolled in the study.

E.5.c. Confirmation of qualifying mood disorder diagnosis

After informed consent, major depressive disorder or bipolar I or bipolar II depression will be confirmed by the study coordinator utilizing the Depression Module of the Structured Clinical Interview for DSM-IV (SCID) [53]. The utilization of the SCID will be to define entry into the study.

E.5.d. Additional screening procedures

Assessments will also be used to confirm severity of depression and suicidal ideation, and will also serve as baseline scores at initial infusion of study drug. The PHQ-9 will be used to define a threshold score of $\geq$15 for enrollment into the study. All qualifying subjects will be administered the Montgomery-Åsberg Depression Scale (MÅDRS) [54] as an additional measure of depressive psychopathology, and the Suicide Status Form (SSF II-R) [55] as a measure of suicide potential. Pregnancy tests will be administered for women < 55 years of age, and urine drug of abuse screens will be reviewed for all participants. The study entry criterion of having failed at least two previous antidepressant or mood stabilizing treatments within the current depressive episode will be assessed using an Antidepressant Treatment History Form (ATHF).

E.5.e. Post-screening visit procedures

Patients enrolled who continue to meet inclusion criteria will be scheduled for their first IV ketamine treatment. At this point, the research pharmacy will be contacted to prepare the ketamine infusion, and the investigator anesthesiologist will be notified of the date and time of the infusion.

E.6. Acute-Phase Administration of Ketamine

All subjects at all sites will be given 3 acute-phase IV ketamine infusions (**Fig. 3**). In the acute phase, the first infusion will be provided at a 40-minute timespan; in considering patient preference information and with investigator consultation for subsequent infusions, subsequent infusions can be provided at either a 40-minute or 100-minute timespan. An IV ketamine drip will be initiated at a rate of 0.5 mg/kg for the 40-minute infusion or 0.3 mg/kg for the 100-minute infusion, for a total dose of 0.5 mg/kg. For example, an enrolled patient that weighs 75kg would receive a dose of 0.938mg/per minute in the 40-minute infusion, and in the 100-minute infusion, would receive a dose of .375 mg/per minute, for a total dose of 37.5mg in either 40-minute or 100-minute infusion. At Mayo Clinic (Rochester, MN), these infusions will be performed either in the

recovery room of the ECT suite for inpatients or at the Center for Clinical and Translational Science Clinical Research Unit (CRU) for outpatient infusions. CRU staff will be key to facilitating the execution of IV infusion procedures. CRU staff will collaborate to prepare infusion logistics, establish venipuncture site, monitor infusion and vital signs during and after infusion (60 minutes). These infusions will be given at least every other day (minimum of 1 day between infusions, and a maximum of 4 days between infusions). All infusions will be completed within a window of 5 to 11 days where the window includes the first infusion day (t_0). The allowable time window is defined in quantitative terms as [t_0, t_0 + 11]. If the final infusion date is designated as t_x, then a given date for t_x is allowable if $t_x < t_0 + 11$.

For example, if the first infusion day was January 11, 2016 (a Monday), the participant could complete their second infusion on January 13, 2016 (Wednesday) and their final infusion on January 15, 2016 (a Friday). Friday's infusion date corresponds to a t_x value of 5, and is allowable. The final 24 hour post-infusion assessment would take place on January 16, 2016 (Saturday).

Alternatively, a participant who receives infusion 1 on a Tuesday could receive infusions 2 and 3 on Thursday during the same calendar week and Monday the following calendar week. In this case, t_x takes a value of 7, which is within the allowable 11-day time window including the first infusion.

E.6.a. Procedures on the day of acute-phase IV ketamine infusions

On the morning of each infusion, the study coordinator (or designated member of the study team) will measure patient's mood using the Montgomery-Åsberg Depression Rating Scale (MÅDRS), the Beck Scale for Suicide Ideation (BSS), the Snaith-Hamilton Pleasure Scale (SHAPS), and the Young Mania Rating Scale (YMRS). The study investigator will complete a Clinical Global Impression (CGI) severity subscale assessment [56]. For Mayo Clinic inpatient infusions, the subject will be escorted to the ECT suite recovery room for IV ketamine infusion. For Mayo Clinic outpatient infusions, the subject will be instructed to check-in at the CRU where the infusion will occur in a private room. The CRU staff will facilitate the execution of IV infusion procedures. CRU staff will set the venipuncture site, proceed with blood draws, monitor infusion and vital signs during and after infusion (60 minutes). For the other 7 sites, the patient will be escorted to the IV ketamine infusion area by the study coordinator, whereupon designated study staff will start the intravenous access. Participants will also be connected to the ECG leads, blood pressure cuff, and pulse oximeter (which measures blood oxygenation non-invasively). These vital signs will also be monitored by the study staff to ensure the participant's safety.

Infusion may be stopped for any marked abnormality in vital signs, defined by floor and ceiling values of the following parameters: heart rate > 130, systolic blood pressure > 160 or < 90, diastolic blood pressure > 95 or < 60, excessive sedation, or development of a heart arrhythmia.

E.6.b. Administration and monitoring of IV ketamine

Table 1 shows the schedule of assessments for this study.

Table 1. Schedule of assessments (acute phase treatment)

		Infusion number 1			Infusion number 2			Infusion number 3		
	Screening	Baseline	40 or 100 min	24h post	Before	40 or 100 min	24h post	Before	40 or 100 min	24h post

SCID	X									
PHQ-9	X	X								
SSF-II	X									
MADRS	X	X	X	X	X	X	X	X	X	X
BSS		X	X	X	X	X	X	X	X	X
SHPS		X		X						X
CGI-S		X			X			X		
CGI-C			X	X		X	X		X	X

SBP	Recorded at baseline, at Q15 minute intervals during infusions, at 30 minutes after end of each infusion, and at 60 minutes after end of each infusion
DBP	
HR & RR	
SaO2	
TEAE	

	Infusion number 1			Infusion number 2			Infusion number 3		
	Baseline	40 or 100 min	24h post	Baseline	40 or 100 min	24h post	Baseline	40 or 100 min	24h post
KSES	For 40 min infusion, recorded at baseline, at 30 min during infusion, at 20 min post infusion stop, at 60 min post infusion stop. For 100 min, recorded at baseline; at 30, 60 and 100 minutes after start of infusion; and 60 minutes post infusion stop.								
YMRS									

RBANS	X								X

Key: BSS = Beck Scale for Suicidal Ideation; CGI-C = Clinical Global Impression, change subscale; CGI-S = Clinical Global Impression, illness severity subscale; DBP = diastolic blood pressure; HR = heart rate; RR = respiratory rate; KSES = ketamine side-effects scale; MADRS = Montgomery-Åsberg Depression Rating Scale; PHQ-9 = 9-item Patient Health Questionnaire; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status; SaO2 = oxygen saturation; SBP = systolic blood pressure; SHPS = Snaith-Hamilton Pleasure Scale; TEAE = open-ended questionnaire to assess for treatment-emergent side-effects; YMRS = Young Mania Rating Scale.

Administration of study drug.

In the acute phase, the first infusion will be provided at a 40-minute timespan; in considering patient preference information and with investigator consultation for subsequent infusions, subsequent infusions can be provided at a 40-minute timespan. An IV ketamine drip will be initiated at a rate of 0.5 mg/kg for the 40-minute infusion or 0.3 mg/kg for the 100-minute infusion, for a total dose of 0.5 mg/kg. For example, an enrolled patient that weighs 75kg would receive a dose of 0.938mg/per minute in the 40-minute infusion, and in the 100-minute infusion, would receive a dose of .375mg/per minute, for a total ketamine dose of 37.5mg in either 40-minute or 100-minute infusion. Any patient preference information for infusion will be considered in consultation with investigator; for example, a patient that describes any side effect as unendurable subject to the 40-minute infusion, may express that they would instead prefer subsequent 100-minute infusions. Any subject enrolled that weighs ≥100kg will be dosed at 50mg maximum of ketamine. This dosage and rate of ketamine infusion are low enough that the presence of an anesthesiologist should not be required, although each site may have the option for an anesthesiologist to be present if desired. Instead, ketamine infusions at this low dose and rate can be administered by nursing staff. A study psychiatrist will be on site during all infusions, and will thus be immediately available as required for clinical ratings and for problems that may arise during the course of infusion. After the 40-minute or 100-minute infusion is completed, the participant's IV access will be discontinued and the participant will be monitored for an additional 60 minutes by study staff and/or other qualified site-specific designees (see §E.5.a). The study psychiatrist will be on site during this 60 minute period following ketamine infusion, as well.

Monitoring of vital signs during administration.
ECG, blood pressure, heart rate, respiratory rate and pulse oximetry will be measured at 15 minute intervals (or more frequently as needed, with values recorded every 15 minutes) from start of infusion. Vital signs will also be recorded 30- and 60 minutes after infusion has stopped. Again, infusion may be stopped for any marked vital abnormality, the parameters being defined as: heart rate > 130, systolic blood pressure > 160 or < 90, diastolic blood pressure > 95 or < 60, excessive sedation, or development of a heart arrhythmia. In the event of a marked vital abnormality resulting in discontinuation of study drug, the site lead investigator will notify the sponsor using the UPIRTSO form provided as a separate document, and must also notify their local IRB/IEC of the event.

Clinical Assessments.
Mood (MÅDRS) and suicide measures (BSS) will be obtained by the study coordinator at baseline, at the end of infusion (40-min or 100-min); and at 24 hours post-infusion time. Thus, there will be three clinical outcome assessment times for these measures per infusion. The Snaith-Hamilton Pleasure Scale (SHAPS) will be administered by the study coordinator at baseline, 24 hours following the first infusion, and 24 hours following the third (final) acute phase infusion. An investigator will complete a CGI-Severity of Illness (CGI-S) assessment at baseline before each infusion, and an investigator will complete a CGI-Global Improvement (CGI-C) assessment at the end of each infusion, and at 24 hours post-infusion for each infusion. See §E.8 for description of assessment measures.

E.6.c. Post-ketamine administration procedures
Inpatients will be escorted back to their inpatient unit by the study coordinator. Participants receiving IV ketamine infusions as outpatients must either have a known responsible adult that will stay for the entirety of the study visit, or have a known responsible adult that will coordinate with research staff the approximate pickup time and an on-campus location where they will pick-up the patient; upon discharge, a member of the research team may escort patient to the pick-up site determined by the participant to meet their known responsible adult.

After each infusion, investigators will complete a procedure note describing (a) all physical symptoms experienced during and through end of infusion, (b) vital signs taken immediately before start of infusion (BP, SpO2, RR and Pulse), and (c) vital signs taken at infusion stop time, and at 30- and 60- minutes after end of infusion.

Whether patient withdraws from study or completes the course of study process, study teams will correspond with inpatient clinical teams (for subjects who are inpatients) or referring clinicians as applicable to each patient at each study site. At minimum, this communication must specify: (a) Participant has withdrawn or completed study process and is no longer participating in this study, and (b) As a result of no longer participating in this study, patient should continue to receive the standard level of care respective to the patient's diagnosis.

Regardless of whether patient withdraws from study or completes the course of study process, the participant will be offered one optional research follow-up visit by the study coordinator or designated study staff. The research follow-up visit will occur within 5 working days following the 24-hour follow-up of the participant's last infusion (infusions 1 or 2 for those who do not complete the acute phase, or infusion 3 for those who complete the acute phase). For participants who enter into the optional continuation phase of the study (see §E.7), the research follow-up visit will occur 5 working days following the 24-hour follow-up of the subject's final continuation phase infusion.

Qualitative Study (Talk-K):

Each recruiting site will contact participants that were enrolled in the Bio-K study. Participants will be contacted 12-36 months after their last infusion and invited to answer additional questions related to their experience in the ketamine trial as well as follow-up information since their last study treatment. Research staff from the University of Michigan will be conducting the research that includes informed consent, a 5-minute questionnaire and 45-60 minute interview. Answering these additional qualitative questions will help providers better understand the participant's experience and improve future research.

The 5-minute questionnaire will ask about their mood since treatment and their current status. The interview will be conducted using a HIPAA compliant and secure Zoom video conference method that participants can join by phone or a website link. These interviews will be recorded, transcribed and then the recording will be destroyed. The interview will ask open-ended questions about their experience during the IV ketamine treatments and since the last study treatment.

E.6.d. Hospitalized patients who are discharged prior to study completion

For hospitalized participants, if dismissed from the hospital before completing all 3 acute phase infusions, then remaining infusions will be conducted on an outpatient basis. Specific procedures will be site-specific.

The patients in need of receiving outpatient infusions will be scheduled per the standard process at each site, at which point they will be greeted by the study coordinator and the process from there will be identical to that for inpatients. The 1-2 hours post-infusion outcome measures, as well as the 24-hour post infusion assessments, will be conducted in the study coordinator's office or by telephone. When receiving infusions as outpatient, Site Lead Investigator or Site Co-Investigator must evaluate the patient before patient discharges from outpatient infusion visit, and discharge will occur only when all physical symptoms are completely resolved. Evaluation must include review of vitals for possible side effects of study drug. For additional patient safety, patient must either have a known responsible adult that will stay for the entirety of the study visit, or have a known responsible adult that will coordinate with research staff the approximate pickup time and an on-campus location where they will pick-up the patient; upon discharge, a member of the research team may escort patient to the pick-up site determined by the participant to meet their known responsible adult.

E.6.e. Treatment as usual

All enrolled subjects will receive treatment as usual while participating in this protocol, at the discretion of study clinicians. This can include psychotherapy, other forms of psychosocial treatment, and pharmacotherapy, as appropriate. The only exceptions will be ECT, TMS, or DBS, which will not be allowed during participation in this protocol. Further restrictions are any ongoing prescription of ≥4mg Lorazepam equivalents total daily on date of infusion, or any morning dosing of any benzodiazepine at time of assessment on date of infusion.

E.7. Biomarker Sampling

E.7.a. Overview and timeline

Recall that our initial biomarker development efforts will focus on a peripheral WBC marker of mTOR/GSK3 target engagement; however, additional sample will be taken to develop additional biomarkers as part of future work. A list of additional specific biomarkers is provided below in **Table 2**. For all 100 subjects, there are biomarker blood draws and serum collections at the following time points, collected by CRU staff.

- Before and after the first acute phase ketamine infusion;
- After the third (final) acute phase ketamine infusion;

Table 2. Summary of biomarker sampling, by type of biological specimen

Biomarker	Sample type	When collected	Processing details
mTOR/GSK3 (primary)	Whole blood (WBCs)	Before infusion 1, End infusion 1, End infusion 3	Sodium heparin tube Keep room temp. Ship overnight to Mayo
DNA/GWAS	Whole blood (DNA)	Before infusion 1	Paxtube, frozen -80 C
RNA/RNAseq	Whole blood (RNA)	Before infusion 1, End infusion 3	Paxtube, frozen -80 C
Telomere length	Whole blood (WBCs)	Before infusion 1, End infusion 1, End infusion 3	DNA prep from whole blood, frozen -80 C
Telomerase	Whole blood (WBCs)	Before infusion 1, End infusion 1, End infusion 3	WBCs lysed in CHAPS buffer, frozen -80 C
Ketamine metabolites	Plasma	End infusion 1, End infusion 3	Plasma, frozen -80 C
Cytokines + CRP	Plasma	Before infusion 1, End infusion 1, End infusion 3	
F2-isoprostanes	Plasma	Before infusion 1, End infusion 1, End infusion 3	
KYN, QUIN acids	Plasma	Before infusion 1, End infusion 1, and infusion 3	Plasma, frozen -80 C
Orexin A	Plasma	Before infusion 1, End infusion 1, End infusion 3	Plasma, frozen -80 C
Cortisol, DHEA, DHEAS	Serum	Before infusion 1, End infusion 1, End infusion 3	Serum, frozen -80 C
BDNF	Serum	Before infusion 1, End infusion 1, End infusion 3	Serum, frozen -80 C

E.7.b. Biomarker samples (core biomarker development)

Biomarker sampling and initial processing details are provided in this section. As shown above, there are three general types of blood samples that we will take. These samples will be drawn according to the schedules presented above in **Table 2**, and will be aliquoted as shown below in

Table 3. Blood sample aliquot and preparation summary

Sample aliquot	Tube	Volume (per time point)
Whole blood (DNA/other)	ACD yellow	10 mL
Whole blood (RNA)	PAXgene tube	10 mL
Whole blood (PMBCs)	Sodium heparin	30 mL
Plasma	EDTA (Lavender)	20 mL
Serum	SST (Red)	10 mL

In sum, this equates to a total of 80 mL of blood drawn per sampling. Over the 11 day duration of acute phase ketamine treatment, this equates to a total of 160 mL of blood drawn on treatment day 1 (one blood drawing before and one at the end of the first ketamine infusion), and an additional 80 mL of blood drawn after the third (final) acute phase ketamine infusion.

Heparin tube samples will be placed on ice and centrifuged cold (at 4 degrees C) within 30-45 minutes of being drawn. The samples will be frozen immediately after aliquoting at –80 degrees C. None of the samples will be drawn in the fasting state.

The samples will be shipped to Mayo Clinic for storage. A standard operating procedure for biomarker procurement and shipment to the Mayo Clinic Depression Center Neuroscience

Translational Laboratory (Attention Dr. Sue Tye) has been developed (see supplement instructions for preparing the plasma and buffy coat samples, and shipping instructions). Biomarker analysis (for primary initial aim) will occur at Mayo Clinic (Rochester, MN), and will be further supported at the University of Michigan. As the occasion arises, some samples might be shipped to other study sites for processing.

E.7.c. Biomarker sampling procedures for mTOR/GSK3 target engagement biomarker
For the primary biomarker, 10 mL blood will be collected before the first ketamine infusion, and the end of the first infusion, and at the end of the third (final) infusion. At each time point, 10 mL will be collected into a heparinized tube. The 10cc heparinized tube sample will be mixed with an equivalent amount of phosphate buffered saline (PBS). This mixture will be slowly overlaid onto Ficoll, the mononuclear layer recovered, and cells washed with PBS. Mononuclear cells will then be viably frozen for cells to be cultured for in vitro biomarker assays. All tubes will be labeled with a study identifier, collection date, and time of draw. As the occasion arises, some samples for mTOR/GSK3 target engagement biomarker might be shipped to the University of Michigan for processing.

E.8. Optional Continuation Phase

E.7.a. Overview of continuation phase
Each study site will have the option of determining if they will offer an optional continuation phase of the study for persons who achieve remission during the acute phase of the study. Remission will be defined as a MÅDRS total score of ≤9 at 24 hours following acute phase infusion #3. Participants who meet this criterion for remission will then be scheduled for the first of four weekly continuation phase ketamine infusions (Figure 3).

E.8.b. Scheduling of continuation phase study visits and post ketamine follow-up
The first continuation phase infusion can occur 4-7 days from the date of acute phase infusion #3. The remaining continuation phase infusions can occur 6-8 days apart.

E.8.c. Continuation phase procedures
The procedures for ketamine administration and monitoring are the same as for acute phase infusions. Therefore, procedures defined in §E.5.a., §E.5.b., and §E.5.c for the mandatory acute phase will also apply to the optional continuation phase. Continuation phase participants may receive only up to 4 continuation phase infusions of ketamine.

The schedule of assessments will proceed as shown in **Table 4** for the 4 weekly optional continuation phase IV ketamine infusions.

Table 4. Schedule of assessments (continuation phase)

	Infusion 1			Infusion 2			Infusion 3			Infusion 4		
	Baseline	40 or 100 min	24h post	Before	40 or 100 min	24h post	Before	40 or 100 min	24h post	Baseline	40 or 100 min	24h post
PHQ-9	X	X	X	X	X	X	X	X	X	X	X	X
MADRS	X	X	X	X	X	X	X	X	X	X	X	X
BSS	X	X	X	X	X	X	X	X	X	X	X	X
SHPS	X		X									X
CGI-S	X			X			X			X		
CGI-C		X	X		X	X		X	X		X	X
SBP DBP HR & RR	Recorded at baseline, at Q15 minute intervals during infusions, at 30 minutes after end of each infusion, and at 60 minutes after end of each infusion											

SaO2 TEAE												
KSES YMRS	For 40 min infusion, recorded at baseline, at 30 min during infusion, at 20 min post infusion stop, at 60 min post infusion stop. For 100 min, recorded at baseline; at 30, 60 and 100 minutes after start of infusion; and 60 minutes post infusion stop.											
RBANS												X

Key: BSS = Beck Scale for Suicidal Ideation; CGI-C = Clinical Global Impression, change subscale; CGI-S = Clinical Global Impression, illness severity subscale; DBP = diastolic blood pressure; HR = heart rate, RR = respiratory rate; KSES = ketamine side-effects scale; MADRS = Montgomery-Åsberg Depression Rating Scale; PHQ-9 = 9-item Patient Health Questionnaire; SaO2 = oxygen saturation; SBP = systolic blood pressure; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status; SHPS = Snaith-Hamilton Pleasure Scale; TEAE = open-ended questionnaire to assess for treatment-emergent side-effects; YMRS = Young Mania Rating Scale.

E.9. Measures

- **9-item Patient Health Questionnaire (PHQ-9):** A self -report screening measure of depressive symptoms, directly correlating with depression symptoms used for diagnosis and widely used in all care settings by all medical specialties [64]. Used only as initial screening into study.
- **Depression Module of the SCID:** The Structured Clinical Interview for DSM-IV is a diagnostic exam used in this case to determine depressive disorder diagnoses [67]. The SCID is thus performed to define entry into study.
- **Montgomery Åsberg Depression Rating Scale (MÅDRS):** A 10-item clinician rating of depressive symptoms used in the vast majority of previous ketamine research in treatment resistant depression. Each item is scored on a 7-point scale (0 to 6) (range 0–60). Anchors are provided for even numbered scale points. Higher scores represent higher levels of depression. Its psychometric properties have been studied extensively in adults [68]. The MÅDRS in adults has high interrater reliability, with estimates as high as 0.97 between a psychiatrist and general practitioner [18]. Correlation between each item and total score ranges between 0.12 (reduced appetite) and 0.84 (apparent sadness). The MADRS correlates with global ratings (0.70) and the HRSD17 (0.59). The MÅDRS has established scores for varying levels of symptom severity. Scores of 44, 32, 23, 15, and 7 have been suggested to designate very severe, severe, moderate, mild, and recovered depressions, respectively [19]. The scale is unidimensional and not confounded by somatic or psychomotor symptoms [20, 21]. While the MÅDRS does not specify a time frame for rating the symptoms, it is usually used to assess the prior week. Previous analysis has assessed remission scores for depression as ≤9 using the MÅDRS. Used repeatedly as a measure of depression response and remission criteria [73].
- **Beck Scale for Suicidal Ideation (BSS):** The Beck Scale for Suicidal Ideation consists of 19 items which can be used to evaluate a patient's suicidal intentions [69]. It can also be used to monitor a patient's response to interventions over time. Each of the 19 items is rated on a 0-3 point scale (range 0-38, with higher scores indicating greater suicidal ideations or risk), and includes specific items that assess wish to live, wish to die, desire to make an active suicide attempt, passive suicidal desire, duration of suicidal ideations, frequency of suicidal ideations, and subjective level of control over suicidal actions.
- **Suicide Status Form (SSF II-R):** Brief self-report survey by patients of overall suicide risk and subcategories associated with theoretical models of suicide. The data for all aspects—both qualitative and quantitative—will be assessed. Used repeatedly as a measure of suicidal crisis response.
- **Young Mania Rating Scale (YMRS):** The YMRS is an 11-item observer-rating scale assessing symptoms of mania [62]. This scale measures a broad range of manic

symptoms and has been shown to be sensitive to therapeutic effects. This scale will be used at each rating visit to assess for treatment emergent (hypo)mania. Hypomania will be defined as an YMRS >12 and mania will be defined as an YMRS ≥20.

- **Clinical Global Impression (CGI):** Overall clinical judgment symptom severity (I) and change for a preceding phase (II). Dropout criteria will be any 1 rating of CGI II – 5 (much worse) or 6 (very much worse) [70].
- **Open-ended assessment of perceptual changes with ketamine treatment:** Patients will be asked open ended questions related to whether they noticed any visual hallucinations, dizziness, vertigo, or any strange feelings or thoughts.
- **Comprehension of study protocol assessment:** Test to assess understanding of purpose of study given to patients as part of entry process.
- **Antidepressant Treatment History Form (ATHF):** This form will be used at time of enrollment to collect pertinent information on past and present anti-depressant trials, including dosage, length of use, and treatment resistance.
- **Snaith-Hamilton Pleasure Scale (SHPS):** This questionnaire is designed to measure the ability to experience pleasure in the last few days [71]. Used repeatedly to score for anhedonia.
- **Ketamine Side Effects Scale**: This scale was developed to measure side effects to ketamine when administered for antidepressive effects [72].
- **Repeatable Battery for the Assessment of Neuropsychological Status**: The RBANS is a brief, standardized, individually administered battery to measure cognitive change in immediate memory, visuospatial/constructional orientation, language (naming, fluency), attention, and delayed memory. The 12 subtests that comprise the RBANS requires approximately 30 minutes to administer.

E.10. Case Report Forms, Data Capture and Source Documentation

Case report forms are provided for each subject in electronic format. Research Electronic Data Capture (REDCap), developed at Vanderbilt University but widely used internationally, is a secure, web-based application designed to support data capture for research studies. REDCap provides: 1) an intuitive interface for data entry (with data validation); 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages (SPSS, SAS, STATA, R); 4) procedures for importing data from external sources; and 5) advanced features, such as branching logic and calculated fields within electronic forms and the survey feature. Data will be uploaded securely from each research site via REDCap.

Electronic source documentation must be made available to confirm data collected properly. In the event that a paper version CRF was used as the primary medium for data collection at any study site, and subsequently entered into REDCap, the paper CRF will serve as the source document. Source document data includes author initials and date of entry to source documentation. Also, the necessary fields for source documentation review are subject identification code, eligibility, study identification, study discussion and date of signed informed consent, dates of visits, record of all UPIRTSO and non-UPIRTSO and results of reporting, concomitant medications, drug order and dispensing records (de-identified), study drug administration information, date subject completed or discontinued study, date and reason study drug was discontinued early, if applicable. Subject measures completed by subject, investigator or subject personnel will also be considered source documents. It will be the responsibility of the investigator to verify that all data entries in the CRFs are accurate and correct.

E.11. Data Safety Monitoring Plan and Board

Monitoring responsibilities for this study will be assigned to investigators Dr. William Bobo (Mayo Clinic), Dr. Sagar Parikh (University of Michigan), Jose Rico, CCRP (Mayo Clinic) or appropriate designee determined by the sponsor. Neither investigator will serve to monitor their own site. Three on-site monitoring visits will be made: pre-initiation, post-initiation and a close-out visit at each site. In addition, either of the investigator monitors will perform the on-site monitoring visits as frequently as necessary. The designated investigator monitor will record dates of the site visits in a study site visit log that will be kept at the study site. The post-initiation visit will be scheduled after enrollment of the 3rd subject has occurred at each site and the close-out visit will occur once recruitment is completed.

At these visits, the investigator monitor will compare the entered CRF data with the source documents. The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the CRF are known to the investigator monitor and study-site personnel, and are accessible for verification by the investigator monitor and study-site personnel. If electronic records are maintained at the study site, the method of verification must be discussed with the investigator monitor and a report will be generated.

Investigator monitor visit expectations include the availability of the study-site personnel, accessible source documentation, and a suitable environment for review of study-related documents. The investigator monitor will meet or correspond with the Site Lead Investigator during the study to provide feedback on the study conduct.

The Bio-K Data Safety Monitoring Board (DSMB) shall have the responsibility to review on a scheduled basis accumulating data and monitoring reports from all sites. The DSMB advises the sponsor NNDC regarding the continuing safety of trial subjects and potential subjects, and the continuing validity and scientific merit of The Bio-K Study.

E.12. Record Retention

In compliance with the ICH/GCP guidelines, the sponsor, site lead investigator and site institution will maintain all Essential Documents (CRFs and source documents related to each study subject) as well as all study documents as specified in ICH/GCP Section 8 – Essential Documents for the Conduct of a Clinical Trial of Guideline for Good Clinical Practice E6: ICH Harmonised Tripartite Guideline. The site lead investigator and institution will take appropriate measures to prevent misplacement, accidental or premature destruction of study documents. The essential documents are grouped in three sections according to the stage of the trial during which they will normally be generated: 1) before the clinical phase of the trial commences, 2) during the clinical conduct of the trial, and 3) after completion or termination of the trial. Together, these essential documents constitute a trial master file to be established with the sponsor and at each site before it begins the clinical phase of the trial.

Essential documents must be retained until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product related to this study. These documents will be retained for a longer period if required by local IRB/IEC or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

In the event that the responsible site lead investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The sponsor must be notified in writing of the name and address of the new custodian at that site. Under no circumstance shall the investigator relocate or dispose of any study documents before having obtained written approval from the sponsor.

If it becomes necessary for the sponsor or the appropriate regulatory authority to review any documentation relating to this study, the site lead investigator/institution must permit access to such reports.

E.13. Study Completion and Termination

The study is considered completed with enrollment of the 100th subject. The final data from the study site will be sent to the sponsor after completion of the final subject assessment at that study site.

The sponsor reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is deemed in closed status when all required study documents have been collected and a study-site closure visit has been performed by the designated investigator monitor.

The sponsor or site investigator monitor may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination. Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to failure of the investigator to comply with the protocol, the requirements of the IRB or local health authorities, the sponsor's procedures, or GCP guidelines, and inadequate recruitment of subjects by the investigator

E.14. Reportable Event – Reporting to Sponsor and Site IRB/IEC

Adverse Effects.

Patients will be asked open ended questions about any adverse perceptual effects of ketamine during the infusion, such as visual hallucinations, dizziness, or vertigo, every 15 minutes during infusions and at 30- and 60 minutes after each infusion. Infusions will be stopped if side effects seem severe or if the patient wishes it to be stopped or infusion may also be stopped for any marked vital abnormality, as noted above. Side effects will also be monitored utilizing the Ketamine Side Effects Scale (KSES) [58] and the YMRS. The KSES and YMRS will be completed for the 40 min infusion, recorded at baseline, at 30 min during infusion, at 20 min post infusion stop (t60), at 60 min post infusion stop (t100). For the 100 min infusion, KSES and YMRS will be recorded at baseline; at 30, 60 and 100 minutes after start of infusion; and 60 minutes post infusion stop. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) is administered in the acute phase at baseline (prior to the first ketamine infusion) and at 24 hours following the 3rd acute phase infusion, and in the optional continuous phase, at 24 hours after the 4th continuous phase infusion, to assess cognitive effects. See §E.8 for description of assessment measures.

The sponsor has established the Standard Operating Procedure in conformity with regulatory requirements to ensure appropriate reporting of Unanticipated Problem Involving Risk to Subjects or Others (UPIRTSO) and non-UPIRTSO events to the sponsor and local IRB/IEC. Including notification to local IRB/IEC of an UPIRTSO or non-UPIRTSO, each site is provided as a separate document the names and telephone numbers of individuals who should be contacted regarding safety issues and/or with questions regarding the study. In addition, each site will be provided an UPIRTSO form to be submitted to the sponsor describing the event, subject identification code, subject date of birth, and date seen.

An UPIRTSO is defined as any problem or event which, in the opinion of the local Investigator, meets all three of the following criteria:

1. Serious: Serious problems or events that result in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or places subjects or others at a greater risk of harm than was previously known or recognized..

2. Unanticipated: A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence and is:
    a. Not already described as a potential risk in the approved informed consent
    b. Not already described as a potential risk in the approved protocol
    c. Not listed in the Investigator's Brochure
    d. Not part of an underlying disease
    e. Occurred at an increased frequency or at an increased severity than expected,
3. Related: A problem or event is "related" if it is possibly related to the research procedures.

An UPIRTSO can include but isn't limited to adverse drug reactions (both serious and unexpected), adverse events, serious adverse events, protocol deviations and protocol violations occurring in this study meeting the criteria that the event was serious, unanticipated and related to study procedures. Reporting to sponsor and local IRB/IEC should occur as soon as possible and in accordance with local IRB/IEC reporting guidelines. In contrast, a non-UPIRTSO, although still reportable, is defined as a reportable event that does not meet all three criteria in the definition of an UPIRTSO.

An Adverse Drug Reaction is defined as an unexpected, unintended, undesired, or excessive response by a patient to a medication that could not have been prevented and having one or more of the following characteristics:

- Requires hospitalization
- Prolongs length of stay
- Requires use of antidote(s), prescription drug therapy, extra diagnostic activities, or other corrective therapeutic measures (e.g. monitoring, discontinue medication)
- Fatality or permanent disability
- Significantly complicates diagnosis
- Negatively affect prognosis
- Medication incompatibility not previously identified
- A serious reaction not listed on the product package insert

An Adverse Event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.
A Serious Adverse Event is any untoward medical occurrence that at any dose results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, a congenital anomaly/birth defect.

A Protocol Violation is defined as any change, divergence or departure from the study design or research procedures that is under the investigator's control and has not been approved by the local IRB/IEC. A Major Protocol Violation/Deviation is defined as any change that affects the rights and welfare of subjects and others, increases risks to subjects and others, decreases potential benefits, compromises the integrity or validity of the research, or represents willful or knowing misconduct to include not limited to enrolling subjects who did not meet the inclusion/exclusion criteria, performing study procedures not approved by the IRB, failure to obtain and/or document informed consent, and receipt of incorrect treatment or dose by a study subject. A Minor Protocol Violation/Deviation is any change that did not increase the risk or decrease the benefit or significantly affect the subject's rights, safety or welfare and/or the integrity of research data (e.g. a routine lab missed at a visit and re-drawn, shortening the duration between a planned study visit, using an outdated HIPAA form or consent form when there are no differences between the two forms other than the approval date).

A Protocol Deviation is defined as a limited prospective exception to the protocol (e.g. agreement between sponsor and investigator to enroll a single subject who does not meet all inclusion/exclusion criteria). Like protocol amendments, deviations initiated by the clinical investigator must be reviewed and approved by the local IRB/IEC and the sponsor prior to implementation, unless the change is necessary to eliminate apparent immediate hazards to the human subjects (21 CFR 312.66), or to protect the life or physical well-being of the subject (21 CFR 812.35(a) (2)).

E.15. On-Site Audits

At any time, representatives of the sponsor's steering committee may schedule a visit of a study site during or after completion of the study. Purpose of such a visit is to conduct an audit of the study for ensuring compliance with regulatory guidelines and subject privacy. Such an audit visit would require access to all study records, including source documents, for review and comparison to the CRFs. The site lead investigator and study-site personnel would need to be made available for query during each audit visit conducted by the sponsor. Similar auditing procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The site investigator should immediately notify the sponsor if contacted by a regulatory agency concerning an upcoming inspection.

E.16. Statistical Analysis

The sample size of 100 has been selected based on feasibility of completion across the 7 study sites, and as larger pilot expansion of clinical practice feasibility. The intent is to enroll 100 subjects (70 unipolar/ 30 bipolar) over 4 sites which would estimate 25 subjects per site. Given potential delays in obtaining IRB approval, some sites may over-recruit. For most data elements, descriptive statistics such as means and standard deviations will be reported. Comparisons of means before and after treatment will be based on *t* tests. Statistical support will be provided from designated psychiatry and psychology department statistician time.

For Aim 1, we will compare baseline WBC markers of impaired cellular energy regulation (described above, see §B.5.c) between remitters and non-remitters using ANCOVA models adjusted for study site, age, and sex. Analyses comparing the baseline biomarkers of responders and non-responders will be performed using similar models, and the association of biomarkers with percent change in MADRS score will be evaluated using linear regression mixed models.

For Aim 2, similar analyses will be performed comparing the other biomarker measures between remitters and non-remitters (and responders and non-responders), and testing for association between percent change in MADRS score and the other biomarker measures.

For Aim 3, we will perform logistic regression analyses to evaluate the additive effects of WBC markers of impaired cellular energy regulation and the other biomarker measures, as predictors of remission (or response). Model selection will be performed using LASSO, and area under the receiver operating characteristic curve (AUC) will be used to evaluate the combined predictive potential of the WBC-based and other biomarkers.

Additionally, we will evaluate whether changes in the biomarker levels (for both WBC-based biomarkers and other biomarkers) are associated with clinical response to ketamine, using similar statistical methods to those described for the analysis of baseline biomarkers. Finally, to supplement the traditional regression-based approaches, statistical learning classification approaches, including random forests, will be utilized to predict remission (and response) using the different biomarkers and clinical predictors. These exploratory analyses will develop predictive models that will need to be evaluated in independent samples collected in future studies.

E.17. Suicide Assessment and Safety Plan

This research study specifically is targeted at identifying participants with treatment resistant unipolar or bipolar major depression, a population of patients who are at high risk for having thoughts of suicide, and may be at high risk for suicide or attempted suicide in the future. Ketamine treatment has been shown to result in a rapid resolution of suicidality and suicide rating scores in previous studies.

Suicide risk will be assessed in multiple domains both with brief surveys filled out by patient along with clinical interview and assessment. Patients will continue with their usual clinical assessment of suicide risk from their inpatient or outpatient care teams and will continue with treatment as usual related to clinical care.

Suicide specific items in outcomes measures include suicide ratings measured by the MADRS and the PHQ-9. For more extensive assessment and analysis, the BSS will also be used to determine immediate changes. Finally, patients will be asked in an open-ended manner if they have concerns related to their safety.

While patients are hospitalized, concerns will be relayed to their primary inpatient team which provides coverage seven days a week, 24 hours a day, and provides monitoring on inpatient hospital in the same manner. Patients will continue to receive their usual care and a safe discharge from the hospital and resolution of patient's acute suicidal crisis will be determined by patient's primary clinical team and not research staff.

If there are concerns raised that are new related to suicide risk assessed by investigator/research assistants while patients are in the hospital, this change in status will be communicated to the inpatient care team. If the patient is an outpatient, this will be communicated to their treating psychiatrist or home medical team.

For patients who are assessed by study coordinator during a repeat visit to have new onset or worsening of suicidal ideation, the study coordinator will contact the site lead investigator at their clinical site or a board certified psychiatrist study team member. This individual will assess patient and make determination of whether the patient needs more in depth assessment, closer outpatient follow-up with their team, or emergent hospitalization. This will also apply if patients are having a worsening of depressive symptoms without increase in thoughts of suicide. In such an event, study site will inform the sponsor of the event of repeat or new onset or worsening of suicidal ideation using the UPIRTSO form provided as a separate document, and must also notify their local IRB/IEC of the event.

Outside the hospital as outpatients, subjects will be given listings of numbers to call if there are concerns in between assessments by research coordinators. These numbers will include study coordinator, local Emergency Department contact information, and the National Suicide Prevention Lifeline at 1-800-273-TALK.

E.18. Dropout Criteria and Pregnancy

Dropout criteria will be any 1 rating of CGI II change in severity score of either 5 (much worse) or 6 (very much worse). Dropout criteria will also include inability to tolerate ketamine treatment as defined by patient and/or treating clinician or other co-investigators. Inability to tolerate treatment may include significant adverse effects during infusion (physiologic, emotional or cognitive) or emergence of worsening psychotic or perceptual changes or mania. Clinical assessment by primary psychiatrist of worsening of symptoms may also constitute reason for study dropout. Subjects may elect to dropout of study.

An existing pregnancy will serve to exclude pregnant patients from enrolling in the study. Further, any initial reports of pregnancy occurring during the study must be reported to the sponsor by the study-site personnel within 24 hours of their knowledge of the event. Each site will be provided with a pregnancy notification form. Abnormal pregnancy outcomes (eg, spontaneous abortion, stillbirth, and congenital anomaly) are considered serious adverse events and must be reported using the provided UPIRTSO form. Any subject who becomes pregnant during the study must be promptly withdrawn from the study and discontinue further study treatment. In addition to submitting an UPIRTSO form to sponsor, local IRB/IEC must notified per established IRB/IEC procedures.

Because the effect of the study drug on sperm is unknown, pregnancies in partners of male subjects included in the study will be reported by the study-site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy notification form to the sponsor. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

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