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II. TITLE:

Examining the Impact of Sirolimus on Ketamine's Antidepressant Effects

III. PURPOSE:

Ketamine is an anesthetic agent, recently found to exert a rapid and potent antidepressant effect in severely depressed patients. Controversy exists in the field regarding how ketamine works in the brain and thus what underlies its rapid and profound antidepressant effects. A better understanding of the mechanism of the neuropsychiatric effects of ketamine are critically important, as pharmacological modulation of these effects can assist in the development of novel medications for the treatment of multiple psychiatric disorders including depression and schizophrenia.

The aim of the study is to provide insight into the impact of the immunosuppressant drug sirolimus (also known as rapamycin; known to have impact on depressive symptoms from molecular and preclinical studies), on the antidepressant effects of the prototypal rapid-acting antidepressant medication, ketamine. More specifically, the study will examine whether sirolimus shows significant interactive effects (i.e., synergy or antagonism) with ketamine in participants with treatment resistant depression as measured by the use of the clinician-administered Montgomery Asberg Depression Rating Scale (MADRS). We will also test the tolerability of the combined ketamine and sirolimus treatment. Secondary outcome measures are designed to measure the effects of sirolimus on the tolerability of ketamine.

- **Aim 1:** To characterize the effects of a single dose of sirolimus prior to a low dose of ketamine in subjects with antidepressant-resistant depressive symptoms.
Design: 30 participants will be randomized into one of two groups at Time/Infusion 1 — ketamine+sirolimus (with placebo at Time 2) or ketamine+placebo (with sirolimus dose at Time 2).
Hypothesis: Sirolimus will interact with ketamine by attenuating or potentiating the antidepressant effects.
- **Aim 2:** To examine the safety and tolerability of combined ketamine-sirolimus treatment.
Design: The Systematic Assessment for Treatment Emergent Effects (SAFTEE) scale will be completed during each visit to compare the adverse events profile in the sirolimus and the placebo groups.

Hypothesis: Participants will tolerate the combined treatment with no residual adverse effects.

- **Aim 3:** To identify biological markers of the combined ketamine-sirolimus treatment.

Design: Inflammatory biomarkers will be measured in 18 depressed participants who will receive open-label sirolimus+ketamine.

Hypothesis: Twenty-four hours post-treatment, sirolimus+ketamine will significantly reduce the inflammatory biomarkers.

IV. BACKGROUND

Despite 50 years of scientific progress, major depression remains among the leading causes of distress and disability in the world^{1, 2} with a 21-fold increase in suicide attempts during major depressive episodes.³ In the United States, the estimated lifetime prevalence is approximately seventeen percent.⁴ One potential reason for this sad state of affairs is that depression is treated today in basically the same way as it was in the early 1960's, when the prescription of tricyclic antidepressants became widespread. Depression is treated predominately by medications that enhance the impact of two brain chemical messengers (serotonin, norepinephrine). While more than 20 antidepressant medications are currently available, the efficacy of these medications is limited.⁵⁻⁸ These antidepressants only produce clinical remission in approximately 30% of patients in their initial clinical trial, after two drugs are tried, each new attempt at treatment only 10%-15% of patients would remit, and after multiple trials over a year only 60%-70% of patients would have a satisfactory response to treatment. Further, among those patients who do respond, clinical response only emerges, on average, after 7 weeks of treatment.⁹ From these data, it is evident that there is tremendous societal need for a novel class of antidepressants that can offer (1) rapid acting effects and (2) efficacy in patients not achieving adequate benefit from existing medications. Over the last decade, accumulating evidence has shown that low dose of ketamine, a *N*-methyl-D-aspartate (NMDA) receptor antagonist, may possess both of these properties.¹⁰

Members of our research group produced the first hint of a new approach that might address some of the limitations of existing antidepressant treatments. We have studied ketamine, a drug that acts on brain systems that use glutamate as a chemical messenger¹¹ and noted a very interesting clinical report from the late 1950s¹² and later basic animal research studies¹³ that suggested that drugs that blocked elements of glutamate signaling in the brain might have antidepressant effects. In 2000, we published a report suggesting that ketamine might have remarkable antidepressant effects.¹⁴ First, rather than taking 7 weeks to respond, patients began to respond to treatment as early as within several hours of the first dose of ketamine. Second, rather than producing response in 10%-15% of patients "treatment-resistant" depression symptoms, 50% of patients met response criteria within 24 hours of their first ketamine. Subsequent studies by other groups using ketamine and other ketamine-like drugs essentially replicated these observations in patients with unipolar and bipolar (manic-depressive) depression.¹⁵⁻¹⁹ Other groups, for example, showed that suicidal thoughts or feelings that had failed to respond to other medication and psychotherapy treatments responded rapidly to ketamine.²⁰ In addition, ketamine worked even in patients who failed to respond to electroconvulsive therapy.²¹ Most importantly, the antidepressant effects of ketamine appear to be sustained by repeated ketamine administration.^{22, 23} To date, more than 15 studies have investigated the efficacy of 0.5 mg/kg ketamine infusion in more than 300 depressed

patients.^{14, 15, 20, 24-36} These studies consistently showed a rapid antidepressant action within 4-hours of ketamine's administration, with a short-term response rate ranging from 43% to 90%.^{31, 37}

The mechanisms of action and underlying neurobiology of ketamine's antidepressant effects are not yet fully known or understood. We believe that advancing our understanding of how ketamine acts on the brain to produce its antidepressant effects is critical for improving the effectiveness and safety of ketamine and/or novel drugs development from the ketamine model. The safety concerns related to ketamine emerge from its abuse liability and its propensity to produce transient changes in cardiorespiratory function as well as perception and mentation in humans. In order to limit the risks of ketamine or to engineer a better ketamine, we need to better understand how it works.

There is controversy in the field regarding how ketamine produces its antidepressant effects. Specifically, investigators argue whether the rapid-antidepressant effects of the drug are secondary to direct action or indirect consequence of ketamine's effects on brain networks. Stress and depression appear to strip the brain of fine connections between nerve cells (dendritic spines) within brain circuits that regulate mood. Ketamine appears to cause these fine connections to regrow rapidly in animals for a period that lasts as long as the antidepressant effects of a single ketamine injection last in humans, i.e., up to a few weeks.³⁸

The indirect network effects of ketamine, sometimes described as "stimulating the go pathway" described in figure 1,³⁹ is central to our proposal. One line of research suggests that the ability of ketamine to stimulate glutamate release and to thereby activate a signaling pathway in nerve cells involving a protein called "the mammalian target of rapamycin" (mTOR). According to this theory, the co-administration of sirolimus, an immunosuppressant drug commonly prescribed for prophylaxis of renal transplant rejection, should *reduce* the antidepressant effectiveness of ketamine.

However, the other major hypothesis is related to the ability of ketamine to directly block signaling via the "stop pathway", i.e., to remove an inhibition on dendritic spine growth.⁴⁰ By blocking a very specific sub-type of receptor target for glutamate in the brain (NR2B subunit-containing NMDA glutamate receptor), ketamine may directly help dendritic spines to regrow in a manner that is independent of mTOR. Sirolimus has been repeatedly shown to have the potential

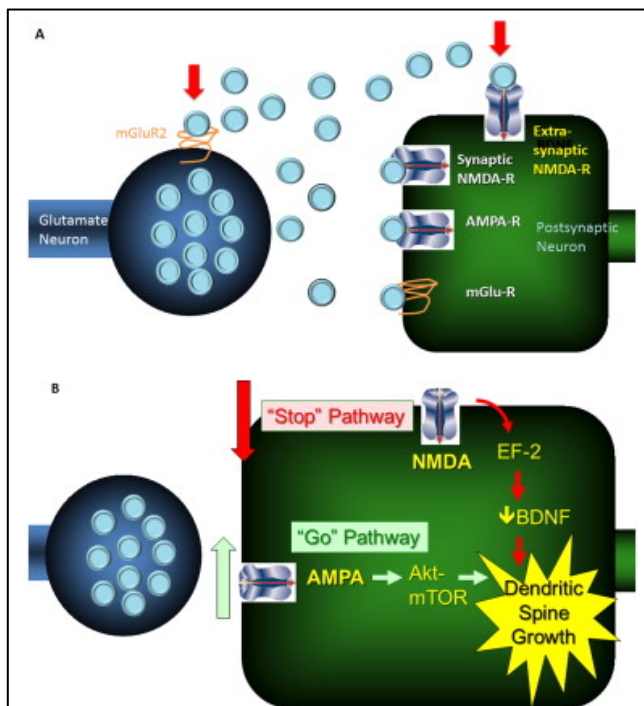


Figure 1. The top figure illustrates the presence of synaptic (receives directly released glutamate) and extrasynaptic (receives glutamate "spillover" from the synapse) glutamate receptors. Ketamine may exert its treatment effects either indirectly by increasing glutamate release and stimulating the "go pathway" (synaptic AMPA glutamate receptors) or by blocking the "stop pathway" (extrasynaptic NR2B-containing NMDA glutamate receptors).

to produce anti-depressant effects in animals^{41, 42} and to have beneficial effects in animal models of other neuropsychiatric disorders,⁴³ perhaps by having anti-inflammatory effects in the brain. A study suggests that standard antidepressant administration reduces mTOR levels, an effect that would mimic the acute effects of sirolimus.⁴⁴ Thus, if the direct effects of ketamine on the “stop pathway” pertain, then the antidepressant effects of sirolimus might *increase* the efficacy of ketamine.

Molecular studies in animals have found that synaptic deficits are precipitated by reduction in neurotrophins such as BDNF,⁴⁵ and by inhibition of mTOR signaling pathway.⁴⁶ Inhibition of mTOR signaling or reduction of BDNF leads to depressive-like behavior and blocks the effect of antidepressants in animal models of depression.^{45, 46} Enhancing mTOR signaling or increasing BDNF produces antidepressant effects in preclinical studies.^{45, 46} In humans, reduced central and peripheral BDNF levels were found in depressed patients^{45, 47} and a functional variant of BDNF polymorphism (Val66Met) has been related to depression, especially in males.⁴⁸ Together these data posit that enhancing BDNF and mTOR signaling leading to prefrontal synaptic formation (synaptogenesis), and reversal of stress/depression-induced neuronal atrophy and synaptic dysconnectivity is a required step for efficacious antidepressant treatment. Traditional antidepressants, targeting the monoaminergic system, were found to increase BDNF and synaptogenesis.^{45, 49} However, these effects were only evident following chronic treatment, which is in line with the delayed antidepressant response to these drugs in humans. Therefore, it is proposed that rapid acting antidepressants would need to directly target the induction of mTOR signaling, the increase of BDNF levels, and the ultimate enhancement of prefrontal synaptogenesis.

Thus, we have the basis for proposing to test the interactive effects of ketamine and sirolimus, a simple clinical trial that has: 1) the potential to shed fundamental light on how ketamine produces rapid clinical improvement and 2) to suggest a strategy for increasing the effectiveness of ketamine. If sirolimus reduces the antidepressant effects of ketamine, then we will know that stimulation of mTOR is critical to the antidepressant effects of ketamine, leading us to test other drugs that directly stimulate this signaling pathway.

The results of Phase 1 & 2 of this study have shown sirolimus pretreatment significantly prolong the antidepressant effects of ketamine and triples the antidepressant response rate at 2 weeks following treatment (see Fig. 2)⁵⁰. Given the role of the immune system in the pathology and treatment of depression, it was proposed that the anti-inflammatory effects of the combined treatment of sirolimus+ketamine underlie the robust enhancement in treatment response rate. Therefore, we will conduct a pilot study to determine the effects of sirolimus+ketamine on a range of inflammatory biomarkers implicated in the pathology of depression. The aim of this Phase 3 of the study is to provide preliminary data about target engagement of the sirolimus+ketamine treatment. The treatment and follow-ups will be comparable to the Phase 2 study, but only open-label sirolimus+ketamine will be administered and the weekly follow-ups will be extended to 4 weeks post-treatment.

V. SIGNIFICANCE:

The proposed project has potential to yield results critical to developing rapid-acting antidepressant medications based on the ketamine model. Specifically, if our results indicate

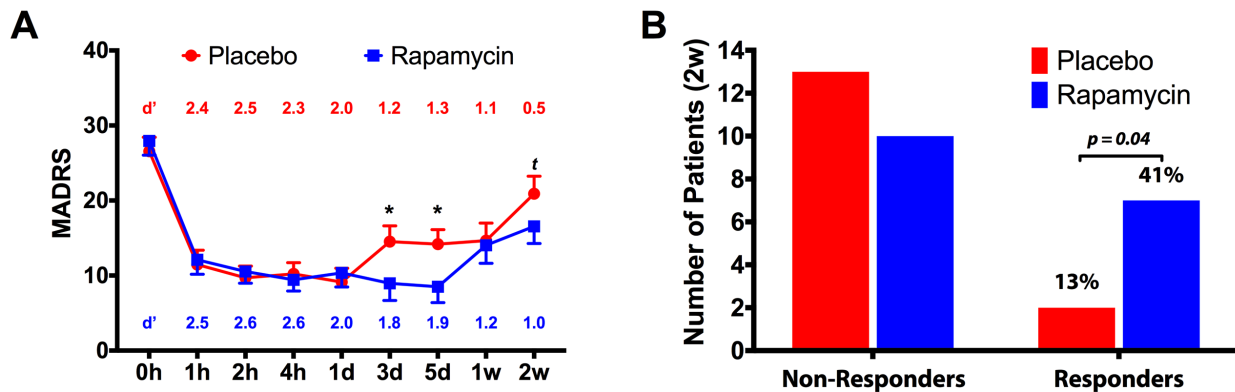


Figure 1. The Study Drug Effect on Montgomery Åsberg Depression Rating Scale (MADRS). (A) There is a significant main effect of time ($F_{(8,245)} = 43.5, p < 0.0001$), demonstrating significant decrease in MADRS scores from baseline. There is also significant interaction between treatment and time ($F_{(8,245)} = 2.0, p = 0.04$), with overall reduction in depression scores following treatment with rapamycin+ketamine (Rapamycin; blue line), compared to post placebo+ketamine (Placebo; red line). (B) Response rate was significantly higher following treatment with rapamycin+ketamine (Rapamycin; blue), compared to post placebo+ketamine (Placebo; red). **NOTES:** Response was defined as 50% improvement in MADRS from baseline; Error bars are standard errors of mean (SEM); d' = Cohen's d' effect size compared to pretreatment MADRS scores; Comparison at each time point is marked with * for $p \leq 0.05$ and t for $p = 0.12$;

synergistic antidepressant effects, then we can pursue the combination of immunosuppressant and antidepressant therapy as a new approach to the treatment of depression. If we see antagonist effects of sirolimus on ketamine, then we will have learned that stimulating the mTOR signaling pathway is critical to the antidepressant effects of ketamine, resolving a major controversy in the field and implicating drugs facilitating mTOR signaling in the treatment of depression.

This proposal has potential to provide critical insights into the mechanisms of ketamine's antidepressant effects thus advancing our understanding of the neurobiology of depression and informing novel drug development for a new generation of rapid-acting antidepressants.

VI. RESEARCH PLAN:

This project will be divided into three phases as described below:

Phase One: Open Label

We anticipate recruiting 3 male or female Veterans or civilians, between the ages of 21-65 years, for an open-label, extended-observation trial in order to first evaluate the safety of combining Ketamine with Sirolimus (same inclusion/exclusion criteria, recruitment procedures, and assessments as detailed below under Phase two of the study). Specifically, we will implement a 10- hour post-Sirolimus observation period that will take place in the Biostudies Unit (9th floor, building 1). Participants will then be asked to return to Biostudies on the morning following the administration of Rapamycin and Ketamine for additional assessment. Transportation will be arranged from Biostudies to the participants' homes and back to Biostudies the following day. In addition, the participants will be provided with a direct telephone number that they can use at any time between the end of the 10-hour observation phase and their appointment on the following day so they can contact a study psychiatrist in case of emergency or adverse effects. For 7 days after the treatment, a study clinician will contact subjects daily to inquire about side effects or interaction

effects that participants may be experiencing as a result of the study medications. The Phase One procedures are further detailed below:

Phase One Research Procedures

Day 1:

T-130 min: Subjects will present to the VACHS to complete vitals and provide a urine sample for drug and pregnancy testing.

T-120 min: Subjects will receive a single 6 mg oral dose of sirolimus. They will be monitored for any acute allergic reaction or other adverse event. Clinical assessments administered including PSWQ, PSQI, MADRS, QIDS-SR, CGI, HAM-A, CADSS, PANSS (assessment measures are described below).

T-60: Intravenous lines started.

T 0*: Blood draw for sirolimus level. Ketamine infusion begins (0.5 mg/kg infused over approximately 40 minutes). Subjects will be monitored closely by study physician and nurse.

T+15: Blood draw for sirolimus and ketamine levels.

T+30: CADSS, PANSS.

T+40: Blood draw for sirolimus and ketamine levels.

T+60**: MADRS, QIDS-SR, CGI.

T+120: CADSS, PANSS, MADRS, QIDS-SR, CGI.

T+230: MADRS, QIDS-SR, CGI, HAM-A.

Ten hours post sirolimus administration, subjects will be evaluated by a study physician or APRN and will be medically cleared for discharge. Subjects will be provided with a study physician's direct telephone number that they can use at any time in case of emergency or adverse effects.

*Blood pressure, heart rate, and oxygen saturation will be recorded every 10 minutes during infusion (T0-40 minutes).

Ketamine Administration Notes:

Subjects in Phase One will receive one infusion of ketamine (0.5 mg/kg infusion over approximately 40 minutes). The ketamine dose and route of administration are similar to that seen in previous investigations and has demonstrated high safety and tolerability.

A physician who is trained and has significant experience with both ketamine and infusion studies will oversee and administer the ketamine infusions. A nurse will also be present with the subject from the insertion of bilateral cannula for drug infusion and blood sampling, through to recovery following ketamine infusion.

Sirolimus Administration Notes:

Subjects will receive a single 6 mg oral dose via oral solution of sirolimus approximately two hours prior to the Ketamine infusion. The sirolimus dose will be given in 2 ounces of orange juice with 4oz of water. As for the tolerability of single dose of sirolimus, based on data in healthy subjects⁵¹ and consultation with Dr. Richard Formica, Director of Transplant Medicine, Yale University, an expert with sirolimus treatment, it is expected that a single 6 mg oral dose will produce minimal adverse effects.

Day 2 /Follow-Up:

Subjects will present to the VACHS to complete clinical assessments and a brief cognitive assessment. Procedures for this 24-hour follow-up will be identical to Phase Two follow-up procedures described below; however, subjects who participate in this 24-hour follow-up for Phase One will not receive any medications or infusions at follow-up. They will be asked about any residual side effects of other adverse events (AEs) and any appropriate referral for treatment will be made as necessary. Transportation to and from Biostudies will be arranged.

Days 3-7/ Phone Follow-Up:

After the Day 2 in-person follow-up, to further assess the safety of combined ketamine and sirolimus, a study clinician will contact Phase One participants by telephone, daily until Day 7. During these daily telephone follow-ups, subjects will be asked about potential side effects or interaction effects they may be experiencing related to the study medications.

After three participants undergo Phase One of this study, The PI will report findings and any issues to the Data and Safety Monitoring Committee (DSMC). The PI will not proceed to Phase Two of the study without a review and recommendation of continuation by the DSMC and subsequent full HSS review and approval of the DSMC's report.

Note: Participation in Phase One will not exclude subjects from participating in Phase Two if they so choose.

A. Phase Two Research Procedures (following DSMC approval) Subjects:

We anticipate recruiting and screening approximately 75 male and female Veterans and non-Veterans/civilians, between the ages of 21-65 years, in order to meet our target enrollment goals. In order to account for subject dropouts and the potential need to discontinue subjects before study completion (see below for discontinuation criteria) and retain a sample of 30 subjects completing the double-blind treatment phase, 45 eligible subjects who meet criteria for Major Depressive Episode as determined by the Mini International Neuropsychiatric Interview (MINI) the study inclusion criteria outlines below, will be randomized into the study. Note, this study will not include a healthy control group as we are interested specifically in the antidepressant effects of ketamine and sirolimus and therefore require subjects with current depression.

B. Inclusion and Exclusion Criteria:

Inclusion Criteria:

1. Veterans and non-Veterans between the ages of 21-65.
2. Diagnosis of Major Depressive Episode (unipolar or bipolar) as determined by the Mini International Neuropsychiatric Interview (MINI).
3. Antidepressant-resistant depressive symptoms, defined by a history of failure of one or more adequate antidepressant trials.
4. Stable doses of antidepressants (if prescribed) for a period of four weeks or longer at the time of randomization, except for MAOIs which are prohibited (see Table 1 for exclusionary medications).

5. Stable course of psychotherapy (if engaged in) for a period of four weeks or longer at the time of randomization.
6. Females will be included if they are not pregnant or breastfeeding and agree to utilize a medically accepted birth control method (to include oral, injectable, or implant birth control, condom, diaphragm with spermicide, intrauterine device, tubal ligation, abstinence, or partner with vasectomy) or if post-menopausal for at least 1 year, or surgically sterile. For those women who are taking an oral contraceptive, we will also ask that they use (or ask their partners to use) a barrier method contraceptive.
7. Able to provide written informed consent according to VA HSS guidelines.
8. Ability to read and write in English.
9. A score greater than or equal to 18 on the Montgomery Åsberg Depression Rating Scale (MADRS).

Exclusion Criteria:

1. Subjects with a diagnostic history of schizophrenia or schizoaffective disorder, or currently exhibiting manic or mixed episodes or psychotic features as confirmed by the Mini International Neuropsychiatric Inventory.
2. Current, ongoing serious suicidal risk as assessed by evaluating investigator or by scoring 5 or more on the item-10 of the MADRS.
3. Patients with unstable or inadequately controlled medical conditions.
4. Patient requiring prohibited medication (see Table 1).
5. Patient with history of organ transplant.
6. Meet criteria for a diagnosis of substance dependence (amphetamines, cocaine, hallucinogens, inhalants, opioids, sedatives/hypnotics/anxiolytics) within the three months prior to screening date.
7. Positive urine drug screen for cannabis, cocaine, PCP, or barbiturates.
8. Positive pregnancy test at screening at any screen given during the study.
9. Known sensitivity to sirolimus or ketamine.
10. History of sensitivity to heparin or heparin-induced thrombocytopenia.
11. Resting blood pressure lower than 85/55 or higher than 150/95, or resting heart rate lower than 45/min or higher than 100/min.

Table 1. Concomitant Treatments that are prohibited

Use category	Type of medication	Details
Prohibited	MAOIs	Prohibited 4-week prior to randomization.
	VNS, ECT, deep brain stimulation	VNS, ECT, or within 6 months at randomization is exclusionary.
	Memantine	Prohibited 4-week prior to randomization.
	Barbiturates	Prohibited 2-week prior to randomization.
	Cidofovir, Mifepristone, Posaconazole, Streptozocin, Ketoconazole, Voriconazole	Prohibited 2-weeks prior to randomization and throughout the study.

Table 1. Concomitant Treatments that are prohibited

Use category	Type of medication	Details
Permitted with restrictions*	Adenovirus vaccine, live; BCG live intravesical; Influenza nasal vaccine, live; Measles/Mumps/Rubella vaccine, live; Rotavirus vaccine, live; Smallpox vaccine (live vaccinia virus); Typhoid vaccine, live; Varicella vaccine, live; Yellow fever vaccine, live; Zoster vaccine, live	Prohibited 2-weeks prior to randomization and for 3 months after completing the study.
	Strong inducers (e.g., rifampin, rifabutin) and strong inhibitors (e.g., itraconazole, erythromycin, telithromycin, clarithromycin) of CYP3A4 and P-gp	Prohibited 2-weeks prior to randomization and throughout the study.
	Benzodiazepines (stable dose)	Benzodiazepines are permitted only if the patient has been taking a stable regimen and dose at least 4 weeks prior to randomization. The dosage prior to randomization should be no more than Clonazepam maximum dose 3 mg/day or equivalent. For patients reporting significant treatment-emergent nervousness, restlessness, and/or akathisia, study clinicians are allowed to increase the dose of the concomitant benzodiazepine anxiolytic up to additional clonazepam 1 mg/day equivalents.
	Modulators of CYP3A4 and P-gp; drugs that could increase sirolimus blood concentrations (e.g., bromocriptine, cimetidine, cisapride, clotrizamole, danazol, diltiazem, fluconazole, protease inhibitors, metoclopramide, nicardipine, troleandomycin, verapamil); drugs and other agents that could decrease sirolimus levels (e.g., carbamazepine, phenobarbital, phenytoin, rifapentine, St. John's Wort); drugs with concentrations that could increase when given sirolimus (e.g., verapamil).	Permitted only at the discretion of the prescriber after careful review of concomitant medications.

Notes: Sedatives, hypnotics, benzodiazepines, sedating antihistamines or other psychotropic medications are not permitted within 8 hours of treatment sessions; except – at the discretion of the investigator – for medications that will result in discontinuation/withdrawal symptoms or that may alter the risk benefit ratio.

*All prescribers will be provided with the list of prohibited and restricted medications as detailed in the sirolimus manufacturer information and made aware that medications should be used with caution.

Privacy:

All reports generated from the data obtained through this study will protect the confidentiality of the subjects who participate in this study. In case of a medical emergency, the medication group blinding can be broken by the principal investigator or a designated covering staff member, in order to supply information required for emergency medical care of research subjects. All subjects will be given a “wallet card” which identifies them as a study participant and lists the emergency contact numbers. If a subject shows clinical deterioration (worsening of depressive symptoms that is higher than 25% of baseline MADRS scores), the participant will exit the study and a study psychiatrist (or APRN) will determine whether a higher level of care (e.g. referral to treating psychiatrist or referral to emergency or inpatient care) is needed.

Selection:

Subject eligibility will first be determined via telephone screening and if records are available, by a preliminary medical record review. A waiver of HIPAA authorization and written informed consent will be obtained for a brief initial phone pre-screen (see Appendix 1) and preliminary medical record review. The phone pre-screen and preliminary medical record review will be done to determine obvious exclusions from the study protocol and to prevent subjects from making unnecessary travel for screening. A separate Phone Pre-screen Identification Page (see Appendix 2) will be completed during each telephone pre-screen so the research staff will have the necessary information to access potential participants’ medical records (with the proper verbal assent) and re-contact them for purposes of appointment scheduling. This identification page will be stored in a separate location from the screening form that will only list the subject ID.

Recruitment:

Subjects will be recruited by flyers, public advertisements (e.g., print, newspaper, radio, bus, billboards, online postings (including Trialfacts/Rewards for Research online marketing and recruitment service and Craigslist.com), social media (e.g., Facebook, Twitter, Instagram), digital media (e.g., TV, online radio, podcasts), by word of mouth, clinician referral, contact with community service groups and clinics, and through IHR0010, Screening for Depressive, Anxiety, and Trauma and Stress-Related Disorders. We intend to register our study on clinicaltrials.gov. We may also utilize the National Center for PTSD newsletter to advertise the study. Subjects will be identified by their response to advertisements and/or internal recruiting. The subjects will be asked to call us if they are interested in participating in the research study. A waiver of HIPAA authorization and written informed consent will be requested for a phone screen and preliminary medical record review in advance of scheduling the in-office screening procedures. There will not be any web-based questionnaires utilized for pre-screening subjects in this study. All available research staff are responsible for recruiting potential subjects.

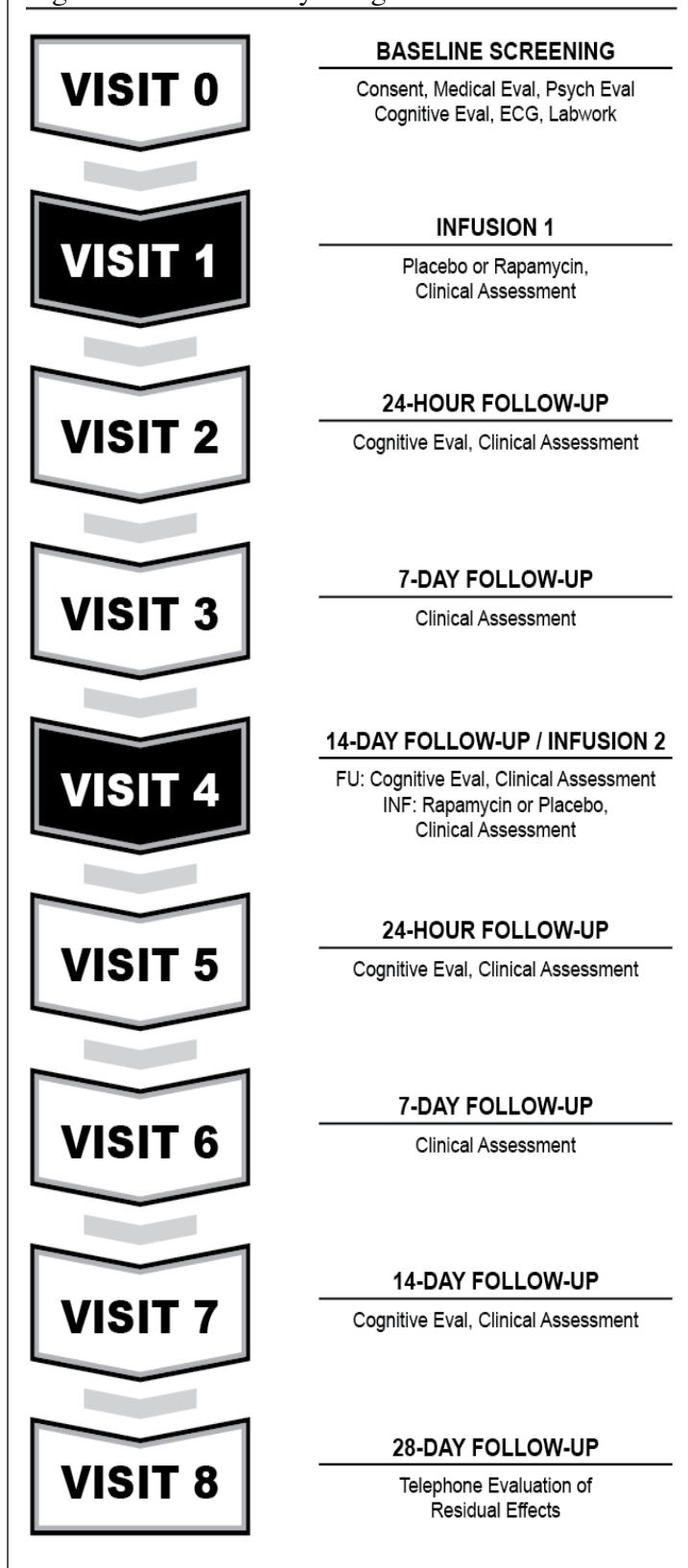
Research Plan and Study Procedures:

This is a double blind, placebo-controlled, crossover, randomized controlled trial investigating the impact of sirolimus on ketamine's antidepressant effects in participants with antidepressant-resistant depressive symptoms. Participants will be treated twice with ketamine 0.5 mg/kg infused over 40 minutes, combined with a single dose of sirolimus 6 mg orally or placebo. The 2 infusions of ketamine are separated by at least 2 weeks interval because based on previous studies the majority of patients return to their baseline depression severity within this time period. A description of each study visit is provided here and summarized in Figure 3. See Table 1 for detail regarding the schedule of study events.

Day 0-Baseline Screening:

Individuals deemed eligible after the telephone pre-screening will undergo a full in-person screening appointment at the VA Connecticut Healthcare System (VACHS), West Haven campus. During the evaluation, the study procedures will be described and the subject's questions will be answered prior to obtaining an informed consent. The investigator or research staff member obtaining consent will ask the participant to provide a brief summary of the study to ensure they understand what is being asked of them and any potential risks and benefits. Once consent is obtained, subjects will participate in the following procedures: a biopsychosocial interview, psychological evaluation, a brief cognitive evaluation, a physical examination, ECG, breathalyzer and a blood sample

Figure 3. Phase 2 study design.



for routine testing. A urine sample will be collected for urine toxicology, pregnancy, and urinalysis.

In addition, because this is a clinical trial for medications for increased safety pre-cautions, female subjects of childbearing age will have a blood sample drawn for a pregnancy test. If a female subject is pregnant, breast-feeding, intending to become pregnant, or does not agree to use an approved birth control method (i.e., oral, injectable, or implant birth control, condom, diaphragm with spermicide, intrauterine device, tubal ligation, abstinence, or partner with vasectomy) she will be excluded.

After completing the baseline screening and being deemed eligible for participation in the study, subjects will be randomized to one of two conditions at Time/Infusion 1 — ketamine+sirolimus (with placebo at Time 2) or ketamine+placebo (with sirolimus dose at Time 2).

Day 1 / Infusion 1:

First, the MADRS assessment will be completed to confirm that the participant continues to have significant depressive symptoms (i.e. MADRS \geq 18) prior to proceeding with Infusion 1. Participants will be rescheduled if depression severity was not met[‡]. If depression severity is confirmed, Infusion 1 procedures will be completed as follows:

- Subjects will present to the VACHS to complete vitals and provide a urine sample for drug and pregnancy testing.
- Subjects will receive a single 6 mg oral dose of sirolimus or placebo, dependent on their randomization. They will be monitored for any acute allergic reaction or other adverse event. Clinical assessments will be administered throughout the infusion visit, including PSWQ, PSQI, MADRS, QIDS-SR, SAFTEE, CGI, HAM-A, CADSS, PANSS** (assessment measures are described below).
- Intravenous lines will be placed.
- A blood draw for sirolimus level will occur prior to the beginning of the ketamine infusion.
- Ketamine 0.5 mg/kg will be infused over approximately 40 minutes. Subjects will be monitored closely by study physician and nurse.
- A blood draw for ketamine will occur during the ketamine infusion.
- One additional blood draw for sirolimus will occur shortly before the participants are discharged from their study visit.
- Subjects may be discharged home after being cleared by a study physician.

[‡] Screening procedures will be repeated if participant was not randomized within 60 days of screening. *Blood pressure, heart rate, and oxygen saturation will be recorded every 10 minutes during infusion (T0-40 minutes). ** Items that could not change within a session, e.g. sleep and appetite, will be carried forward from the initial ratings.

Day 2+ / 24-hour Follow-Up:

Subjects will present to the VACHS to complete clinical assessments and a brief cognitive assessment. They will be asked about any residual side effects of other adverse events (AEs) and any appropriate referral for treatment will be made as necessary.

Days 3-7+ / Phone Follow-Up:

A study staff member will contact participants by telephone every other day after the 24-hour follow-up until day 7 of the study to track symptom trajectory using QIDS and MADRS. During these telephone follow-ups, subjects will also be asked about potential side effects or interaction effects they may be experiencing related to the study medications.

Day 8+ / 7-Day Follow-Up:

The procedures for this visit are identical to those described above for Day 2. For special circumstances (e.g., inclement weather, transportation issues, considerable travel burden, etc.) this visit may occur over the phone. In the cases where a phone visit is required, we will forego the cognitive testing as there is not a way to administer this remotely, but all clinical measures and review of AEs will take place.

Day 15+ / 14-Day Follow-Up and Infusion 2:

First, the MADRS assessment will be completed to confirm that the participant continues to have significant depressive symptoms (i.e. within 20% of their baseline MADRS) prior to proceeding with Infusion 2. If depression severity is confirmed, Infusion 2 procedures will be identical to Infusion 1. As this is a crossover study, those subjects who received sirolimus on Day 1 will receive placebo in the second infusion and those who received placebo will receive sirolimus.

Based on several studies, we predict the vast majority of subjects will have returned to within 20% of their baseline depressive symptoms within the 2 weeks period. Should a subject not have returned to within 20% of their baseline depressive symptoms, Infusion 2 will be rescheduled. We will continue to monitor the participant weekly with full assessments identical to Day 2. Participants will be discharged from the study should they maintain these benefits for more than 5 weeks after Infusion 1.

Day 16+ / 24-Hour Follow-Up to Infusion 2:

The procedures for this visit are identical to those described above for Day 2.

Days 17-21+ / Phone Follow-Up:

A study staff member will contact participants by telephone every other day after the 24-hour follow-up until day 21 of the study to track symptom trajectory using QIDS and MADRS. During these telephone follow-ups, subjects will also be asked about potential side effects or interaction effects they may be experiencing related to the study medications.

Day 22+ / 7-Day Follow-Up to Infusion 2:

The procedures for this visit are identical to those described above for Day 2. For special circumstances (e.g., inclement weather, transportation issues, considerable travel burden, etc.) this visit may occur over the phone. In the cases where a phone visit is required, we will forego the cognitive testing as there is not a way to administer this remotely, but all clinical measures and review of AEs will take place.

Day 29+ / 14-Day Follow-Up to Infusion 2:

The procedures for this visit are identical to those described above for Day 2. In addition, participants will be discharged from the study with appropriate referral. For special

circumstances (e.g., inclement weather, transportation issues, considerable travel burden, etc.) this visit may occur over the phone. In the cases where a phone visit is required, we will forego the cognitive testing as there is not a way to administer this remotely, but all clinical measures and review of AEs will take place.

Day 43+ / 28-Day Telephone Call:

A telephone call will be made to all subjects to inquire about any potential residual effects of ketamine or sirolimus and other AEs. Any additional follow-up and/or referrals will be arranged as clinically indicated.

Phase Three Research Procedures:

We anticipate recruiting and screening approximately 35 male and female Veterans and non-Veterans/civilians, between the ages of 21-65 years, in order to meet our target sample of 18 subjects. Screening, enrollment and discontinuation criteria are identical to Phase 1 and 2. Treatment and study procedures are identical to Phase 2, except that participants will have only 1 treatment day (i.e., Infusion 1) with open-label sirolimus+ketamine (i.e., no Infusion 2 of placebo+ketamine). The weekly follow-up post Infusion 1, will be up to Day 29+ (i.e., 4 weeks follow-ups instead of 2 weeks). The follow-up period was extended considering the prolonged antidepressant effects of the combined sirolimus+ketamine treatment. The collection, processing and analysis of peripheral inflammatory biomarkers will be completed under a separate protocol and consent (i.e., CA0008). Participants in Phase 2, who consented to be contacted for future studies, will be invited to participate in Phase 3. This approach will both facilitate recruitment and reduce study risk as these participants who already received and well tolerated the study drug.

Ketamine Administration:

Subjects will complete two test days separated by at least two weeks. Subjects will receive an infusion of ketamine (0.5 mg/kg infusion over approximately 40 minutes). All subjects will receive two ketamine infusions—once with a placebo and once with a single dose of sirolimus (6 mg, oral administration). The order of placebo and sirolimus is randomized. Note, the ketamine dose and route of administration are similar to that seen in previous investigations and has demonstrated high safety and tolerability.

A physician who is trained and has significant experience with both ketamine and infusion studies will oversee and administer the ketamine infusions. A nurse will accompany the subject throughout the study sessions, from the insertion of bilateral cannula for drug infusion and blood sampling, through to recovery following ketamine infusion.

Sirolimus or Placebo Dosing:

Subjects will receive a single 6 mg oral dose via oral solution of sirolimus or a dose of placebo approximately two hours prior to the infusions. As above, the order of placebo and sirolimus is randomized. The sirolimus dose as well as the placebo solution will be given in 2 ounces of orange juice, followed by 4 oz of water. As for the tolerability of single dose of sirolimus, based on data in healthy subjects⁵¹ and consultation with Dr. Richard Formica, Director of Transplant Medicine, Yale University, an expert with sirolimus treatment, it is expected that a single 6 mg oral dose will produce minimal adverse effects.

Screening Measures:

Assessments that will be used during the psychological evaluation are listed below.

Psychological Evaluation:

1. *Alcohol and Consumption Habits*: This is a brief measure that documents alcohol, caffeine, and nicotine habits.
2. *Beck Suicide Ideation Scale (BSI)*: The BSI is a 21-item self-report questionnaire that may be used to identify the presence and severity of suicidal ideation. Items on this measure also assess the respondent's suicidal plans, deterrents to suicide, and the level of openness to revealing suicidal thoughts.
3. *Behavioral Inhibition & Activation Scales (BIS/BAS)*: The BIS/BAS is a self-report scale designed to assess dispositional sensitivity to the behavioral inhibition system (BIS) and the behavioral activation or behavioral approach system (BAS).
4. *Big Five Inventory (BFI)*: The BFI measures an individual on the Big Five Factors (dimensions) of personality (Goldberg, 1993). Each of the factors is then further divided into personality facets.
5. *Brief TBI Screen*: The Brief Traumatic Brain Injury Screen, also called the DVBIC TBI Screening Tool, is a 3-item instrument used to evaluate the presence of head injury and related symptomatology. It was validated in a small, initial study conducted with active duty service members who served in Iraq/Afghanistan between January 2004 and January 2005.
6. *Clinical Global Impressions Scale (CGI)* and the *Patient Global Impressions Scale (PGI)*: The CGI and PGI are widely used instruments, which assess overall severity of illness on a 1 to 7 point scale with 1 indicating "normal, not at all ill" and 7 indicating "among the most extremely ill patients." These instruments also assess global improvement on a 1-to-7 point scale with 1 indicating "very much improved," 4 indicating "no change" and 7 indicating "very much worse."
7. *Clinician Administered Dissociative States Scale (CADSS)*: The CADSS has self and interviewer-administered items including 5 subscales, generated a priori, evaluating dissociation including altered environmental perception, time perception, spatial/body perception, derealization and memory impairment.
8. *Cognitive tasks*: This will include CogState, a well-validated short computerized cognitive tasks. These short computerized tasks along with a brief neuropsychological battery will assess cognitive functions.
9. *Columbia-Suicide Severity Rating Scale (C-SSRS)*: The C-SSRS is a brief clinician administered and standardized measure that uniquely assesses essential information about suicide behavior, ideation, lethality and severity, and distinguishes between suicidal occurrences and non-suicidal self-injury.
10. *Early Trauma Inventory (ETI-SR)*: The ETI-SR is a self-report instrument to assess childhood trauma and includes physical, emotional and sexual abuse as well as general traumas.
11. *Global Perceived Early Life Stress (GPELS)*: The GPELS is a self-report of perceived stress during childhood.
12. *Hamilton Anxiety Rating Scale (HAM-A)*: The HAM-A is a standardized clinician-rated instrument to evaluate the severity of anxiety symptoms.
13. *Klein Loss Scale (KLS)*: The KLS is a self-report of parental loss or separation during childhood.

14. *Life Events Checklist (LEC)*: The LEC is a self-report instrument that measures reports of traumatic life events.
15. *Perceived Stress Scale (PSS)*: This is a 10-item self-report questionnaire to assess the severity of perceived stress over the past month.
16. *Cumulative Adversity Interview (CAI)*: A multifaceted semi-structured assessment of stressful life events and chronic subjective stress used in research on stress and psychopathology.
17. *Massachusetts General Hospital Antidepressant Treatment History Questionnaire (MGH-ATRQ)*: This is a self-rated questionnaire used to determine treatment resistance in major depressive disorder.
18. *Montgomery-Asberg Depression Rating Scale (MADRS)*: The MADRS is a standardized instrument to ascertain depressed mood and neurovegetative signs and symptoms of depression.
19. *Penn State Worry Questionnaire (PSWQ)*: The PSWQ is a self-report questionnaire to assess for 'worry' symptoms that are typical of generalized anxiety.
20. *Pittsburgh Sleep Quality Index (PSQI)*: The PSQI is a self-report questionnaire to assess sleep quality and sleep disturbance.
21. *Positive and Negative Symptom Scale (PANSS)*: The PANSS is commonly used to measure the severity of symptoms in psychotic disorders. It is a clinician-administered scale and includes three categories of symptoms: (1) positive symptoms, such as hallucination and delusion; (2) negative symptoms, such as flat affect and difficulty in abstract thinking; (3) general psychopathology, such as mannerisms and posturing.
22. *PTSD Checklist (PCL)*: The PCL is used to measure PTSD symptoms and is a self-report questionnaire that has high reliability.
23. *Quick Inventory of Depressive Symptoms – Self-Report (QIDS-SR)*: The QIDS-SR is a patient-rated depression instrument.
24. *Sheehan Disability Scale (SDS)*: The SDS is a brief self-rated measure of disability and impairment.
25. *Systematic Assessment for Treatment Emergent Events (SAFTEE)*: The SAFTEE is a commonly used instrument originally developed by NIMH and adapted into a self-report instrument. It examines, in systematic fashion, possible treatment-emergent side effects and probes for specific adverse symptoms, including suicidal thoughts and behaviors, and self-injurious behavior.
26. The Mini International Neuropsychiatric Interview (MINI) will be used to provide current and past diagnoses of psychiatric disorders, notably the inclusion/exclusion criteria of a current major depressive episode.
27. *Socio-demographic/General Information*: At intake, demographic data and medical history will be assessed with interviews and self-report forms that provide data on age, race, socioeconomic status, marital status, educational and occupational levels, and significant medical history. These are adapted from previous diagnostic and clinical studies at this center.

Medical Assessments:

1. Physical exam by a licensed physician, or Advanced Practice Registered Nurses (APRN's).
2. Routine laboratory studies including a complete blood count (CBC) w/ differential, a comprehensive metabolic panel, HCG, TSH, fT4, CRP, and ESR, in addition to urinalysis and urine toxicology screen. Additional tests will be requested as clinically indicated.

3. Urine toxicology screens will be performed at the screening appointment and will be administered more frequently if the clinician or research staff becomes concerned about possible illicit drug use. Patients will be informed of random urine drug screens.
4. An ECG will be performed.
5. Pregnancy Tests- female subjects will have a blood sample drawn for a pregnancy test.

Audio Recording:

Clinician administered interviews may be audio recorded for inter-rater reliability and staff training purposes. Subject identifying information will not be on the audio recording. Recordings will be stored in a locked cabinet when not in use. Subjects will be asked on the consent form to indicate whether or not they agree to having their clinical interviews audio recorded. If subjects disagree to having their clinical interview audio recorded, it will not affect their eligibility to participate in study procedures.

Data Storage:

Data will be housed at VACHS West Haven campus in Building 1, where the PI's office is located. The computers where data will be stored are password protected and behind the VA firewall. Only approved research staff will have access to the screening data.

Table 2. Schedule of Study Events

<i>Visit</i>	<i>1- Baseline</i>	<i>2- Infusion **</i>	<i>3- 24-hr</i>	<i>4- 7-day</i>	<i>5- 14-day Infusion **</i>	<i>6- 24-hr</i>	<i>7- 7-day</i>	<i>8- 14-day</i>	<i>9- 28-day</i>
<i>Day</i>	<i>-60-0</i>	<i>1</i>	<i>2</i>	<i>8</i>	<i>15+</i>	<i>16+</i>	<i>23+</i>	<i>30+</i>	<i>44+</i>
Alcohol & Consumption	x								
BFI	x								
Blood draw/labs	x								
BIS/BAS	x								
Breathalyzer	x	x			x				
BSI	x	x	x	x	x	x	x	x	
B-TBI-S	x								
Perceived Stress Scale	x								
Cumulative Anxiety Inter.	x								
CADSS		x			x				
CGI-S/I and PGI-S/I	x	x	x	x	x	x	x	x	
Collect AEs and SAEs	x	x	x	x	x	x	x	x	x
Cognitive Tasks	x		x	x		x	x		
Concomitant Meds	x	x	x	x	x	x	x	x	
C-SSRS	x								
Demographics	x								
ECG	x								
ETI-SR	x								
GPELS	x								
HAM-A	x	x	x	x	x	x	x	x	
*Informed Consent	x								
Ketamine Infusion		x			x				
KLS	x								
LEC	x								

MADRS	x	x	x	x	x	x	x	x	
M-MACE	x								
MGH-ATRQ	x								
Medical Evaluation	x								
PANSS		x			x				
PCL	x	x	x	x	x	x	x	x	
PSQI	x	x	x	x	x	x	x	x	
PSWQ	x	x	x	x	x	x	x	x	
QIDS-SR	x	x	x	x	x	x	x	x	
Sirolimus/Placebo Dose		x			x				
SAFTEE	x	x	x	x	x	x	x	x	x
MINI	x								
SDS	x								
Serum Pregnancy	x								
Urinalysis	x	x			x				
Urine Toxicology/Pregnancy		x			x				
Vital Signs	x	x			x				

*Informed consent will be obtained prior to any study procedure.

Abbreviations: Big Five Inventory (BFI), Behavioral Inhibition & Activation Scales (BIS/BAS), Beck Suicide Inventory (BSI), Brief TBI Screen (B-TBI-S), Clinician Administered Dissociative States Scale (CADSS), Clinical Global Impression Severity/Improvement (CGI-S/I), Patient Global Impression-Severity/Improvement (PGI-S/I), Cogstate and brief neuropsychological battery comprises the cognitive testing portion, Columbia-Suicide Severity Rating Scale (C-SSRS), electrocardiogram (ECG), Early Trauma Inventory – Self Report (ETI-SR), Global Perceived Early-Life Stress (GPELS), Hamilton Anxiety Rating Scale (HAM-A), Klein Loss Scale (KLS), Life Events Checklist (LEC), Montgomery-Asberg Depression Rating Scale (MADRS), Modified Military Acute Concussion Evaluation (M-MACE), MGH Antidepressant Treatment Response Questionnaire (MGH-ATRP), Positive and Negative Symptom Scale (PANSS), Pittsburgh Sleep Quality Index (PSQI), Penn State Worry Questionnaire (PSWQ), Quick Inventory of Depressive Symptomatology – Self Report (QIDS-SR), Systematic Assessment for Treatment Emergent Events (SAFTEE), Structured Clinical Interview for DSM (SCID), Sheehan Disability Scale (SDS). **Phone call follow-ups will be conducted every other day for five days following both infusion days.

Criteria for Discontinuation

Participants may be discontinued from this proposed study at any time. Specific reasons for discontinuing a participant include:

- Voluntary discontinuation by the participant, who is at any time free to discontinue his/her participation in the study without prejudice to further treatment.
- Clinical deterioration: The following are objective criteria for clinical deterioration, (1) a 25% increase in MADRS scores at any time during the study, and (2) the onset of active suicidality as assessed by the study physicians or by scoring 5 or more on the item-10 of MADRS.
- Stopping birth control or positive pregnancy test.

- Safety reasons as judged by the investigator.
- Severe non-compliance to protocol as judged by the investigator.
- Incorrect enrollment i.e., the subject does not meet the required inclusion/exclusion criteria for the study.

A subject that discontinues will always be asked about the reason(s) for discontinuation and the presence of any adverse events. If possible, they will be seen and assessed by the investigator. Adverse events will be followed up as medically necessary.

Participant Compensation:

All participants will be compensated for their participation in this study and completion of all study visits is not required for receipt compensation. For Phase One, subjects will be paid \$10/hour, not to exceed \$70 for the baseline-screening visit. They will receive \$100 for the medication visit, and they will receive \$50 for the 24-hour follow-up visit. They will also be reimbursed for transportation based on mileage or receipts provided up to \$50 per visit. At the discretion of the investigator, subjects may be reimbursed for additional costs including travel, parking, transportation, meals, or other expenses. Subjects will be asked to provide receipts so that they can be reimbursed for these expenses, as applicable. For Phase Two, subjects will be paid \$10/hour, not to exceed \$70 for the baseline-screening visit. They will receive \$50 per visit for all remaining visits for a possible of 7 visits or \$350 (i.e., infusion 1, 24-hour follow-up, 7-day follow-up, 14-day follow-up/infusion 2, 24-hour follow-up, 7-day follow-up, 14-day follow-up) and will be reimbursed for transportation based on mileage or receipts provided up to \$50 per visit. Payments for this study are processed through Yale University. As such, participants' names, addresses and social security numbers will be provided to the Yale University Accounts Payable department in order to process subject payment for participating in this study. Participants may be asked to complete a W-9 form (federal request for taxpayer identification and certification), which allows Yale to process their payment and potentially report their earnings to the IRS. Payment may be made to participants in cash on the day of each visit, at the discretion of the PI. For any cash payments, participants will be asked to sign a receipt acknowledging payment and the amount they were given. Most participants will receive payments in the form of a check or through Yale University's debit card system. The debit cards will be offered through the YCCI OnCore ePayments system and will be our preferred payment option. Subjects will be informed that they will receive payment via a Bank of America pre-paid debit card. We will provide their name, address, and telephone number to the Bank of America for processing and their card will be related to their name and number. Their SSN or other information will not be provided. All compensation information will be discussed in the informed consent process. Yale will also send subjects an IRS 1099 form reporting the amount of money paid to them.

For Phase Three, subjects will be paid \$10/hour, not to exceed \$70 for the baseline-screening visit. They will receive \$50 per visit for all remaining visits for a possible of 6 visits or \$300 (i.e., infusion 1, 24-hour follow-up, 7-day follow-up, 14-day follow-up, 21-day follow-up, 28-day follow-up). At the principal investigator's discretion, further subject payment may be made for travel, parking, lodging, food, or transportation. Payment will be received through the mail in the form of a check from the VA, unless participants have arranged for direct deposit through the VA. Payment by check may take up to 6 weeks and is subject to withholding for outstanding debts; for

example, defaulted student loans, child support, or back taxes. If participants have any federal debt, there is a possibility that they will not receive any money after their participation.

There will be no charge to participants for any aspects of this study including services, testing, evaluation, or medications. However, some Veterans are required to pay a co-payment for medical and other services provided by the VA Connecticut Healthcare System that are not part of the study. These co-pay requirements will continue to apply to medical care and services provided by VA that are not part of this study.

Data Analysis:

This study, investigating the impact of sirolimus on ketamine's rapid antidepressant effects, is the first of its kind. In order to assess study utility and to determine if medication dose adjustments may be useful, we plan to do interim analyses after completion of the first 6 subjects, with subjects randomized according to block randomization (block of six). We propose to study 30 patients, overall. This sample size is feasible within the funding available for this project and will provide 80% power for detecting ketamine-sirolimus interaction effects of moderate size ($d'=.53$), assuming a two-tailed $\alpha=.05$. The data analysis plan is provided only for the primary outcome variable, the MADRS score. The normality of the data will be tested initially using probability plots and the Kolmogorov-Smirnov tests. These data are frequently skewed and so we plan to analyze them using a non-parametric approach to repeated measures data.⁵¹ The data will be first ranked and then fitted with a mixed-effects model with an unstructured variance-covariance matrix and p values adjusted for ANOVA-type statistics. In these models sirolimus (active vs. placebo) and time will be fitted as within subjects variables. All interactions will be fitted. Age and sex will be treated as covariates/grouping factors. Post-hoc analyses will be conducted to interpret interactive effects significant at the $p=0.05$ level. Subject will be used as the clustering variable. Plasma ketamine and ketamine metabolite levels will be analyzed using linear mixed models. All secondary measures will be assessed using similar methodology. Behavioral measures of Phase 3 will use similar methodology. The collection, processing and analysis of peripheral inflammatory biomarkers will be completed under a separate protocol and consent (i.e., CA0008).

IX. POTENTIAL RISKS:

Although precautions will be taken to minimize risk to participants, some risks and inconveniences remain. The risks and inconveniences involved with this study include those associated with: (a) ketamine administration, (b) sirolimus administration, (c) phlebotomy and intravenous line placement, (d) psychiatric and cognitive evaluation to include clinical and neuropsychological assessments, (e) clinical deterioration, (f) ECG, (g) HIV and Hepatitis testing, and (h) pregnancy and breast-feeding.

Ketamine Administration:

Ketamine is a dissociative anesthetic that has been used clinically since the late 1960s. Despite extensive experience, there is no clear and compelling evidence of long-term toxicity associated with ketamine administration in medically supervised settings. However, there are acute medical and neuropsychiatric sequelae that deserve special consideration. The ketamine doses employed in this study have been specifically selected to produce behavioral effects without significant sedation. At these doses, ketamine produces a transient alteration of consciousness including altered sensory processing, and thought processes. Initially, subjects frequently feel "drunk" and giddiness is common. As blood levels increase, blood pressure and heart rate increase moderately.

This increase is transient. Subjects report differences in complex problem solving evident on frontal lobe and delayed memory tasks, some subjects may report a narrowing of their concentration, feeling distant from surroundings, and enhanced perception of some sensory stimuli. Subjects sometimes report blurred vision and nystagmus. Alterations in the perception of time, body boundaries, and illusions occur. Subjects may experience visual distortions, altered perception of orientation in space, and inability to control thought processes. Subjects may report feeling quite distanced from their surroundings, describe altered awareness of their bodies, and they may close their eyes. During this period, they are still oriented to time and place. They can complete ratings scales testing memory without impairment, their rate of finger tapping is unchanged and the latency of their response on continuous performance tasks of attention is not increased. However, some individuals feel that they cannot control the experience and find it frightening. Vivid dreams and poor sleep quality after infusion of ketamine has also been reported, although dream content was not necessarily unusual and alterations in sleep were not reported on subsequent nights. The doses used in this protocol produce blood levels that are 1/6 to 1/3 of those produced clinically when ketamine is used as an anesthetic. Short-term safety data showed that adverse events in response to ketamine infusion have been mild and transient, with no evidence of any clinically significant or persistent adverse effects. Adverse events included nausea and vomiting, sedation, anxiety, hypotension, insomnia and nightmares and transient pain in the infusion arm.

Sirolimus Administration:

Sirolimus is a commonly prescribed immunosuppressant (maximum daily dose is 40 mg); FDA approved for prophylaxis of renal transplant rejection. The limited administration and low dose proposed in this study was selected to yield efficacy but minimize side effects.^{51, 52} Short-term administration (i.e., a single 6 mg dose) is anticipated to have limited, if any, side effects. Potential side effects include nausea, vomiting, diarrhea, constipation, stomach pain, headache, skin rash, and joint pain. Other adverse reactions related to treatment with sirolimus include: peripheral edema, hypertriglyceridemia, hypertension, hypercholesterolemia, increased creatinine, fever, urinary tract infection, anemia, pain, thrombocytopenia, susceptibility to infection, lymphoma, and malignancy, hypersensitivity reactions, exfoliative dermatitis, angioedema, fluid accumulation and wound healing, proteinuria, interstitial lung disease, and increased risk of calcineurin inhibitor-induced hemolytic uremic syndrome/thrombotic thrombocytopenic purpura/thrombotic microangiopathy (HUS/TTP/TMA).

Also uncommon, but possible, is an allergic reaction to sirolimus. Signs of such a reaction include the acute onset of hives, difficulty breathing, swelling of the face lips, tongue, or throat. As described above, a trained physician and nurse will be in attendance throughout the drug administration and infusion to monitor subjects for any side effects or other adverse events.

Blood drawing & intravenous line placement:

For the intake appointment, a butterfly needle will be inserted into a vein to retrieve blood samples. The placement of a butterfly needle in the vein can be associated with bruising, infection, or clot formation. These risks are minimized by proper techniques. Sometimes people also experience a feeling of lightheadedness during or following blood withdrawal. Subjects will have no more than 8 ounces of blood drawn. Blood samples are drawn for routine labs and drug screening.

During the intravenous line placement for the ketamine administration, a bruise may occur at the puncture site, and very rarely, an infection may develop. If this occurs, appropriate treatment will be instituted immediately. To reduce any risk of large amount of air bubbles getting into the veins, all air bubbles are out of the IV tubing before connecting the tubing to the IV lines. Also, the infusion pumps used automatically detect air bubbles going through the pump and stops infusion.

Risks associated with blood loss are minimal. Less than 200 mL will be drawn, and this represents 40% of a 500 mL blood donation.

Psychiatric Screening and evaluation: The risks and discomforts of the screening and evaluations are minimal. The discomfort associated with the physical examination or intake interview with the study clinician includes the possible stress of answering personal questions. Subjects will be answering questions about their symptoms and filling out questionnaires. They may find this process to be inconvenient, uncomfortable or upsetting. The psychological testing may include personal questions about previous experiences. The questions will be asked in a private room. Subjects will be informed that they do not have to answer any question that they do not want to answer. Subjects will also have the option to discuss their concerns with someone on the research staff. One or more individuals will be available to talk to the subjects should they become distressed during an interview or while filling out questionnaires. The cognitive testing portion may be frustrating or boring for some subjects, yet it shown to be well tolerated.

Clinical Deterioration: There is a risk of increasing depressive symptoms due to the natural history of the illness and/or poor response to ketamine and sirolimus administration.

ECG: Sometimes the adhesive pads used to attach the leads for recording the electrical activity of the heart (ECG) can cause skin irritation. Such irritation usually clears without treatment.

HIV, Hepatitis B and C testing: as part of the screening assessment, may yield information that we are legally required to disclose to the State of Connecticut and/or CDC as reportable infections. Any identifiable information obtained in connection with this protocol remains confidential. Information is disclosed only with the subject's prior authorization or as required by U.S. and State law. Information that we are legally required to disclose includes abuse of a child or elderly person, or certain reportable infectious disease.

Pregnancy and Breast Feeding: Women will be tested for pregnancy as part of the routine laboratory tests the day of the screening appointment. If the test is positive, the woman will not be included in the study. Before starting the study, we will ask women to avoid becoming pregnant and ask what precautions will be taken. We will ask that participants use contraception throughout the duration of the study and for 12 weeks following the study. We ask that they agree to use an approved birth control method including oral, injectable, or implant birth control, condom, diaphragm with spermicide, intrauterine device, tubal ligation, abstinence, or partner with vasectomy) or if post-menopausal for at least 1 year, or surgically sterile. If they are taking a contraceptive medication, we ask that they also use (or ask that their partner use) a barrier method contraceptive (e.g, condom, diaphragm with spermicide). If the participant changes her mind about becoming pregnant or how to avoid becoming pregnant, we ask that she tell us immediately.

In addition, women who are breastfeeding will be excluded. Urine pregnancy testing will be completed prior to each infusion.

MINIMIZING RISKS

General risk-minimizing strategies: (1) Effective screening to exclude subjects who would be placed at a greater risk. This includes a comprehensive psychiatric and medical evaluation, physical examination, and the screening studies performed before starting studies (see above). Trained staff, under the supervision of the PI, will conduct all screening procedures. (2) The investigator or a designated person will explain the benefits and risks of participation in the study to each subject. Subjects will be asked to verbalize their understanding of all aspects of the consent, including risks, benefit and alternatives. The voluntary nature of research studies is always emphasized. (3) All subject information will be kept confidential and only members of the investigative team with appropriate IRB/HIC and HIPAA training will have access to the study data. Data will be maintained and secured in locked file cabinets or password protected electronic media. A numbering code will be used to assign a unique identifier to each subject. (4) A Data and Safety Monitoring Plan is described below.

Ketamine Effects: As noted above, our group here at the VACHS and Yale pioneered ketamine studies in healthy subjects and in depression, and has been performing these studies since early 1990s. Before participating, subjects undergo careful psychiatric and medical evaluation. A research nurse and study physician are available at all times during test sessions to provide support and consistent “reality testing” for individuals experiencing confusion or transient psychosis. If a subject reports that symptoms cannot be tolerated, the ketamine infusion will be stopped. Subjects will be observed for at least an hour after the termination of testing, and if intolerable physical or behavioral symptoms persist, subjects may be admitted to the inpatient Clinical Neuroscience Research Unit at the Connecticut Mental Health Center for further observation and overnight if necessary. Participants will be informed that they may not drive or operate machinery for 24 hours after end of test day procedures, and study staff will ensure that they are picked up by a responsible adult or safely reach their home on alternate transportation. Subjects will be provided a number to call to reach an on-call research psychiatrist (24 hours/day) should unpleasant effects occur after subjects have left the testing facility. Medication effects will be reviewed and “debriefed” with subjects following each test day by a study clinician. If necessary, subjects will be administered oral diazepam to reduce residual symptoms and their participation in the study will be terminated if necessary. Such subjects will be monitored as found appropriate by the study physicians.

We are also taking a number of precautions to help reduce the chance of having an unpleasant response to ketamine or to reduce the severity of any lingering medication effects. These precautions include:

- 1) A research clinician will be present throughout the study to offer support and to help clarify the progress of the test day in case the medication causes feelings of confusion.
- 2) Medications are available (IV lorazepam) to relieve distress related to the behavioral effects of ketamine.
- 3) We will ask the subject to remain at the VACHCS in a recovery room for several

hours after the behavioral effects of ketamine should have worn off to further evaluate side effects and ensure the subject is feeling well before discharge to home.

- 4) We will review the test day with the subject to deal with their feelings and reactions to each test day before they leave.
- 5) We will ask the subject to contact us at any time if any unpleasant effects occur.
- 6) We will ask the subject not to engage in demanding work in the day following the test sessions and we will work with the subject to schedule your test days accordingly.
- 7) If the subject has any lingering medication effects, such as sedation, we will terminate the remaining test days and work with him/her until these side effects have resolved.
- 8) If the subject develops psychiatric symptoms, we may admit him/her to the hospital. This may be involuntary if he/her are in danger of harming self or others.
- 9) We will conduct 24-hour, 7-day, 14-day, and 28-day follow-up assessments for residual effects.

Clinical Deterioration: Subjects will be closely monitored with serial depression rating scales. In the event of clinical deterioration, the subject will be discharged from the study protocols and the study physicians will initiate treatment as clinically recommended. As noted above, the following are objective criteria for acute decompensation: (1) a 25% increase in MADRS scores at any time during the study, or (2) the onset of active suicidality as assessed by the study physicians or by scoring 5 or more on the item-10 of MADRS. Study participants will be informed that a decision to initiate standardized psychotropic and/or psychotherapeutic regimens will not adversely affect their ability to participate in future protocols or receive treatment at VACHS, Yale Depression Research Program (YDRP), CMHC, or private psychiatric practice in the community. All participants will be treated by a study physician until appropriate referral is arranged.

IV Irritation:

If there is irritation due to IV blood sampling, the physician present during the study will examine the subject, and the study will be terminated if the subject is in pain.

Data Safety Monitoring Plan:

An independent data and safety monitoring board (DSMB) will be established for the clinical trial (i.e., phase 1 & 2, not the mechanistic phase 3 study).

The investigator's assessment of the overall risk for subjects participating in this study is moderate.

Moderate Risk DSMP

1. Personnel responsible for the safety review and its frequency:

The principal investigator, Chadi Abdallah, M.D., 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 (monitor) will evaluate whether the study should continue unchanged, require modification/amendment, continue or close to enrollment. Furthermore, a data and safety monitoring committee (DSMC) of independent researchers/clinicians (including an ethicist and statistician) will review adverse events. The DSMC committee will review the study prior to the initiation of Phase One subject enrollment. The DSMC will be consulted again prior to Phase Two of the study, at which point the committee members will determine whether this study can be safely conducted on an outpatient basis. In addition, the DSMC will conduct a safety assessment twice yearly. Either the principal investigator, the DSMB (Phase 1 & 2), or the VA IRB have the authority to stop or suspend the study or require modifications.

2. The risks associated with the current study are deemed moderate for the following reasons:

1. We do not view the risks associated with ketamine infusion and sirolimus dosing as minimal.
2. Given the established safety and validity of the use of ketamine and sirolimus, we do not view the proposed study as high risk.

Although we have assessed the proposed study as one of moderate risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. In order to assess safety in this patient group, we will conduct an interim data analysis after the first 5 subjects are completed.

Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

3. Attribution of Adverse Events:

Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design according to the following categories:

- a. Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).
- b. Probable: Adverse event is likely related to investigational procedures(s)/agent(s).
- c. Possible: Adverse event may be related to investigational procedures(s)/agent(s).
- d. Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).
- e. Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

4. Plan for Grading Adverse Events:

The following scale will be used in grading the severity of adverse events noted during the study:

1. Mild adverse event
2. Moderate adverse event
3. Severe

5. Plan for Determining Seriousness of Adverse Events:

Serious Adverse Events:

In addition to grading the adverse event, adverse events are evaluated to determine whether they meet the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it:

1. is life-threatening
2. results in in-patient hospitalization or prolongation of existing hospitalization
3. results in persistent or significant disability or incapacity
4. results in a congenital anomaly or birth defect OR
5. results in death
6. 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, or
7. adversely affects the risk/benefit ratio of the study

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 IRBs is necessary.

6. Plan for reporting serious AND unanticipated AND related adverse events, anticipated adverse events occurring at a greater frequency than expected, and other unanticipated problems involving risks to subjects or others to the IRBs

The investigator will report the following types of adverse events to the IRBs: a) serious AND unanticipated events; b) adverse events occurring with a greater frequency than expected; and c) other unanticipated problems involving risks to subjects or others.

These adverse events or unanticipated problems involving risks to subjects or others will be reported to the IRBs and DSMB (Phase 1 & 2) within 48 hours of it becoming known to the investigator, using the appropriate forms.

7. Plan for reporting adverse events to co-investigators on the study, funding and regulatory agencies

For the current study, the following individuals, funding, and/or regulatory agencies will be notified:

- All Co-Investigators listed on the protocol.
- DSMB (Phase 1 & 2)

The principal investigator, Chadi Abdallah M.D., will conduct a review of all adverse events upon completion of every study subject. The principal investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

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Yale University

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Appendix 1: Initial Phone Pre-screen

Sirolimus and Ketamine Phone Interview

Introduce yourself to all candidates. For candidates who call for information about the Sirolimus and Ketamine study, follow the script below:

If you call someone back and speak with them directly:

"Hello, my name is: _____. I am calling from the National Center for PTSD at the VA in West Haven. I am returning your call indicating that you may be interested in participating in our research study". (Follow with script from the next section).

If you answer a call from someone interested in the Sirolimus and Ketamine study, start here:

"Thank you for your interest in our study. May I tell you a little about the study? We are currently enrolling eligible Veterans and civilians in a research project to study the impact of sirolimus on ketamine's antidepressant effects. Sirolimus is an FDA-approved drug commonly used in cancer treatment and following organ transplants. Ketamine is an FDA-approved anesthetic drug shown to have rapid antidepressant effects. As this study is exploring the interaction between these two medications and their impact on depressive symptoms, we will be recruiting individuals who have current depression and have tried at least one antidepressant in the past with little to no success at improving symptoms. This study is a randomized, controlled trial in which it is expected most participants will receive two ketamine infusions, one with sirolimus and one with placebo, an inactive "sugar pill." The order in which you receive these will be based on the way you are randomized into the study. The infusion days will happen here at the VA and will be separated by approximately two-weeks. There will be follow-up assessments after each infusion to assess any changes in symptoms you may experience and to address any question or concerns you may have.

Does this sound like something you may be interested in? If yes, continue.

If the candidate does not want to pursue the interview, thank them for their time and end the phone call.

May I ask you some questions about your physical and mental health, to determine if you may be eligible for the study? Some of these questions may be of a confidential nature. Is this an appropriate time for you to talk? We expect these questions to take approximately 10 minutes to complete."

If the candidate agrees: *"Thank you. Please understand that your participation is voluntary and it is optional to answer these questions. You do not have to answer any question that makes you uncomfortable. Your decision to participate in this research will not affect your medical care or benefits."*

Document Subject Assent:

Subject Agrees ☐

Subject Disagrees ☐

Date: _____

Subject Id #: _____

Screened by: _____

Are you available for weekly appointments during the day? Yes ☐ No ☐

Are you available for appointments on more than one day? Yes ☐ No ☐

Demographics

1. What is your primary language? _____

2. What is your age? _____

Physical Health

3. Do you have any current or ongoing medical problems? ☐ Yes ☐ No

If yes, what are they: _____

4. Have you ever had an organ transplant? ☐ Yes ☐ No

If yes, what and when: _____

5. Have you had any recent vaccines? (e.g., flu shot, MMR, small pox, typhoid, etc.) ☐ Yes ☐ No

If yes, what and when: _____

6. Have you ever taken a medication called ketamine? ☐ Yes ☐ No

If yes, when, what was it for, did you have any adverse reaction: _____

7. Have you ever taken a medication called sirolimus? ☐ Yes ☐ No

If yes, when, what was it for, did you have any adverse reaction: _____

8. Do you have a history of heparin sensitivity or heparin-related thrombocytopenia? ☐ Yes ☐ No

If yes, when, what was it for, did you have any adverse reaction: _____

9. If female, are you currently pregnant or breastfeeding? ☐ Yes ☐ No ☐ N/A male subject

10. Are you currently taking any medications, vitamins, herbal or dietary supplements?

Name of Medication, Vitamin, etc.	Reason	Dose	Regimen/length of time on medication/% improvement of symptoms
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11. Have you smoked marijuana (cannabis) at any time during the last 30 days? _____ If yes, when was the last time you smoked marijuana? _____

12. Have you participated in any research studies that involved taking any medications, even if just for one day? _____ If yes, when were those studies and what medications may have been involved? _____

MDD Symptom Screening

IN THE PAST 2 WEEKS ...

13. Have you been feeling down, depressed, or hopeless? ☐ Yes ☐ No
14. Have you experienced decreased interest or pleasure in doing things? ☐ Yes ☐ No
15. Have you had trouble falling or staying asleep, or sleeping too much? ☐ Yes ☐ No
16. Have you been feeling tired or having low energy? ☐ Yes ☐ No
17. Have you had a poor appetite or been over eating? ☐ Yes ☐ No
18. Have you been feeling bad about yourself (e.g., like a failure, like you've let yourself or others down, etc.)? ☐ Yes ☐ No
19. Have you had problems with concentration? ☐ Yes ☐ No
20. Have you been moving or speaking so slowly that others may have noticed? Or on the other hand, been so restless that you were moving around much more than usual. ☐ Yes ☐ No
21. How have the symptoms you reported above have affected your functioning over the last two weeks? _____

Other Disorders Screening

22. Have you been diagnosed with bipolar disorder or schizophrenia? ☐ Yes ☐ No
23. Have you experienced a traumatic event in your life that continues to bother you (e.g., you think about it, dream about it, feel anxious when reminded of it, etc.) ☐ Yes ☐ No
24. Have you been having trouble in your personal life, or at school/work, because of problems with alcohol within the past 3 months? ☐ Yes ☐ No
25. Have you been having trouble in your personal life, or at school/work, because of problems with drugs within the past 3 months? ☐ Yes ☐ No

Treatment

If prior questions regarding psychological symptoms were answered affirmatively ask the following:

26. Are you currently receiving psychotherapy for depression? ☐ Yes ☐ No
(If yes, how long have you been in treatment? : _____)

27. (you may know this already from above)
Are you currently receiving medication for MDD or another psychological condition? ☐ Yes ☐ No
If yes, what is it (name & dose) and how long have you been taking the medication? _____

28. **If you are eligible for the study and have symptoms of MDD:** Would you be willing to take medication as part of this study? ☐ Yes ☐ No

29. **Do you have a medical chart at the VA?** ☐ Yes ☐ No

30. **If yes, may we have permission to access your chart to look at your laboratory tests and other medical records?**

Document Subject's verbal consent:

Subject Agrees ☐ Subject Disagrees ☐

At the conclusion of the phone interview: "Thank you for answering my questions. If you have given us permission to do so, we will review your medical records. I will also review your responses to my questions with the study physician, and call you back. If it appears that you may be eligible for our research, we will also schedule an in-person screening evaluation at that time. Do you have any questions for me?"

Document Participant Disposition

Candidate is eligible for a face-to-face screening for the study based on phone screen: ☐ Yes ☐ No

Candidate's medical records have been reviewed: ☐ Yes ☐ No

If the candidate is not eligible for the study list the reason(s) why: _____

Contact the candidate following review and confirmation of available material and either schedule a face-to-face interview and review of the informed consent and HIPAA or inform him/her of ineligibility.

CONTINUE ON TO NEXT PAGE →

Appendix 2: Phone Pre-screen Identification Page

Phone Pre-Screen Identifier Page (if participant agrees to us review their medical records)
(to be stored separately from the telephone pre-screening form)

1. What is your full name: _____
2. What are the last four digits of your social security number? _____
3. What is your date of birth? _____
4. What is the best contact number for us to reach you regarding scheduling and reminder calls?
