A Double-Blind, Placebo-Controlled Study of Brexpiprazole plus Ketamine in Treatment-Resistant Depression (TRD)

May 10, 2019

PRINCIPAL INVESTIGATOR: Maurizio Fava, M.D.
Executive Director, MGH Clinical Trials Network and Institute (CTNI)
Executive Vice-Chair, Department of Psychiatry, Massachusetts General Hospital
55 Fruit Street – Bulfinch 351
Boston MA 02114
Phone: 617-724-0838
Fax: 617-726-2688
Email: MFava@Partners.org

STUDY SPONSOR: Otsuka Pharmaceuticals

A. TABLE OF CONTENTS

B. LIST OF TABLES

C. STUDY SYNOPSIS

Title of Study:	**A Double-Blind, Placebo-Controlled Study of Brexpiprazole plus Ketamine in Treatment-Resistant Depression (TRD)**
Objective:	To evaluate the efficacy, safety, and tolerability of brexpiprazole in combination with intranasal ketamine (40 mg biweekly for 2 weeks and weekly for 2 weeks), compared to placebo, as a rapidly acting treatment for TRD
Methodology:	This is a multi-site, double-blind, placebo-controlled study of the acute efficacy of brexpiprazole or placebo in combination with intranasal ketamine added to ongoing, stable, and adequate antidepressant therapy (ADT) in the treatment of adults with MDD with TRD.
Number of Patients:	52 Patients
Diagnosis and Main Criteria for Inclusion:	Male and female outpatients (aged 18 to 65 years) who meet DSM-5 (*Diagnostic and Statistical Manual of Mental Disorders*, Fifth Edition) criteria for MDD, have a current depressive episode of at least 8 weeks, and meet all of the following criteria: • <50% response to current antidepressant treatment (at least 8 weeks at an adequate dose, stable dose for at least 4 weeks) • >1 and <8 failed trials in the current episode as per MGH ATRQ • MADRS ≥ 20 at screening and at baseline • Good general health • BMI= 18-35 kg/m^2 • Willing to use birth control if the patient is of child bearing potential
Test Product, Dosage, and Mode of Administration:	Subjects will be randomly assigned to one of two arms in a 1:1 fashion: brexpiprazole 0.5-3 mg/day or placebo for four weeks in combination with bi-weekly administration of intranasal ketamine (40 mg) for two weeks, and then weekly administration of intranasal ketamine (40 mg) for two weeks.
Visit Schedule:	Screening Visit, Baseline visit (Visit 1), Visits 1 and 2 days after the Baseline Visit (Visits 2 and 3), and then every 3 days after that for 26 days, with end of study visit one week after the last double-blind visit.
Primary Outcome Measure:	Symptom improvement will be evaluated by assessing changes in the SDQ.
Secondary Outcome Measures:	MADRS, HAMD-6, CGI-S, CGI-I.

Safety and Tolerability Measures:	Adverse event (AE) recording, clinical laboratory measures, vital sign parameters, electrocardiograms (ECGs), physical examinations, CHRT.
Statistical Justification:	The sample size was chosen to ensure 80% power to detect an effect size of 0.8 on the SDQ between brexpiprazole and placebo at a two-sided 0.05 significance level. This assumes 52 subjects (26 subjects per arm).

D. BACKGROUND AND INTRODUCTION

Public Health Significance of Major Depressive Disorder (MDD)

Major Depressive Disorder (MDD) is a serious, debilitating, life-shortening illness that affects many persons of all ages and backgrounds. The point prevalence of MDD is high (2.3-3.2% in men; 4.5-9.3% in women) and the lifetime risk for MDD is 7.0-12.0% for men and 20.0-25.0% for women (Kessler et al, 2003). MDD is a disabling disorder that costs the U.S. over $80 billion per year in direct and indirect costs (Greenberg et al, 2003). Specifically, the annual cost of depression (approximately $83.1 billion in 2000) includes $26.1 billion (31%) in direct medical costs (e.g., diagnosis, treatments, and overall cost to the community), $5.4 billion (7%) in suicide-related mortality costs, and $51.5 billion (62%) in workplace costs (Greenberg et al, 2003). Depression substantially impairs work productivity, increasing work absence and reducing work performance (Stewart et al, 2003). Depressed workers report significantly more total health-related Lost Productive Time (LPT) than non-depressed workers (a mean 5.6 hours/week versus an expected 1.5 hours/week) (Stewart et al, 2003). Depression is also associated with increased health care utilization for general medical conditions, and at least a 50% increase in total health services costs (Simon et al, 2003, 1995; Unützer et al, 1997). Depression also has detrimental effects on all aspects of social functioning (e.g., self-care, social role, and family life, including household, marital, kinship, and parental roles) – costs not traditionally calculated (Fergusson et al, 1995; Wiersma, 1996; Paykel, 1999). Finally, recovery from depression is associated with lower primary care costs and less missed work time due to illness (Simon et al, 2002).

Modest Efficacy of Currently Available Therapies for MDD

All FDA-approved antidepressants used as monotherapies have shown only modest benefits. In fact, in acute (6-8 week) studies, typically with relatively uncomplicated, non-chronic forms of MDD, response rates (e.g., 50.0% or greater reduction in depressive symptoms) range between 45.0-55.0% and remission rates (e.g., minimal or no depressive symptoms) range between 30.0-35.0% (Steffens et al, 1997; Anderson, 2000; Anderson, 2001; Entsuah et al, 2001). Similarly, the efficacy of psychotherapy for the treatment of depression has been found to be modest as well, with effect sizes comparable to those of standard antidepressants (Cuijpers et al, 2010). Although the combination of antidepressants and psychotherapy appears to be somewhat more efficacious than either one approach alone, the efficacy of this combination treatment also remains limited (Cuijpers et al, 2009). Even after multiple, sequential interventions, only about 50-60% of patients achieve complete remission (Rush et al, 2006; Nelson, 2006; Insel and Wang, 2009).

Treatment-Resistant Depression (TRD)

Treatment-resistant depression (TRD) typically refers to the occurrence of an inadequate response following adequate antidepressant therapy among patients suffering from MDD (Fava, 2003). What constitutes inadequate response has been an object of considerable debate in the field, and most

experts would currently argue that inadequate response is the failure to achieve remission (Fava, 2003). Although the more traditional view of treatment resistance has focused on nonresponse (e.g., patients who have reported minimal or no improvement with drug therapy), failure to achieve remission despite adequate treatment represents a significant challenge for both clinicians and patients/consumers. In addition, response without remission has a potentially poorer outlook, as residual symptoms are associated with poorer outcome and increased relapse risk (Judd et al, 1998; Van Londen, et al 1998). Finally, response without remission following antidepressant treatment is associated with a significantly greater number of somatic symptoms than remission and with impaired social functioning (Denninger, et al 2006; Paykel, 2002). For these reasons, it has been argued that "complete remission should be the goal in the treatment of patients with MDD, because it leads to a symptom-free state and a return to premorbid levels of functioning" (Bakish, 2001). With this treatment approach in mind, inadequate response implies that the treatment failed to achieve remission. Several operational definitions of remission have been proposed, but from the clinician and patient perspective, remission typically implies achieving a relatively asymptomatic state (Frank et al, 1991). Since treatment resistance in depression typically concerns the occurrence of an inadequate response following adequate antidepressant therapy, it is apparent that the number of failed adequate antidepressant trials may range from one to countless. However, the most common definition of TRD is that of inadequate response (e.g., failure to achieve remission) to at least one antidepressant trial of adequate dose and duration (Fava and Davidson, 1996). The findings of the largest clinical trial in MDD, the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial, have shown that approximately two thirds of MDD patients do not achieve remission following an initial adequate trial of antidepressant therapy, suggesting that TRD is highly common in practice (Trivedi et al, 2006).

There are two approaches to the study of treatments for TRD. One uses the prospective ascertaining of treatment resistance, with the goal of minimizing the possible confounding effect of the patients' recall bias. Patients who are currently symptomatic may underestimate the degree of improvement experienced in the course of the index episode, particularly with prior treatments. As a result of recall biases, relapses during antidepressant treatment may be misdiagnosed as nonresponse. When treatment resistance is assessed prospectively, patients are followed by the study clinicians throughout sequential trials of antidepressant medications, using standard measures of depressive symptoms to monitor clinical progress. When a retrospective assessment is used, researchers use one of three instruments to assess treatment resistance in depression. Two are clinician-rated [the Antidepressant Treatment History Form (ATHF) (Sackeim, 2001) and the Harvard Antidepressant Treatment History (HATH) (Nierenberg et al, 1991)], and one is self-rated [the Massachusetts General Hospital Antidepressant Treatment Response Questionnaire (MGH ATRQ) (Fava and Davidson, 1996)). Of these three instruments, only two, the ATHF and the MGH ATRQ, have been empirically validated (Sackeim, 2001; Chandler et al, 2010).

There is substantial mortality and morbidity associated with TRD, and both depression and general medical health care expenditures tend to increase with the degree of TRD (Russell et al, 2004). TRD is associated with poorer clinical outcome, particularly among those who require multiple antidepressant medications (Fekadu et al, 2009). The scope of this problem is therefore highly significant both in terms of the numbers of affected individuals and the resultant societal and economic burdens. Despite the enormous public health significance of this problem, systematic research on TRD has yielded only limited success (Nierenberg et al, 2007).

Lag Time Limitations of Current Therapies for TRD

There are only three pharmacological treatments approved by the FDA for adjunctive treatment in patients with TRD: aripiprazole, quetiapine, and olanzapine (Papakostas et al, 2007; Bauer et al, 2010; Nelson and Papakostas, 2009; Tohen et al, 2010). In addition, only three non-pharmacological therapies have been approved for TRD: transcranial magnetic stimulation (TMS) (O'Reardon et al, 2007), vagus nerve stimulation (VNS), and electroconvulsive therapy (ECT) (Bajbouj et al, 2010; Trevino et al, 2010). However, as currently delivered, none of these pharmacologic and non-pharmacologic treatments has been shown to result in rapid symptom resolution (defined as a sizeable and statistically significant treatment effect versus placebo that is apparent as early as 24 to 72 hours post-initiation of therapy), despite the tremendous need for rapid antidepressant therapies that would allow for meaningful clinical improvements within the context of very short hospital admissions for TRD patients.

Promising Preliminary Results with Ketamine Therapy

Over the last decade, a series of studies has demonstrated the ability of novel approaches – such as ketamine, an N-Methyl-D-aspartate (NMDA) antagonist,– to provide significant amelioration of symptoms within a few hours, with symptoms typically returning within a period of days after discontinuation of the acute intervention (Machado-Vieira et al, 2009). The exact mechanism of action of ketamine (see below) is not clear (Machado-Vieira et al, 2009). However, the promising results seen in these trials with ketamine therapy highly suggest that a more ambitious program of research might accelerate development of rapid-onset antidepressant treatment.

D.1.a. TOXICOLOGY - KETAMINE

D.1.a.1. SINGLE DOSE TOXICITY STUDIES

The LD50 for ketamine ranges between 140.0 mg/kg (rat, intraperitoneal injection) and 616.0 mg/kg (mouse, oral administration) (CVMP, 1997). These high LD50-values highly exceed the dose level of ketamine in the proposed study.

D.1.a.2. REPEAT-DOSE TOXICITY STUDIES

In rats daily intravenous doses of 2.5, 5.0 or 10.0 mg/kg for six weeks provoked a slight decrease in food intake and moderate body weight gain suppression (CVMP, 1997). In a 6-week dog study, ketamine was injected intramuscularly at 4.0, 20.0 or 40.0 mg/kg/day. At all dose levels there was some degree of weight loss and anorexia. Some blood parameters were dose-dependently elevated. Histological changes in the liver were minor (CVMP, 1997). The magnitude of the dose levels associated with these effects, and the duration of treatment in these rat and dog studies, highly exceed the dose level of ketamine in this study.

D.1.a.3. NEUROTOXICITY

Ketamine has been shown to induce neuronal vacuolation and apoptotic cell death in the posterior cingulate and retrosplenial cortex of adult rats (40.0-60.0 mg/kg, single subcutaneous or intraperitoneal dose) and adult mice (50.0 mg/kg/day intraperitoneally for 7 days) (Olney 1989; Jevtovic-Torodovic 2000, 2001, 2005; Zuo 2007). In the hippocampus of adult mice treated with ketamine at 5.0 mg/kg intraperitoneally for 5 days, increased cell death was reported (Majewski-Tiedeken 2008). The relevance to adult humans is unknown. The magnitude of the dose levels associated with these rodent findings highly exceed the dose level of ketamine in the proposed study.

D.1.a.4. DEVELOPMENTAL OR REPRODUCTIVE TOXICITY STUDIES

An embryo-fetal developmental toxicity study in rats given intraperitoneal doses of 25.0, 50.0 or 100.0 mg/kg during days 1-15 or 5-15 of gestation showed pathological degenerative changes in the fetuses at histological examination (focal nuclear hypochromatosis and interfibrillary edema of the heart; parenchymal liver cell degeneration; proximal convoluted tubule degeneration). This degenerative effect was observed at all dose levels, and was dependent upon the dose and the duration of treatment (CVMP, 1997; Kochhar 1986). In an embryo-fetal developmental toxicity study mice were injected with ketamine (50.0 mg/kg/day) intravenously during the period of organogenesis. No treatment-related effects were noted (Abdel-Rahman and Ismail 2000).

Reproduction studies in dogs, injected with 25.0 mg/kg ketamine intramuscularly six times during one trimester of pregnancy (twice a week over a three-week period), did not show apparent adverse effects. Rats were injected during the premating period (10.0 mg/kg intravenously), the period of organogenesis (20.0 mg/kg intramuscularly) and the perinatal period (20.0 mg intramuscularly), and rabbits were injected during the period of organogenesis (20.0 mg/kg intramuscularly). Ketamine did not affect reproduction (CVMP, 1997). From these studies, no definitive conclusions can be drawn on the reproductive toxicity potential of ketamine. The magnitude of the dose levels associated with the rat fetal toxicity findings highly exceed the dose level of ketamine in the proposed study.

D.1.a.5. GENETIC TOXICITY STUDIES

In an Ames test, ketamine did not show a mutagenic effect. Ketamine has been reported to show genotoxic potential in an in vitro sister chromatic exchange assay in Chinese Hamster ovary cells (Adhvaryu et al., 1986; CVMP, 1997) and an in vitro micronucleus test in Chinese Hamster lung

fibroblasts (Toyama 2006). From these studies, no definitive conclusions can be drawn on the genotoxic potential of ketamine.

D.1.b. Toxicology – Brexpiprazole

D.1.b.1. Carcinogenesis

Lifetime carcinogenicity studies were conducted in ICR mice and SD rats. Brexpiprazole was administered orally for two years to male and female mice at doses of 0.75, 2 and 5 mg/kg/day (0.9 to 6.1 times the oral MRHD of 4 mg/day based on mg/m2 body surface area) and to male and female rats at doses of 1, 3, and 10 mg/kg and 3, 10, and 30 mg/kg/day, respectively (2.4 to 24 and 7.3 to 73 times the oral MRHD, males and females). In female mice, the incidence of mammary gland adenocarcinoma was increased at all doses and the incidence of adenosquamous carcinoma was increased at 2.4 and 6.1 times the MRHD. No increase in the incidence of tumors was observed in male mice. In the rat study, brexpiprazole was not carcinogenic in either sex at doses up to 73 times the MRHD. Proliferative and/or neoplastic changes in the mammary and pituitary glands of rodents have been observed following chronic administration of antipsychotic drugs and are considered to be prolactin mediated. The potential for increasing serum prolactin level of brexpiprazole was shown in both mice and rats. The relevance for human risk of the findings of prolactin-mediated endocrine tumors in rodents is unknown.

D.1.b.2. Mutagenesis

Brexpiprazole was not mutagenic when tested in the in vitro bacterial reverse mutation assay (Ames test). Brexpiprazole was negative for clastogenic activity in the in vivo micronucleus assay in rats, and was not genotoxic in the in vivo/in vitro unscheduled DNA synthesis assay in rats. In vitro with mammalian cells brexpiprazole was clastogenic but only at doses that induced cytotoxicity. Based on a weight of evidence, brexpiprazole is not considered to present a genotoxic risk to humans.

D.1.b.3. Impairment of Fertility

Female rats were treated with oral doses of 0.3, 3 or 30 mg/kg/day (0.7, 7.3, and 73 times the oral MRHD on a mg/m2 basis) prior to mating with untreated males and continuing through conception and implantation. Estrus cycle irregularities and decreased fertility were observed at 3 and 30 mg/kg/day. Prolonged duration of pairing and increased preimplantation losses were observed at 30 mg/kg/day. Male rats were treated with oral doses of 3, 10, or 100 mg/kg/day (7.3, 24 and 240 times the oral MRHD on a mg/m2 basis) for 63 days prior to mating with untreated females and throughout the 14 days of mating. No differences were observed in the duration of mating or fertility indices in males at any dose of brexpiprazole

D.2. Clinical Experience – Ketamine and Brexpiprazole

D.2.a. Ketamine

Ketamine is a noncompetitive antagonist of NMDA glutamate receptors and it has been safely used for anesthesia and analgesia for many years. Early case reports indicated a possible antidepressant effect of ketamine administered as an anesthetic to patients with depression undergoing surgical procedures (Kudoh, 2002, Correll, 2006). In a small double-blind trial of a single IV ketamine infusion, patients with TRD (defined as at least two failed trials of an antidepressant) showed a dramatic decrease in depressive symptoms, which was sustained for approximately 3 days (Berman, 2000). More recent reports have confirmed the antidepressant effects of intravenous ketamine. In a double-blind, crossover study in 18 patients with TRD, the IV administration of a single dose of ketamine (0.5 mg/kg) led to a significant rapid improvement in depressive symptoms sustained up to seven days after the infusion (Zarate, 2006). In two open-label trials on patients with TRD, ketamine infusion rapidly reduced depressive symptoms (Phelps, 2009; Price, 2009). Most importantly, ketamine produced a rapid and robust reduction not only in global depression scores, but in severity of suicidal ideation, supporting the use and safety of ketamine in acutely suicidal depressed patients. The patients enrolled in those studies had a depression rated as moderate to severe, were on no other psychotropic medications and were followed for a short period of time. Another recent open label study supported the safety of repeated intravenous ketamine administrations (6 infusions over 2 weeks) for patients with TRD who had significantly improved after a single infusion (Rot, 2010).

More recently, clinical researchers have examined the utility of a more user-friendly administration route, intranasal, for ketamine. In a randomized, double-blind, crossover study (Lapidus et al, 2014) designed to test the safety, tolerability, and efficacy of intranasal ketamine in patients with depression who had failed at least one prior antidepressant trial, 20 patients with major depression were randomly assigned, and 18 completed 2 treatment days with intranasal ketamine hydrochloride (40 mg) or saline solution. The primary efficacy outcome measure was change in depression severity 24 hours after ketamine or placebo, measured using the Montgomery-Åsberg Depression Rating Scale. Secondary outcomes included persistence of benefit, changes in self-reports of depression, changes in anxiety, and proportion of responders. Potential psychotomimetic, dissociative, hemodynamic, and general adverse effects associated with ketamine were also measured. Patients showed significant improvement in depressive symptoms at 24 hours after ketamine compared to placebo ($t = 4.39$, $p < .001$; estimated mean Montgomery-Åsberg Depression Rating Scale score difference of 7.6 ± 3.7; 95% confidence interval, 3.9-11.3). Response criteria were met by 8 of 18 patients (44%) 24 hours after ketamine administration compared with 1 of 18 (6%) after placebo ($p = .033$). Intranasal ketamine was well tolerated with minimal psychotomimetic or dissociative effects and was not associated with clinically significant changes in hemodynamic parameters. This study provided the first controlled evidence for the rapid antidepressant effects and safety of intranasal ketamine.

A recent meta-analysis (Xu et al, 2016) identified nine trials, including 201 patients (52% female, mean age 46 years), with six trials assessing low-dose ketamine (0.5mg/kg i.v.) and 3 testing very

low-dose ketamine (one trial assessed 50mg intra-nasal spray, another assessed 0.1-0.4mg/kg i.v., and another assessed 0.1-0.5mg/kg i.v., intramuscular, or s.c.). At day 3, the reduction in depression severity score was less marked in the very low-dose trials (P homogeneity <.05). The absolute benefits were large, with day 7 remission rates of 24% vs 6% (P=.02). There is substantial heterogeneity in clinical response, with remission among one-fifth of patients at 1 week but most others having benefits that are less durable.

D.2.a.1. KETAMINE SAFETY

For a summary of clinical information regarding the efficacy (for anesthesia) and safety of ketamine, refer to the latest version of the U.S. package insert.

Ketamine administration at sub-anesthetic doses appears to present an acceptable level of risk for carefully screened populations of medically healthy human subjects in the context of clinical research programs that intensively monitor subjects throughout their study participation (Perry, 2007).

D.2.a.2. KETAMINE PHARMACOKINETIC STUDIES AND DRUG-DRUG INTERACTIONS

Bioavailability following an intramuscular dose is 93.0%, intranasal dose 25.0-50.0%, and oral dose 20±7.0%. Ketamine is rapidly distributed into brain and other highly perfused tissues, and is 12.0% bound in plasma. The plasma half-life is 2.3 ± 0.5 hours. Oral administration produces lower peak concentrations of ketamine, but increased amounts of the metabolites norketamine and dehydronorketamine. Ketamine and its metabolites undergo hydroxylation and conjugation. Norketamine produces effects similar to those of ketamine. There are no significant differences between the pharmacokinetic properties of the S-(+) and R-(-)-isomers.

Drug Interactions: Midazolam attenuates altered perception and thought processes. Lorazepam may decrease ketamine-associated emotional distress but does not decrease cognitive or behavioral effects of ketamine. Acute administration of diazepam increases the half-life of ketamine. Lamotrigine significantly decreases ketamine-induced perceptual abnormalities, but increases the mood elevating effects. Haloperidol may decrease impairment by ketamine in executive control functions, but does not affect psychosis, perceptual changes, negative schizophrenic-like symptoms, or euphoria. Alfentanil is additive to ketamine in decreasing pain and increasing cognitive impairment. Physostigmine and 4-aminopyridine can antagonize some pharmacodynamic effects of ketamine.

D.2.b. BREXPIPRAZOLE

Brexpiprazole (Rexulti(®)) is a serotonin-dopamine activity modulator, with a unique receptor binding profile and low intrinsic D2 activity suggestive of a lower potential than aripiprazole to cause activation-like adverse effects, such as akathisia (McKeage K, 2016). The drug was recently approved by the US FDA for adjunctive therapy with antidepressant treatment (ADT) in patients with major depressive disorder (MDD) and inadequate response to ADT. In two phase III trials, adjunctive oral

brexpiprazole 2 or 3 mg once daily was more effective than monotherapy with ADT in improving depressive symptoms in adults with MDD who demonstrated an incomplete response to previous treatment with ADT (McKeage K, 2016). Adjunctive brexpiprazole was generally well tolerated in clinical trials, which included treatment periods of up to 52 weeks (McKeage K, 2016). There is preliminary evidence that adjunctive treatment with brexpiprazole may represent a strategy for patients with MDD and inadequate response to antidepressant treatment who have symptoms of irritability (Fava M et al, 2016) and anxiety (McIntyre RS et al, 2016).

E. STUDY RATIONALE

Primary Aim: The primary objective is to investigate whether brexpiprazole (initial dose .5 mg/day titrating to 3 mg/day over 21 days) is superior to placebo when combined with intranasal ketamine in the acute treatment of patients with TRD at two weeks (Day 14), when added to ongoing and stable antidepressant therapy with respect to the study primary outcome measure [change in the SDQ total score].

Secondary Aim 1: The secondary objective #1 is to investigate whether brexpiprazole (initial dose .5 mg/day titrating to 3 mg/day over 21 days) is superior to placebo when combined with intranasal ketamine in the acute treatment of patients with TRD within 48 hours (Day 2), when added to ongoing and stable antidepressant therapy with respect to the study primary outcome measure [change in the SDQ total score].

Secondary Aim 2: The secondary objective #2 is to investigate whether brexpiprazole (initial dose .5 mg/day titrating to 3 mg/day over 21 days) is superior to placebo when combined with intranasal ketamine in the acute treatment of patients with TRD at four weeks (Day 28), when added to ongoing and stable antidepressant therapy with respect to the study primary outcome measure [change in the SDQ total score].

Secondary Aim 3: The secondary objective #3 is to investigate whether brexpiprazole (initial dose .5 mg/day titrating to 3 mg/day over 21 days) is superior to placebo when combined with intranasal ketamine in the acute treatment of patients with TRD at four weeks (Day 28), when added to ongoing and stable antidepressant therapy with respect to the rates of sustained response [50% or greater reduction in the MADRS total score].

Secondary Aim 4: The secondary objective #4 is to investigate whether brexpiprazole (initial dose .5 mg/day titrating to 3 mg/day over 21 days) is superior to placebo when combined with intranasal ketamine in the acute treatment of patients with TRD at Day 2, Day 14, and Day 28, when added to ongoing and stable antidepressant therapy with respect to the study secondary outcome measures [MADRS, HAMD-6, CGI-S, CGI-I].

Secondary Aim 5: The secondary objective #5 is to characterize the safety and tolerability of brexpiprazole or placebo treatment during the acute, 4-week treatment period.

F. OBJECTIVES

F.1. PRIMARY AIM OF THE KETAMINE-BREXPIPRAZOLE TRIAL

The primary objective is to investigate whether the degree of improvement in SDQ total score on brexpiprazole (initial dose .5 mg/day titrating to 3 mg/day over 21 days is superior to placebo when combined with intranasal ketamine in the acute treatment of patients with TRD at two weeks (Day 14), when added to ongoing and stable antidepressant therapy with respect to the study primary outcome measure. Superiority will be demonstrated by a statistically significant greater decrease ($p<0.05$, 2-sided) on the SDQ total score for patients receiving brexpiprazole versus placebo therapy.

F.2. SECONDARY AIMS OF THE KETAMINE-BREXPIPRAZOLE TRIAL

The secondary objectives are to:

- Investigate whether brexpiprazole (initial dose .5 mg/day titrating to 3 mg/day over 21 days) is superior to placebo when combined with intranasal ketamine in the acute treatment of patients with TRD within 48 hours (Day 2), when added to ongoing and stable antidepressant therapy with respect to the study primary outcome measure [change in the SDQ total score]. Superiority will be demonstrated by a statistically significant greater decrease ($p<0.05$, 2-sided) on the SDQ total score for patients receiving brexpiprazole versus placebo therapy.

- Investigate whether the degree of improvement in SDQ total score on brexpiprazole (initial dose .5 mg/day titrating to 3 mg/day over 21 days) is superior to placebo when combined with intranasal ketamine in the acute treatment of patients with TRD at four weeks (Day 28), when added to ongoing and stable antidepressant therapy with respect to the study primary outcome measure. Superiority will be demonstrated by a statistically significant greater decrease ($p<0.05$, 2-sided) on the SDQ total score for patients receiving brexpiprazole versus placebo therapy.
- Investigate whether brexpiprazole (initial dose .5 mg/day titrating to 3 mg/day over 21 days) is superior to placebo when combined with intranasal ketamine in the acute treatment of patients with TRD at 4 weeks (Day 28), when added to ongoing and stable antidepressant therapy with respect to the rates of sustained response [50% or greater reduction in the MADRS total score]. Superiority will be demonstrated by a statistically significant greater ($p<0.05$, 2-sided) of sustained response for patients receiving brexpiprazole versus placebo therapy.

- Investigate whether the degrees of improvement in secondary outcome measures [MADRS, HAMD-6, CGI-S, CGI-I] total scores on brexpiprazole (initial dose .5 mg/day titrating to 3 mg/day over 21 days) are superior to placebo when combined with intranasal ketamine in the acute treatment of patients with TRD at Day 2, Day 14, and Day 28, when added to ongoing and stable antidepressant therapy with respect to the study primary outcome measure. Superiority will be demonstrated by a statistically significant greater decrease ($p<0.05$, 2-sided) on the total scores of these secondary measures for patients receiving brexpiprazole versus placebo therapy.
- Characterize the safety and tolerability of brexpiprazole compared to placebo using the following information: vital signs, treatment-emergent adverse events, the CHRT, 12-lead electrocardiogram (ECG), and other laboratory analyses.

G. STUDY DESIGN

G.1. OVERVIEW

This is a multi-site, double-blind, placebo-controlled study of the acute efficacy of oral brexpiprazole or placebo combined with intranasal ketamine added to ongoing, stable, and adequate antidepressant therapy (ADT) in the treatment of adults with MDD with TRD. Adequate ADT is defined as a therapeutically sufficient dose for a sufficient treatment period, which would be expected to be effective as listed in the MGH ATRQ.

G.2. METHODS

Following a washout period for patients on prohibited psychotropic agents, 52 eligible subjects will be randomly assigned to one of two arms in a 1:1 fashion: brexpiprazole (initial dose .5 mg/day titrating to 3 mg/day over 21 days) or placebo for four weeks in combination with bi-weekly administration of intranasal ketamine (40 mg) for two weeks, followed by weekly administration of intranasal ketamine (40 mg) for two weeks. All subjects will be followed for 28 days to examine the efficacy and tolerability of the study drugs' effects.

G.3. STUDY DESIGN

The study will consist of a screening period and a randomization period. Patients who meet eligibility during the screening period will be randomized to double-blind treatment with brexpiprazole plus intranasal ketamine treatment or placebo as described above.

H. STUDY POPULATION

H.1. NUMBER OF SUBJECTS

52 subjects will enter the double-blind treatment phase of the study. This trial will be conducted according to the U.S. FDA guidelines and the Declaration of Helsinki. IRB- approved written informed consent will be obtained from all patients before any protocol-specified procedures are carried out. The subjects will be drawn from an outpatient sample of patients with MDD, diagnosed with the use of the Structured Clinical Interview for DSM-5 Axis I Disorders–Patient Edition (SCID-5), currently on an adequate dose of antidepressant therapy, as defined by the MGH ATRQ, for at least 8 weeks, with the dose being stable for at least 4 weeks, and with TRD (see definition below). For entry into the study, all inclusion criteria must be met, and none of the exclusion criteria can be met.

H.2. SUBJECT ELIGIBILITY

H.2.a. INCLUSION CRITERIA

A subject will be eligible for inclusion only if all of the following criteria are met:

1. Male or female, 18 to 65 years of age, inclusive, at screening.
2. Able to read, understand, and provide written, dated informed consent prior to screening. Participants will be deemed likely to comply with study protocol and communicate with study personnel about adverse events and other clinically important information.
3. Diagnosed with MDD, single or recurrent, and currently experiencing a major depressive episode (MDE) of at least eight weeks in duration, prior to screening, according to the criteria defined in the Diagnosis and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). The diagnosis of MDD will be made by a site psychiatrist and supported by the SCID-5. The diagnosis will be confirmed by remote, independent raters from the MGH CTNI (Clinical Trials Network and Institute) with a SAFER interview.
4. Has a history of TRD during the current MDE, as assessed by the investigator and remote centralized rater using the MGH ATRQ. TRD is defined as failure to achieve a satisfactory response (e.g., less than 50% improvement of depression symptoms), as perceived by the participant, to at least 2 “treatment courses” during the current episode of a therapeutic dose of an antidepressant therapy of at least 8 weeks duration (including the current ADT). The adequacy of dose and duration of the antidepressant therapy will be determined as per the MGH ATRQ criteria. The TRD status will be confirmed by remote, independent raters from the MGH CTNI who will administer the MGH ATRQ, via teleconference, between the screening visit and the baseline visit. Participants must currently be on a stable (for at least 4 weeks) and adequate (according to the MGH ATRQ) dose of ongoing antidepressant therapy (any antidepressant therapy, with the exception of MAOIs or antipsychotics), of which total duration must be at least 8 weeks.
5. Meet the threshold on the total MADRS score of $\geq$20 at both the screen visit and the baseline visit (Day -7/-28 and Day 0), and as confirmed by the remote centralized MGH CTNI rater between the screen visit and the baseline visit.

6. In good general health, as ascertained by medical history, physical examination (PE) (including measurement of supine and standing vital signs), clinical laboratory evaluations, and ECG.
7. If female, a status of non-childbearing potential or use of an acceptable form of birth control per the following specific criteria:
    a. Non-childbearing potential (e.g., physiologically incapable of becoming pregnant, i.e., permanently sterilized (status post hysterectomy, bilateral tubal ligation), or is post-menopausal with her last menses at least one year prior to screening); or
    b. Childbearing potential, and meets the following criteria:
        i. Childbearing potential, including women using any form of hormonal birth control, on hormone replacement therapy started prior to 12 months of amenorrhea, using an intrauterine device (IUD), having a monogamous relationship with a partner who has had a vasectomy, or is sexually abstinent.
        ii. Negative urinary pregnancy test at screening, confirmed by a negative urinary pregnancy test at randomization prior to receiving study treatment.
        iii. Willing and able to continuously use one of the following methods of birth control during the course of the study, defined as those which result in a low failure rate (i.e., less than 1% per year) when used consistently and correctly: implants, injectable or patch hormonal contraception, oral contraceptives, IUD, double-barrier contraception, sexual abstinence. The form of birth control will be documented at screening and baseline.
8. Body mass index between 18-35 kg/m^2.
9. Concurrent psychotherapy will be allowed if the type (e.g., supportive, cognitive behavioral, insight-oriented, et al.) and frequency (e.g., weekly or monthly) of the therapy has been stable for at least three months prior to screening and if the type and frequency of the therapy is expected to remain stable during the course of the subject's participation in the study.
10. Concurrent hypnotic therapy (e.g., with zolpidem, zaleplon, melatonin, benzodiazepines or trazodone) will be allowed if the therapy has been stable for at least 4 weeks prior to screening and if it is expected to remain stable during the course of the subject's participation in the study. Patients can also continue treatment with benzodiazepines used for anxiety if therapy has been stable for at least 4 weeks prior to screening and if it is expected to remain stable during the course of the subject's participation in the study.

H.2.b. EXCLUSION CRITERIA

A potential participant will NOT be eligible for participation in this study if any of the following criteria are met:

1. Female of childbearing potential who is not willing to use one of the specified forms of birth control during the study.
2. Female that is pregnant or breastfeeding.
3. Female with a positive pregnancy test at screening or baseline.
4. History during the *current* MDE of failure to achieve satisfactory response (e.g., less than 50% improvement of depression symptoms) to >7 treatment courses of a therapeutic dose of an antidepressant therapy of at least 8 weeks duration, according to the MGH ATRQ, as confirmed by the remote, independent MGH CTNI rater.
5. Total MADRS score of <20 at the screen visit or the baseline visit, or as assessed by the remote, independent MGH CTNI rater and reported to the site.
6. Current diagnosis of a substance use disorder (abuse or dependence, as defined by DSM V-TR™), with the exception of nicotine dependence, at screening or within 6 months prior to screening.
7. Current Axis I disorder, diagnosed at screening with the use of the Structured Clinical Interview for DSM-5 AXIS I Disorders (SCID-5), that is the principal focus of treatment and MDD the secondary focus of treatment for the past 6 months or more.
8. History of bipolar disorder, schizophrenia or schizoaffective disorders, or any history of psychotic symptoms in the current or previous depressive episodes.
9. History of anorexia nervosa, bulimia nervosa, or eating disorder not otherwise specified, within 5 years of screening.
10. Any Axis I or Axis II Disorder, which at screening is clinically predominant to their MDD or has been predominant to their MDD at any time within 6 months prior to screening.
11. In the judgment of the investigator, the subject is considered at significant risk for suicidal behavior during the course of his/her participation in the study.
12. Has failed to respond to ECT during the current depressive episode.
13. Has received VNS at any time prior to screening.
14. Has dementia, delirium, amnestic, or any other cognitive disorder.
15. Has a clinically significant abnormality on the screening physical examination that might affect safety, study participation, or confound interpretation of study results according to the study clinician.
16. Participation in any clinical trial with an investigational drug or device within the past month or concurrent to study participation.
17. Current episode of:
    a. Hypertension, Stage 1 as defined by a systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥100 mHg at the Screening and Baseline Visits within 1.5

hours prior to randomization on two of three measurements (standing and supine) at least 15 minutes apart.

b. Recent myocardial infarction (within one year) or a history of myocardial infarction.
c. Syncopal event within the past year.
d. Congestive heart failure (CHF) New York Heart Association Criteria >Stage 2
e. Angina pectoris.
f. Heart rate <45 or >110 beats per minute at screening or randomization (Baseline Visit).
g. QTcF (Fridericia-corrected) ≥450 msec at screening or randomization (Baseline Visit).

18. Chronic lung disease excluding asthma.
19. Lifetime history of surgical procedures involving the brain or meninges, encephalitis, meningitis, degenerative central nervous system (CNS) disorder (e.g., Alzheimer's or Parkinson's Disease), epilepsy, mental retardation, or any other disease/procedure/accident/intervention which, according to the screening clinician, is deemed associated with significant injury to or malfunction of the CNS, or history of significant head trauma within the past 2 years.
20. Presents with any of the following lab abnormalities:
    a. Thyroid stimulating hormone (TSH) outside of the normal limits and clinically significant as determined by the investigator. Free thyroxine (T4) levels may be measured if TSH level is high. Subject will be excluded if T4 level is clinically significant.
    b. Patients with diabetes mellitus fulfilling any of the following criteria:
        i. Unstable diabetes mellitus defined as glycosylated hemoglobin (HbA1c) >8.5% at screening.
        ii. Admitted to hospital for treatment of diabetes mellitus or diabetes mellitus-related illness in the past 12 weeks.
        iii. Not under physician care for diabetes mellitus.
        iv. Has not been on the same dose of oral hypoglycaemic drug(s) and/or diet for the 4 weeks prior to screening. For thiazolidinediones (glitazones) this period should not be less than 8 weeks.
    c. Any other clinically significant abnormal laboratory result (determined as such by the investigator and MGH CTNI medical monitor) at the time of the screening.
21. History of hypothyroidism and has been on a stable dosage of thyroid replacement medication for less than 2 months prior to screening. (Subjects on a stable dosage of thyroid replacement medication for at least 3 months or more prior to screening are eligible for enrollment.)

22. History of hyperthyroidism which was treated (medically or surgically) less than 6 months prior to screening.
23. History of positive screening urine test for drugs of abuse at screening: cannabinoids (if the patient has a legitimate medical prescription for cannabis, patient must agree to abstain during the entirety of the study and to have a negative test at baseline), cocaine, amphetamines, barbiturates, opiates (unless use is in accordance with guidance provided in table of allowed and excluded medications).
24. Patients with exclusionary laboratory values (see Table 1), or requiring treatment with exclusionary concomitant medications (see Appendix 1).
25. Patients on exclusionary concomitant psychotropic medications, the half-life of which would not allow sufficient time for patients to have been free of the medication post-taper for five half-lives within the maximum screening period (28 days).
26. Patients with a history of narrow angle glaucoma.
27. Liver or renal Function tests which meet the exclusion criteria in Table 1, or a history of hepatic or renal dysfunction.

TABLE 1-EXCLUSIONARY SAFETY VALUES OF POTENTIAL CLINICAL CONCERN

Hematology	
Leukocytes	<2 or >17.5 x $10^3/mm^3$
Platelets	<75 or >700 x $10^3/mm^3$
Chemistry	
Total bilirubin	>2 times the upper limit of the reference range
AST*	>2.5 times upper limit of the reference range
ALT*	>2.5 times upper limit of the reference range
GGT*	>2.5 times upper limit of the reference range
Alk Phosphatase*	>2.5 times upper limit of the reference range
Creatinine	>1.3 times upper limit of the reference range
BUN/Urea	>1.3 times upper limit of the reference range
Glucose	< 70 mg/dl or >2 times the limits of the reference range
Uric acid	>1.5 times upper limit of the reference range

*LFTs higher than 2.5 times ULN will be exclusionary.

H.3. RATIONALE FOR THE INCLUSION/EXCLUSION CRITERIA

Eligibility criteria for this study have been carefully considered to ensure the safety of the study subjects and to ensure that the results of the study are meaningful in support of the research hypotheses. It is imperative that subjects fully meet all eligibility criteria.

I. STUDY ASSESSMENTS – PLAN AND METHODS

I.1. STUDY CONDUCT

An overview of the schedule of visits and procedures is shown in Table 2.

TABLE 2– OVERVIEW OF STUDY SCHEDULE

Screen Visit	Screening Period	V1 / Baseline Visit	V2	V 3	V 4	V 5	V 6	V 7	V 8	V 9	V 10	V 11
Day -28 to Day -7	Taper (opt.) 5 half-lives of excluded psychotropic drug(s)	Day 0	Day 1	Day 2	Day 5	Day 8	Day 11	Day 14	Day 17	Day 21	Day 23	Day 28
		Baseline Assess.	Assess.	Assess.	Assess.	Assess.	Assess.	Assess.	Assess.	Assess.	Assess.	Assess.
		Intranasal Ketamine Admin.	Assess.	Assess.	Intranasal Ketamine Admin.	Intranasal Ketamine Admin.	Intranasal Ketamine Admin.	Intranasal Ketamine Admin.	Assess.	Intranasal Ketamine Admin.	Assess.	Assess.
		Study Drug*	Study Drug*	Study Drug*	Study Drug*	Study Drug*	Study Drug*	Study Drug*	Study Drug*	Study Drug*	Study Drug*	Study Drug*

*Brexpiprazole or Placebo

Subjects will be screened for eligibility as detailed above. Study participants will be consented prior to the administration of any procedure or wash-out. Following a washout period, under the close supervision and monitoring of the study clinicians, for patients on psychotropic drugs (with the exception of ongoing antidepressant therapy and of ongoing hypnotic therapy), baseline assessment, MGH CTNI remote interview, and re-confirmation that all criteria for entry into the study are met, eligible subjects will be randomly assigned to one of two arms: brexpiprazole plus intranasal ketamine (n=25) or placebo plus intranasal ketamine (n=25). The brexpiprazole dose will be titrated in the following manner: 0.5 mg/day during the first week, at the Visit 5/Day 8 visit, the dose will be increased to 1 mg/day , at Visit 7/Day 14 the dose will be increased to 2 mg/day, and at Visit 9,/Day 21, the dose will be increased to 3 mg/day. If the dose escalation is not tolerated, patient can go back to previous dose (fixed-flexible dose escalation). As per Table 2, all subjects will be followed for 28 days to examine the efficacy and tolerability of brexpiprazole plus intranasal ketamine's effects. The study assessments will be performed at Days 0, 1, 2, 5, 8, 11, 14, 17, 21, 23, and 28 to assess the safety and efficacy of brexpiprazole plus intranasal ketamine compared to placebo plus intranasal ketamine treatment therapy in depressed patients who have demonstrated an inadequate response

to at least 2 adequate antidepressant therapies (ADTs) during the current MDE. Intranasal ketamine (40 mg) will be administered after all the assessments are completed on Days 0, 5, 8, 11, 14, and 21.

I.1.a. SCREENING PHASE

The screening period will range from a minimum of 7 days to a maximum 28 days. The purpose is to ensure that only appropriate patients are entered into the study. The investigator will determine that the patients meet eligibility criteria and will collect the demographic and medical data permitting full characterization of the patients. The screening period is also designed to ensure that any prohibited pharmacotherapy is washed out. Benzodiazepine therapy is allowed per the requirements in Appendix I. The screening period begins when informed consent is signed. Screening evaluations are to be conducted as outlined in the Study Schedule (

TABLE 3– STUDY SCHEDULE

).

The screening period will range from a minimum of 7 days to a maximum of 28 days. The screening period begins when informed consent is signed. Only the study physicians will perform the informed consent procedures. Once patients agree to participate in the study by signing the informed consent document, a full medical and psychiatric history will be taken and a physical and laboratory examination will be performed, as outlined in the Table 3.

Only patients currently on stable antidepressant therapy will be eligible for the study. During screening, investigators must document history during the *current* episode of inadequate response to at least 2 trials of antidepressant treatment of adequate dose and duration, as specified by the MGH ATRQ (Fava and Davidson, 1996; Fava, 2003; Chandler et al, 2010). Such determination will be confirmed by the remote, independent MGH CTNI raters.

We have observed in 3 multi-center trials recently completed by the MGH CTNI that the placebo responses among subjects who have not responded to antidepressant therapy and are still on the antidepressant at the time of the screening assessment are comparable or even lower than the placebo response rates reported in trials with the prospective determination of resistance, such as the aripiprazole augmentation trials (Fava et al, 2010; Fava et al, 2012; Nelson et al, 2009).

The screening period is also designed to allow the gradual taper and discontinuation of the ongoing excluded psychotropic drugs so that any prohibited psychotropic medication is washed out under supervision of a study physician. The study physician and/or site staff will maintain ongoing telephone contact with the patient during the washout period in order to ensure patient safety during this time. Patients will discontinue their excluded psychotropic drugs documented during the

screening phase to allow proper wash-out at the baseline/randomization visit (e.g., at least 5 half-lives of the drug). The screening period (between the screen visit and baseline visit) can be extended to allow for a clinically appropriate tapering of such medications, again under the close supervision of the study physician, who will see them weekly during the taper phase and to ensure that the discontinuation of the excluded psychotropic medications occurs at least 5 half-lives prior to the baseline visit. The investigator will determine that patients meet eligibility criteria and will collect the demographic and medical data permitting full characterization of the patient.

TABLE 3– STUDY SCHEDULE

	Screen Visit	**Remote, Indep. SAFER Rater *Only***	**V1 Baseline Visit**	**V 2**	**V 3**	**V 4**	**V 5**	**V 6**	**V 7**	**V 8**	**V 9**	**V 10**	**V 11**
	Day -28 to Day -7		Day 0	Day 1	Day 2	Day 5 +/- 2 days	Day 8 +/- 2 days	Day 11 +/- 2 days	Day 14 +/- 2 days	Day 17 +/- 3 days	Day 21 +/- 3 days	Day 23 +/- 4 days	Day 28 +/- 4 days
Informed Consent	X												
Inclusion/Exclusion	X		X										
SCID-5	X												
SDQ	X		X	X	X	X	X	X	X	X	X	X	X
HAM-D17			X										
HAM-D6				X	X	X	X	X	X	X	X	X	X
MADRS	X	X*	X		X	X	X	X	X	X	X	X	X
CGI-S	X	X*	X	X	X	X	X	X	X	X	X	X	X
CGI-I			X	X	X	X	X	X	X	X	X	X	X
MGH ATRQ	X	X*											
Medical History	X												
Concomitant Medications	X		X	X	X	X	X	X	X	X	X	X	X
CHRT	X		X	X	X	X	X	X	X	X	X	X	X
Menopause and Hormonal Status (RLHQ)††			X										
Vital Signs and AEs**	X		X	X	X	X	X	X	X	X	X	X	X
Physical Exam	X								X				X
12-lead ECG	X												
ECG			X			X	X	X	X		X		
Urine tox screen test	X		X										

Pregnancy test (urine)	X		X										
Chemistry, CBC **	X								X				X
TSH, HbA1c	X												
Randomization			X										
Intranasal ketamine administration			X			X	X	X	X		X		
Study Drug Dispensation			X				X		X		X		

*Performed by MGH CTNI remote, independent rater. **Refer to Table 4 for specific tests to be performed. ††Menopause and hormonal status questionnaire (RLHLQ, Modules 1 and 2 in all women, and Module 3 (Day 30) in pre-menopausal women only).

I.1.b. Remote Interview

To ensure that the appropriate patients are entered into the trial, remote diagnostic assessments (SAFER Interview), and baseline depression severity and treatment history assessments (MGH ATRQ, MADRS, and CGI-S) will be performed remotely by the independent MGH CTNI rater, and the patient will be contacted by telephone while they are off-site between the screen visit and the baseline visit, during which call the above assessments will be performed. Sites will be notified of the results within 24 hours of the interview.

I.1.c. Randomization

For entry into the study and randomization, all eligibility criteria MUST be met (e.g., all inclusion criteria and no exclusion criteria). Patients who meet inclusion/exclusion criteria at the baseline visit (Day 0) will be randomized in a 1:1 fashion to either brexpiprazole plus intranasal ketamine treatment or placebo plus intranasal ketamine. All subjects will be followed for up to 28 days post randomization in order to examine the efficacy, safety, and tolerability of the study drug as well as the durability of the study drug's effects.

Specifically, patients will return for outcome assessments 24 hours after initiating treatment (Day 1), 48 hours after initiating treatment (Day 2), and then every 3 days after that for 26 days, with end of study visit one week after the last double-blind visit. (See Table 3 for the Study Schedule of the events.)

Patients with a MADRS score below 20 at either the screening or baseline visits will be excluded from further participation in the study. Upon determining at the baseline visit (Day 0) that a patient still meets all entry criteria for the study, the patient will be assigned (by study subject number assigned at consent) to the next randomization slot from the randomization list that will have been generated by the MGH Biostatistician.

I.1.d. Baseline Visit (Visit 1) (Day 0)

The patient should be instructed to refrain from eating and avoid alcohol as well as opioid and narcotic analgesics the day of the baseline visit (after midnight), as well as benzodiazepines within 2 hours of the intranasal administration. For visits in the afternoon, patients may have a light meal in the morning no less than 3 hours before the study visit.

Upon arrival, the patient will be administered the SDQ, , the 17-item Hamilton Rating for Depression (HAM-D-17), the MADRS, the CGI-I, the CGI-S, and the CHRT by the site rater.

Upon completion of all study-related inclusion procedures as listed in Table 3 and upon the investigator's confirmation that the patient still meets all of the study inclusion criteria and none of the exclusion criteria, the patient may be randomized.

All subjects will be dispensed brexpiprazole or placebo, as well as 40 mg of ketamine administered intranasally. The study drug (brexpiprazole or placebo) should be taken by the patient before ketamine administration. Detailed instructions for ketamine dose preparation will be provided to the pharmacist in an additional guidance document. Storage conditions should be followed according to the package insert. Dosing procedures will be provided to the clinical unit in a separate document.

Physiological Monitoring: Medical staff monitoring the ketamine administration will be prepared to treat increases in blood pressure greater than 180/110 mm Hg or heart rate greater than 110 bpm, or follow their institutional guidelines if more conservative. If these elevations resolve spontaneously within a short time period, they will generally not be treated. Heart rate and blood pressure should be recorded at 15 minutes post dose, 30 minutes post dose, 45 minutes post dose, 1 hour post dose, 90 minutes post dose and 2 hours post dose. The ketamine intranasal administration will be discontinued in the event that three consecutive measurements remain above protocol-defined limits, or according to institutional guidelines if more conservative, despite antihypertensive administration. A study psychiatrist will release the subject at the conclusion of the testing period at the end of the visit. A trained research assistant along with a research nurse will be present during the entire administration and monitoring period. All side effects will be recorded during the ketamine treatment and for the 2 hours following. Clinically relevant vital sign changes will be recorded as adverse events.

A study physician (MD or DO) will be present in the unit throughout the administration of ketamine so that potential adverse events can be evaluated and treated promptly, and will assess the patient for AEs, both physical and psychiatric, at Hours 1 and 2 following completion of the ketamine administration. They will remain available in the study site until discharge. The study physician will have a current certificate for ACLS training. An emergency medical cart will be available with oxygen delivery capability. The study physician will remain in the unit for at least one hour following the termination of the ketamine treatment or until the patient meets Aldrete criteria (good respiration, O2 saturation, consciousness, circulation, and activity) for Phase 2 post-anesthesia care, whichever

period is longer. For sites which are not hospital-based, procedures for emergency transfer to the Emergency Department will be described in the Manual of Operations (MOP).

Discharge Procedure: Discharge procedures should not begin earlier than 3 hours following completion of the intranasal administration of ketamine. Before being discharged patients should be awake and alert, feeling well, with no nausea, vertigo, or dizziness. There should be no significant change in vital signs from prior to ketamine administration.

They will be given a small meal before discharge.

Patients will be instructed not to drive. The study coordinator will release them to the care of an adult escort, who will accompany the patient home.

I.1.e. VISIT 2 (DAY 1)

Upon arrival, the patient will be administered the SDQ, the HAM-D6, , the CGI-I, the CGI-S, and the CHRT. Following completion of the scales, the remaining procedures listed in Table 3 should be completed.

I.1.f. VISIT 3 (DAY 2)

Upon arrival, the patient will be administered the SDQ, the HAM-D6, the MADRS, the CGI-I, the CGI-S, and the CHRT. Following completion of the scales, the remaining procedures listed in Table 3 should be completed.

I.1.g. VISIT 4 (DAY 5)

The patient should be instructed to refrain from eating and avoid alcohol as well as opioid and narcotic analgesics the day of the baseline visit (after midnight) , as well as benzodiazepines within 2 hours of the intranasal administration. For visits in the afternoon, patients may have a light meal in the morning no less than 3 hours before the study visit.

Upon arrival, the patient will be administered the SDQ, the HAM-D6, the MADRS, the CGI-I, the CGI-S, and the CHRT. Following completion of these scales the procedures listed in Table 3 should be completed.

The procedures for administration of ketamine and dispensation of blinded study drug will be the same as in section I.1.d and the Manual of Operations for the study.

I.1.h. VISIT 5 (DAY 8)

The patient should be instructed to refrain from eating and avoid alcohol as well as opioid and narcotic analgesics the day of the baseline visit (after midnight), as well as benzodiazepines within 2 hours of the intranasal administration. For visits in the afternoon, patients may have a light meal in the morning no less than 3 hours before the study visit.

Upon arrival, the patient will be administered the SDQ, the HAM-D6, the MADRS, the CGI-I, the CGI-S, and the CHRT. Following completion of these scales, the procedures listed in Table 3 should be completed.

The procedures for administration of ketamine and dispensation of blinded study drug will be the same as in sectionI.1.d. , and the Manual of Operations for the study.

I.1.i. VISIT 6 (DAY 11)

The patient should be instructed to refrain from eating and avoid alcohol as well as opioid and narcotic analgesics the day of the baseline visit (after midnight), as well as benzodiazepines within 2 hours of the intranasal administration. For visits in the afternoon, patients may have a light meal in the morning no less than 3 hours before the study visit.

Upon arrival, the patient will be administered the SDQ, the HAM-D6, the MADRS, the CGI-I, the CGI-S, and the CHRT. Following completion of these scales, the procedures listed in Table 3 should be completed.

The procedures for administration of ketamine and dispensation of blinded study drug will be the same as in section H.1.d. , and the Manual of Operations for the study.

I.1.j. VISIT 7 (DAY 14)

The patient should be instructed to refrain from eating and avoid alcohol as well as opioid and narcotic analgesics the day of the baseline visit (after midnight), as well as benzodiazepines within 2 hours of the intranasal administration. For visits in the afternoon, patients may have a light meal in the morning no less than 3 hours before the study visit.

Upon arrival, the patient will be administered the SDQ, the HAM-D6, the MADRS, the CGI-I, the CGI-S, and the CHRT. Following completion of these scales, the procedures listed in Table 3 should be completed.

The procedures for administration of ketamine and dispensation of blinded study drug will be the same as in section H.1.d. , and the Manual of Operations for the study.

I.1.k. VISIT 8 (DAY 17)

Upon arrival, the patient will be administered the SDQ, the HAM-D6, the MADRS, the CGI-I, the CGI-S, and the CHRT. Following completion of these scales, the procedures listed in Table 3 should be completed.

I.1.l. VISIT 9 (DAY 21)

The patient should be instructed to refrain from eating and avoid alcohol as well as opioid and narcotic analgesics the day of the baseline visit (after midnight), as well as benzodiazepines within 2 hours of the intranasal administration. For visits in the afternoon, patients may have a light meal in the morning no less than 3 hours before the study visit.

Upon arrival, the patient will be administered the SDQ, the HAM-D6, the MADRS, the CGI-I, the CGI-S, and the CHRT. Following completion of these scales, the procedures listed in Table 3 should be completed.

The procedures for administration of ketamine and dispensation of blinded study drug will be the same as in section H.1.d. , and the Manual of Operations for the study.

I.1.m. VISIT 10 (DAY 23)

Upon arrival, the patient will be administered the SDQ, the HAM-D6, the MADRS, the CGI-I, the CGI-S, and the CHRT. Following completion of these scales, the procedures listed in Table 3 should be completed.

I.1.n. VISIT 11 (DAY 28)

Upon arrival, the patient will be administered the SDQ, the HAM-D6, the MADRS, the CGI-I, the CGI-S, and the CHRT. Following completion of these scales, the procedures listed in Table 3 should be completed.

I.1.o. EARLY TERMINATION VISIT

If a patient is terminated from the study before completion, but after at least one dose of ketamine and/or study drug, the procedures listed in Table 3 for Visit 11 (Day 28) should be completed.

I.1.p. END OF STUDY TREATMENT ADMINISTRATION

At the end of double-blind treatment (Day 28), both patients and clinicians will be asked to guess treatment assignment.

I.1.q. POST-STUDY FOLLOW-UP

Patients who are non-responders at the completion of Visit 11 (Day 28) will be offered a referral for treatment.

I.1.r. PATIENT RECRUITMENT

Patients will be recruited through advertisement, as well as clinician referral and self-referral. Self-referred patients will call the study sites and will be screened over the phone by the study coordinators. Those deemed potentially eligible will be scheduled for a screening visit with one of the study physicians at the sites. Based on clinical and human subject protection considerations, a potential subject will, under no circumstance, be advised to taper his/her current medication regimen prior to the screening visit.

Participants will also be recruited through physician referral. Each site will recruit subjects via referral from primary care and specialty clinics from the communities surrounding each study site. Each site will utilize the experience of the MGH CTNI to conduct outreach activities to community organizations, colleges and other resources. Sites will regularly collaborate on strategies to recruit women and minorities, and sites will employ strategies specific to their local community, in ways that are culturally sensitive and specific to the local minority population. Retention will be enhanced with fair reimbursement (as deemed appropriate by the local IRB) for participants' time.

Assessments and procedures within the study visits will occur as shown in Table 3. A summary description of each data collection instrument is provided later in this section.

I.2. PROCEDURES

I.2.a. LABORATORY/DIAGNOSTIC PROCEDURES

Once the patient has signed the informed consent document, the urine pregnancy and toxicity test will be performed at screen and baseline visits. 12 lead ECGs will be measured at the screen visit and at visits 4,5,6,7 and 9 as required by the physiological monitoring procedures in the Manual of Operations . A Chemistry and CBC will also be obtained at the screening visit, and at Visits 7 and 11. If TSH is high, subject may be tested for T4 at the request of the PI. Weight, oral temperature, and seated and supine pulse and blood pressure (Vital Signs) will be recorded at each visit. Height is recorded at the Screening Visit only. (BMI is calculated automatically in the EDC). At the screen visit, Visit 7 and at Visit 11, patients will have a physical examination. The laboratory tests required are listed in Table 4, below. Laboratory tests will be performed at the site, and each site will provide their laboratory normal to the coordinating center.

TABLE 4– LABORATORY TESTS

Laboratory Test	**Frequency**
CBC	Screen, Visit 7, Visit 11
Chemistry (Total bilirubin, AST, ALT, GGT, ALK Phosphatase, Creatinine, BUN/Urea, Glucose, Uric Acid)	Screen, Visit 7, Visit 11
TSH, HbA1c	Screen
Pregnancy Test	Screen, Visit 1
Urine Tox Screen	Screen, Visit 1

I.2.b. BLINDING PROCEDURES

Individual treatment codes, indicating the treatment randomization for each randomized patient, will be available to the investigator(s) or site pharmacists from a member of the MGH CTNI unblinded staff. The treatment code must not be broken except in medical emergencies when the appropriate management of the patient necessitates knowledge of the treatment randomization. The investigator documents and reports the action to MGH CTNI study staff, in accordance with the procedures in the Manual of Operations, without revealing the treatment given to the patient to MGH CTNI blinded staff. Treatment codes will not be broken for the planned analyses of data until all decisions on the evaluability of the data from each individual patient have been made and documented.

I.2.c. Outcome and Safety Measures

I.2.c.1. Outcome Measures

Symptom improvement will be evaluated by assessing changes in the primary outcome measure, the self-rated Symptoms of Depression Questionnaire (SDQ) (Pedrelli et al, 2014). Brexpiprazole has shown in recent studies to have significant benefits in domains such as irritability, anger, anxiety, and nervousness. These domains are not captured or are only partially captured by standard scales such as HAMD or MADRS. In a recent study of NSI-189, the SDQ slightly outperformed the MADRS in detecting a change over placebo because of the broader symptom net cast by the SDQ.

Secondary outcome measures include the HAM-D6, which has shown greater sensitivity to change and greater effect sizes compared to the longer, multidimensional versions of the HAM-D (e.g., HAM-D-17 and HAM-D-28) (Faries et al, 2000; Bech et al, 2010), the MADRS and the CGI-S and CGI-I (Guy, 1976).

I.2.c.2. Safety Measures

Vital signs will be obtained at every visit. At screening and at visits 1, 4, 5, 6, 7 and 9, digital ECGs will be obtained after the patient has been resting in a semi-recumbent position for at least 10 minutes. All digital ECGs will be documented by recording date, time, heart rate, QRS duration, PR interval, RR interval, QT interval, and QTcF interval. QTcF intervals will be calculated using the Fridericia formula (Puddu et al 1988). If indicated, additional ECG assessments can be made at the discretion of the investigator. The investigator will judge the overall interpretation as normal or abnormal. If abnormal, it will be decided as to whether or not the abnormality is clinically significant or not clinically significant and the reason for the abnormality will be recorded on the eCRF. Abnormal values shall not be recorded as AEs unless deemed clinically significant.

The patient's blood pressure will be measured a total of three times at both the screen and baseline visits (prior to randomization), with each measurement taken at least 15 minutes after the previous measure. If two out of three measurements are within the range for inclusion at each visit (see

Exclusion 17a and 17b), the patient will be considered to have met the enrollment criteria for normal blood pressure. The baseline blood pressure should be measured within 1.5 hours prior to randomization.

At the screen visit and the baseline visit (Visit 1) and at Visit 7 and Visit 11, patients will have their blood drawn for Chemistry and CBC testing. At the screen visit, patients will also have TSH and HbA1c measured. At the screen visit and at Visit 7 and Visit 11, patients will have a physical examination.

The presence of any side effect or adverse event will be carefully documented at screen and at every subsequent visit covering events since the last visit. Reasons for premature discontinuation, including intolerable side effects, will be recorded.

I.2.d. Study Instruments

The following instruments will be administered according to the schedule in Table 3:

I.2.d.1. Diagnostic Instruments

Structured Clinical Interview for DSM-5 (SCID 5): The SCID, administered by the clinician, includes modules aimed at diagnosing possible Axis I disorders. Questions here are asked exactly as written, and each is based on the individual criteria from DSM-5.

Massachusetts General Hospital Antidepressant Treatment Response Questionnaire (MGH ATRQ) (Fava and Davidson, 1996; Fava, 2003): This is a clinician assisted questionnaire that examines the antidepressant treatment history, using specific anchor points to define the adequacy of both dose and duration of each antidepressant trial, as well as the degree of symptomatic improvement obtained with each trial. This questionnaire, which has been validated, allows for the determination of treatment resistance in depression (Chandler et al, 2010).

Reproductive Lifecycle and Hormone Questionnaire (RLHQ) (Freeman et al, 2013). This is a standard brief questionnaire implemented systematically at baseline in clinical trials for MDD to assess reproductive lifecycle status and the use of exogenous hormones. This study will use Modules 1 and 2 in all women, and add Module 3 (menstrual cycle tracking) in applicable women.

I.2.d.2. Efficacy Measures

Symptoms of Depression Questionnaire (SDQ) (Pedrelli et al, 2014): This validated self-rating instrument has 44 items on a scale of 1-6, measuring multiple depressive symptom domains. The time frame for this scale is the past 3 days.

6 and17-item Hamilton Rating Scale for Depression (HAM-D6)(HAM-D17) (Hamilton, 1960, 1967): This instrument is completed with a structured interview guide developed by Per Bech by the clinician based on his/her assessment of the patient's symptoms (Bech et al, 1986). This structured interview has been validated in the DUAG studies for use with time frames shorter than one week

(Gram, 2008). The HAM-D6 has been shown greater sensitivity to change and greater effect sizes compared to the longer, multidimensional versions of the HAM-D (HAMD-17 and HAMD-28) (Faries et al, 2000; Bech et al, 2010). The time frame for this scale is the past 24 hours. Only once, at the baseline visit (Visit 1), will the 17-item version of the HAM-D be administered, in order to examine for the presence of anxious MDD (which is based on the anxiety somatization subscale of the HAM-D17), with the time frame of the past 3 days.

Clinical Global Impressions–Severity (CGI-S) and Clinical Global Impressions–Improvement (CGI-I) scales (Guy, 1976): These clinician-rated scales rate the severity of the disorder and the global improvement since beginning of the study. The time frames for these two scales are the past 3 days.

Montgomery-Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979): This 10-item clinician-rated instrument measures depression severity. It will be administered with a structured interview guide. The time frame for this scale is the past 3 days.

I.2.d.3. Safety Measures

The Concise Health Risk Tracking (CHRT) (Trivedi et al, 2011): The Concise Health Risk Tracking (CHRT) is a validated clinician-rated scale evaluating three independent factors (current suicidal thoughts and plans, perceived lack of social support, and hopelessness). It has excellent psychometric properties and can be used to monitor suicidal risk in clinical practice and research settings. The time scale for the CHRT is the last three days.

I.2.e. Reliability Training

All site study staff chosen to be involved in the assessment of study subjects are very familiar with standardized clinician-rated measures such as the SCID-5, the HAM-D6, the MADRS and the CHRT. They have all received extensive training in the use of these instruments through videotaped and live interviews of mock patients.

I.2.f. Patient Adherence to Protocol

Every effort will be made to encourage patients to comply with the procedures and the assessments involved in study. Patients will be compensated for their time at each visit, at a rate determined appropriate by the IRB (estimated compensation: $20.00/hour). We believe that this level of subject compensation, while non-coercive, facilitates adherence to assessments and procedures that require frequent, inconvenient visits. Subjects and clinicians will be asked to guess which arm of the study the subjects were in at the end of the study to assess if blinding was successful.

I.3. Discontinued Subjects

Sites will follow the procedures in Section I.1.o. for discontinued patients.

I.4. Evaluation of Adverse Events (AEs)

I.4.a. DEFINITION OF AEs

Adverse Event

An adverse event is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An adverse event does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product. (Definition per International Conference on Harmonization [ICH]).

This definition includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

I.4.b. REPORTING OF AEs

Observed and spontaneously reported adverse events will be recorded at each visit. Spontaneously-reported adverse events will be classified as mild, moderate or severe. Patients shall be allowed to contact the investigator or a member of his staff at any time between visits concerning adverse events or worsening of symptoms.

All adverse events and special reporting situations, whether serious or non-serious, will be reported from the time a signed and dated Informed Consent Form (ICF) is obtained until completion of the subject's last study-related procedure (which may include contact for follow-up of safety). Serious adverse events, including those spontaneously reported to the site-investigator from the time a signed and dated ICF is obtained until within 30 days after the last dose of study drug must be reported using the Serious Adverse Event Form. The Investigator-Sponsor will evaluate any safety information that is spontaneously reported by a site- investigator beyond the time frame specified in the protocol.

All events that meet the definition of a serious adverse event will be reported as serious adverse events, regardless of whether they are protocol-specific assessments.

All adverse events, regardless of seriousness, severity, or presumed relationship to study drug, must be recorded using medical terminology in the source document and the Electronic Case Report Form (eCRF). Whenever possible, diagnoses should be given when signs and symptoms can be ascribed to a common medical etiology with reasonable certainty as per site clinician (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection" when such a determination is made with reasonable certainty as per site clinician). Investigators must record in the eCRF their opinion concerning the relationship of the adverse event to study therapy.

All measures required for adverse event management must be recorded in the source document and reported according to Investigator-Sponsor instructions.

The Investigator-Sponsor assumes responsibility for appropriate reporting of adverse events to the regulatory authorities. The Investigator-Sponsor will also report to site investigators all serious adverse events that are unlisted (unexpected) and associated with the use of the study drug. The site investigator will report these events to the appropriate IRB.

I.4.b.1. Time Period for Collection of Adverse Events

Adverse events and SAEs will be collected from the time of signature of informed consent throughout the treatment period and including the observation period. Unsolicited SAEs will be collected for 30 days post last study treatment.

I.4.b.2. Follow-up of Unresolved Adverse Events

Any AEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator for as long as medically indicated, but without further recording in the eCRF. MGH CTNI or its designee retains the right to request additional information for any patient with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

I.4.b.3. VARIABLES

The following variables will be collected for each AE:

- AE (verbatim)
- The date and time when the AE started and stopped
- Maximum intensity or intensity or changes in intensity
- Whether the AE is serious or not
- Investigator causality rating against study drug (yes or no)
- Action taken with regard to IP
- AE caused patient's withdrawal from the study (yes or no)
- Outcome

In addition, the following variables will be collected for SAEs:

- Date AE met criteria for SAE
- Date investigator became aware of SAE
- AE is serious due to [insert reason]
- Date of hospitalization
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to study procedure(s)
- Causality assessment in relation to study drug
- Causality assessment in relation to other medication
- Description of AE

Intensities will be reported for each AE in the following categories: a) Mild (awareness of sign or symptom, but easily tolerated); b) Moderate (discomfort sufficient to cause interference with normal activities); c) Severe (incapacitating, with inability to perform normal activities).

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section H.4.d. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

Worsening symptoms of the primary study condition (i.e., MDD) should not be recorded as an AE. However, worsening of MDD will be reported as an adverse event if, in the opinion of the investigator, clinically significant worsening of depression occurs following intranasal ketamine administration. In addition, hospitalization resulting from worsening psychiatric symptoms should be recorded as an SAE in the eCRF.

Should an overdose of study drug occur, it must be reported in accordance with the procedures described below. An overdose associated with symptoms must be reported as an AE, while an overdose without associated symptoms, must be reported only on the separate MGH CTNI Clinical Study Overdose Report Form. Any overdose will also be considered a protocol violation and reported as such.

I.4.b.4. Suicidal Ideation and Behavior

The documentation and proper management of suicidal ideation by sites will be documented with the use of the CHRT completed by site clinicians during all visits.

Any patient who, based on the investigator's judgment, poses an imminent risk of suicide should be discontinued from the study (see Section J.6). All efforts should be taken to minimize the risk of suicide and the investigator should carefully monitor the patient. If during the course of the study, a patient shows evidence of suicidal behavior, the patient should be evaluated immediately or referred to their attending psychiatrist or a local emergency room for further evaluation to see if they are safe to continue in the study. The site should have the ability to direct any patient that requires emergency hospitalization to an emergency room or inpatient psychiatric unit that is within a 50-mile radius of the site. In addition, the patient should be able to contact someone at the site 24 hours per day should they have serious worsening of their condition.

Suicide and attempted suicide, irrespective of the method, but occurring in connection with the use of IP, should be reported as AEs (all suicides are SAEs; attempted suicides can be either serious or non-serious adverse events). The event should be identified as suicide or attempted suicide, and the method of the suicide or attempt should be provided. If an attempted suicide meets the criteria for

an SAE, the event must be reported according to the guidelines in Section I.4.i.2. If a suicide attempt does not meet the criteria for an SAE, it should be considered a reportable event.

All events of suicidal ideation and behavior will be carefully monitored. These include events of suicide attempts, emergence or significant worsening of suicidal ideation, completed suicides, and suicidal behavior. If a suicidal behavior meets the criteria for a SAE, according to the guidelines in I.4.i.2, investigators are required to report the event according to the guidelines for SAEs described in Sections I.4.i.2

I.4.c. Adverse Event Coding

The latest version of the AE dictionary, Medical Dictionary for Regulatory Activities (MedDRA), will be used by the CRO staff for the classification and analysis of AEs entered in the study database. For regulatory reporting, SAEs will be processed in the electronic data capture (EDC) system and coded using MedDRA.

I.4.d. Pregnancy

If a subject (or subject's partner) becomes pregnant during the study, it must be reported in within 24 business hours of the time the investigator becomes aware of the event and in accordance with the procedures described on the Pregnancy Report Form. Pregnancy in itself is not regarded as an AE unless there is a suspicion that a study drug may have interfered with the effectiveness of a contraceptive medication. Any pregnancy that occurs from Day 1 to 30 days following the last dose given will be followed for gestational outcome and the outcome would be reported to the Sponsor.

I.4.e. Causality Collection

The investigator will assess the causal relationship (i.e., the relationship to study treatment) between the IP and AEs, and answer "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?"

Causal relationship in cases where the disease under study has deteriorated due to lack of effect will be classified as no reasonable possibility.

For SAEs, causal relationship will also be assessed. Note that for SAEs that could be associated with any study procedure, the causal relationship is implied as "yes."

A guide to the interpretation of the causality question is found in the MOP.

I.4.f. Adverse Events Based on Signs and Symptoms

All AEs spontaneously reported by the patient in response to the open question from the study personnel: "Have you had any health problems since the previous visit?" or revealed by observation will be collected and recorded in the eCRF. In the collection of AEs, the recording of diagnoses is preferred (when possible) over the recording of a list of signs and symptoms. However, if a diagnosis

is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

I.4.g. Adverse Events Based on Examinations and Tests

The results from protocol-mandated laboratory tests and vital signs will be summarized in the clinical study report (CSR). Deterioration as compared with baseline in protocol-mandated laboratory values and vital signs should therefore only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the study drug.

If deterioration in a laboratory value/vital sign is associated with clinically significant signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible, the reporting investigator uses the clinical, rather than the laboratory term (e.g., anemia versus low hemoglobin value). In the absence of clinical signs or symptoms, clinically significant deteriorations in non-mandated parameters should be reported as AE(s).

Any new or aggravated clinically significant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

I.4.h. Disease Progression or Worsening Depression

Disease progression can be considered as a worsening of a patient's condition attributable to the disease for which the study drug is being studied. It may be an increase in the severity of the disease under study and/or increases in the symptoms of the disease. The development of worsening depression should be considered as disease progression and not an AE. Events, which are unequivocally due to disease progression, should not be reported as an AE during the study. However, if worsening of depression occurs during ketamine treatment, in the opinion of the investigator, is to be reported as an adverse event.

I.4.i. Serious Adverse Events (SAEs)

The EDC platform will provide a mechanism for sites to report SAEs, and for the Site Investigators and MGH CTNI Medical Monitor to sign off on the SAE reports. Reportable (related and/or unexpected) SAEs will be sent to the Sponsor or their designee within regulatory timelines.

I.4.i.1. Definition of SAEs

The following criteria define a SAE:

- Death
- Life-threatening event
- Hospitalization or prolongation of hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect

- Medically important event, as defined in this protocol*

*Medical and scientific judgment will be exercised in deciding whether expedited reporting is also appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These will usually be considered serious.

I.4.i.2. Reporting of SAEs

When a SAE is discovered at the site, it will be reported immediately (within 24 business hours), as described below. Reportable serious adverse events will be reported according to the central IRB reporting policy and FDA regulations. Reportable events may include (but will not be limited to): (a) changes in the health status of the patient, (b) unexplained or severe psychiatric complications, and (c) clinically significant development or worsening of suicidal ideation or behavior. The Site Investigator will determine whether the event is study-related. All reportable serious adverse events will be reported by CTNI within 48 business hours to the CTNI medical monitor and the study site PIs.

I.5. Protocol Waivers and Violations

I.5.a. Protocol Waivers

Protocol waivers will not be allowed for this study.

I.5.b. Protocol Violations

I.5.b.1. Procedure for Non-Compliant Patients

Patients will be scheduled for the period of study participation when they are available to come in for study visits. Patients who miss one visit will be allowed to make up that visit within a window that depends on the visit. Every effort will be made by the site to adhere to the study visit schedule, in order for assessments to be able to be performed at the stipulated time points. Patients who miss more than one visit will be discontinued from the study. However, patients who either complete the study or discontinue prematurely will be eligible for three months of free care at the clinic where they were enrolled. Patients who withdraw consent will no longer have data collected for the study. Every effort will be made to maintain contact with patients who are discontinued, but who do not withdraw consent, according to the study schedule (Table 3) for 30 days post-treatment in order to collect safety data.

I.5.b.2. PROTOCOL VIOLATION DEFINITIONS

TABLE 5 - LIST OF EXAMPLES OF PROTOCOL VIOLATIONS

	Major Protocol Violation	**Minor Protocol Violation**
The list of examples is intended as a guide and is not all-inclusive.		
Definition	A violation that **<u>may</u>:** Impact subject safety, Affect the integrity of study data, and/ or Affect the subject's willingness to participate in the study.	A violation that **<u>does not</u>:** Impact subject safety, Compromise the integrity of study data, and/ or Affect the subject's willingness to participate in the study.
Examples ***(not all-inclusive)***	Failure to obtain informed consent Informed consent obtained by an unauthorized individual Enrollment of a subject who did not meet eligibility criteria for whom a protocol exception was not obtained Performing a study procedure that is not approved by the IRB and/or is not in the protocol Failure to perform a required lab test that, in the opinion of the Site Investigator, may affect subject safety or data integrity Failure to perform or follow a study procedure that, in the opinion of the Site Investigator, may affect subject safety or data integrity Failure to follow safety (AE) management plan Failure to report a SAE to the IRB and/or Coordination Center	Implementation of unapproved recruitment procedures Only a photocopy of the signed/ dated consent form is available (the original is missing) Pages are missing from the signed/ dated informed consent form Use of invalid consent form (i.e., without IRB approval or outdated/expired form) Failure to perform or follow an approved study procedure that, in the opinion of the Site Investigator, **does not** affect subject safety or data integrity Study procedure conducted out of sequence Failure to perform a required lab test Missing lab results Study Visit out of approved window Over-enrollment

		Enrollment of subjects after IRB approval of the study has expired Failure to submit a continuing review application to the IRB before study expiration
Reporting Requirements	Record the date discovered, date occurred, description of event in the Protocol Deviation Log. Notify the Coordinating Center within 24 business hours.	Record the date discovered, date occurred, description of event in the Protocol Deviation Log. Notify the Coordinating Center

I.5.b.3. PROTOCOL VIOLATION REPORTING

Protocol violations will be reported as described in the MOP.

J. TREATMENT

J.1. DOSING SCHEDULE

Ketamine 40 mg will be administered intranasally six times during the study. Ketamine will be administered at the respective visits, by study staff, onsite. Brexpiprazole or matching placebo will be administered orally daily for 28 days according to the fixed-flexible dose escalation schedule.

J.2. STUDY PRODUCT SUPPLIES AND ADMINISTRATION

Intranasal ketamine will be prepared as described in the Manual of Procedures. The intranasal ketamine will be supplied in a metered MAD Nasal™ Intranasal Mucosal Atomization Device. Each Intranasal Mucosal Atomization Device will contain 1mL of 40mg/mL Ketamine and be used for one administration. A total of two sprays, with one spray (a volume of 0.5 mL) given in each nostril, will be administered. A total of six atomization devices will be prepared for each patient.

Brexpiprazole and matching placebo, in .5 mg and 1 mg tablets, will be supplied to each site by Otsuka Pharmaceuticals. The site pharmacist will package the brexpiprazole and placebo tablets in bottles, and supply 4 bottles for each patient, according to the randomization scheme. Each bottle will contain 10, 20 or 30 pills, according to the dosage requirements listed below, of either brexpiprazole or placebo, and be labeled with the randomization number for that patient.

TABLE 6 BREXPIPRAZOLE DOSING

Visit/Day	Visit 1/Day 0	Visit 5/Day 8	Visit 7/Day 14	Visit 9/Day 21
Dose/day	.5 mg	1mg	2 mg	3 mg
Number of Pills per Dose	1	1	2	3
Number of Pills per Bottle	10	10	20	30

J.3. Packaging and Labeling

J.3.a. Concomitant Open-Label ADT

Open-label ADT will be provided to allow for continuing the same dose of ADT throughout the study. The amount of open-label study medication (ADT) dispensed to a patient at any given visit is contingent upon the prescribed daily dose of ADT. ADT will be provided through prescriptions, and patients will be reimbursed for any out of pocket expenses they incur for filling the prescription.

J.3.b. Blinded Brexpiprazole/Placebo Therapy

Blinded study drug will be provided to each subject at Visits 1, 5, 7 and 9, and a pill count will be performed by study site personnel at each study visit. Patients will be instructed to bring their pill bottles to every visit.

J.4. Dose Modification for Toxicity

Dose modifications will be allowed, if patient cannot tolerate the dose increases. If a dose modification is necessary, patients should return to the previous tolerable dose.

J.5. Concomitant Therapy

All concomitant medications taken during the study will be recorded in the Concomitant Medication Log for each patient, along with dosage information and start and stop dates. Allowed concomitant medications include any prescription or over-the-counter medication not specifically excluded by the protocol (see Appendix 1), as well as stable, ongoing antidepressant therapy and stable allowed hypnotic therapy. Patients requiring excluded drugs will be discontinued from the study.

J.6. Discontinuation from Study Treatment

Every effort will be made to keep the patient in the study for the full study period consisting of one day of acute treatment plus 6 follow-up assessments. Acceptable reasons for early discontinuation include the following: 1) request of patient, 2) decision of physician, 3) serious adverse event, and 4) protocol violation.

J.6.a. Procedures for Discontinuation from Study Treatment

J.6.a.1. MEDICAL REASONS FOR DISCONTINUATION

In the event that the patient's blood pressure rises to an unacceptable level during treatment, in the opinion of the attending physician, the physician will discontinue treatment and initiate appropriate measures according to usual medical practice.

A patient who decides to discontinue participation in the study will always be asked about the reason(s) and the presence of any AEs. If possible, they will be seen and assessed by an investigator. AEs will be followed up until resolution (see Section I.4.b).

If a patient discontinues from the study before randomization, then no further follow-up will be expected. However, if the patient discontinues after randomization, but before receiving any study treatment, the patient will be asked to return for a final study visit, during which the procedures outlined in the Early Termination Visit procedures will be completed, including AEs and concomitant medication assessments.

If a patient discontinues from the study before completion and has received a dose of study drug, the patient will be asked to return for a final study visit, at which the procedures outlined in the Early Termination Visit will be completed. Every effort should be made to follow up with patients who discontinue from the study prior to Visit 11 (Day 28). If patients refuse to return to the clinic for the study-related assessments, a modified follow-up through, for example, regular telephone contact or a contact at study closure should be arranged, if agreed to by the patient and in compliance with local data privacy laws/practices. If the patient refuses follow-up, the reason for the refusal and last contact date should be documented in the eCRF and source documents.

Patients who discontinue from the study will not be replaced.

J.7. UNBLINDING PROCEDURE

J.7.a. METHODS FOR ENSURING BLINDING

The investigator, patient, and study staff will be blinded. Packaging and labeling of the study drugs will be performed in a way to ensure blinding throughout the study.

No members of the study team at study sites will have access to the randomization scheme during the conduct of the study.

The randomization schedule for blinding of randomized treatment will be maintained by MGH CTNI or representative and will not be disclosed until after database lock.

J.7.b. METHODS FOR UNBLINDING THE STUDY

Individual treatment codes, indicating the treatment randomization for each randomized patient, will be available to the investigator(s) or pharmacists from MGH CTNI or its designee.

The treatment code must not be broken except in medical emergencies when the appropriate management of the patient necessitates knowledge of the treatment randomization. The investigator documents and reports the action to MGH CTNI or representative, without revealing the treatment given to the patient to MGH CTNI or representative's staff.

MGH CTNI retains the right to break the code for serious adverse events (SAEs) that are unexpected and are suspected to be causally related to a study drug and that potentially require expedited reporting to regulatory authorities. Treatment codes will not be broken for the planned analyses of data until all decisions on the evaluability of the data from each individual patient have been made and documented.

K. ETHICAL CONSIDERATIONS

K.1. RISK/BENEFIT ASSESSMENT

K.1.a. POTENTIAL RISKS

Participants must be capable of understanding the nature of this study, its potential risks, discomforts and benefits. Study physicians will obtain consent after they have fully explained the study purpose and its procedures, and potential participants have demonstrated an understanding of the protocol, willingness to participate, and competency to consent.

K.1.b. SAFETY

Ketamine was well tolerated after single-dose administration up to 60.0 mg, multiple-dose administration up to 35.0 mg, and single-dose administration up to 25.0 mg. There were no deaths or SAEs reported in any published study. One (1) subject was discontinued due to a treatment-related AE of mild ventricular tachycardia in one study, and no subjects were discontinued due to AEs in two other studies. The most common AEs considered by the investigator to be related to ketamine (occurring >1 incidence) were: headache (6 events), diarrhea (4 events), nausea (3 events), and anxiety (2 events), dyspepsia (5 events), pruritus generalized (3 events), headache (2 events), diarrhea (2 events), and abdominal pain (2 events). In these three studies, there were no clinically significant values, changes, or trends in clinical laboratory data. No clinically significant changes were observed in the hypothalamic-pituitary-adrenal axis hormones of cortisol, prolactin, and luteinizing hormone. There were no clinically significant changes in vital signs and electrocardiograms (ECGs), including no evidence of changes in corrected QT (QTc) prolongation.

The most frequent adverse events with brexpiprazole are akathisia, headache, and weight increase. Mean changes from baseline in Abnormal Involuntary Movement Scale and Barnes Akathisia Rating Scale total scores are significantly greater with brexpiprazole 3 mg versus placebo.

K.1.c. Confidentiality and Loss of Privacy

The risk of loss of privacy is judged to be minimal. Confidentiality will be maintained by numerically coding all data, by disguising identifying information, and by keeping all data in locked file drawers or on password-protected databases on password-protected and encrypted computers in locked offices. Subject information will be accessible only to research staff. Information about study participants will not leave our institution in any form that would identify individual subjects. Sites will enter subject data directly into the study database, which will be managed by the MGH CTNI team. Subjects will be assigned study ID numbers.

K.1.d. Planned procedures for protecting against or minimizing potential risks

Participants must be capable of understanding the nature of this study, its potential risks, discomforts and benefits. Study physicians will obtain consent after they have fully explained the study purpose and its procedures, and the potential participant has demonstrated an understanding of the protocol, willingness to participate, and competency to consent. Confidentiality will be maintained by numerically coding all data, by disguising identifying information, and by keeping all data in locked file drawers or on password-protected databases on password-protected and encrypted computers in locked offices. Subject information will be accessible only to research staff. Information about study participants will not leave our institution in any form that would identify individual subjects. Data will be transmitted with subjects identified by code.

Regarding confidentiality and privacy, the risk of loss of privacy is judged to be minimal. Confidentiality will be maintained by numerically coding all data, by disguising identifying information, and by keeping all data in locked file drawers or on password-protected databases on password-protected and encrypted computers in locked offices. Subject information will be accessible only to research staff. Information about study participants will not leave our institution in any form that would identify individual subjects. Data will be transmitted with subjects identified by code.

K.2. Justification of the Use of Placebo Arm

MDD is a serious illness. The decision to conduct placebo-controlled trials was carefully considered. Randomized trials utilizing a placebo control group are the gold standard for the establishment of treatment efficacy. The use of a placebo treatment is a method of providing a control group in the study design, minimizes investigator bias in assessments, promotes retention of the control group, and controls for the natural course of the disease (Vickers and deCraen, 2000). Therefore, lack of adequate control comparisons would limit the contribution of the data from this trial.

K.2.a. Clinical Monitoring of Subjects and Management of Clinical Deterioration and Emergence or Worsening of Suicidal ideation

All patients will receive monitoring for clinical deterioration, support, attention, and reassurance in the context of a therapeutic alliance. By definition, the target population is one in which subjects have not responded to available antidepressant therapies. Both active treatment and placebo/control groups in each trial will receive comprehensive evaluation and careful monitoring. Close monitoring by a trained psychiatrist is beneficial for individuals with mood disorders. If patients are in need of more intensive psychiatric treatment at any time during the course of the trial, study personnel will assist in needed evaluation and referral to appropriate treatment settings. Subjects will be carefully monitored regarding depressive and other psychiatric symptoms. Non-responders to study treatment will be offered referrals for treatment at the end of the trial.

K.3. Informed Consent

The Investigator must ensure that patients are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical trials in which they volunteer to participate. Preparation of the consent form is the responsibility of the Investigator and must include all elements required by CFR 21 Part 50.25 and the local IRB.

All subjects will receive the consent form for the study. These documents will be read by the patients and also reviewed by the patient with a clinician on the research staff prior to participating in the study. Any questions, concerns, or ambiguities will be clarified by the site's PI or another study clinician prior to the patient signing consent. Patients will sign informed consent and only then will begin participation in the study.

K.4. IRB Review

Before study initiation, the Investigator must have written and dated approval/favorable opinion from the IRB for the protocol, consent form, patient recruitment materials/process (e.g., advertisements), and any other written information to be provided to patients.

The Investigator should provide the IRB with reports, updates, and other information (e.g., Safety Updates, Amendments, and Administrative Letters) according to regulatory requirements and Institution procedures.

A detailed list of required regulatory documents also to be submitted to MGH CTNI will be sent upon final approval of the protocol.

K.5. Potential Benefits to Study Participants and Others

Potential benefits of the research to participants and others: This study will potentially help individuals with TRD. If we discover that this intervention is effective, these results will lead to a full clinical development program of this treatment for TRD.

Risks to subjects relative to the anticipated benefits to research participants and others: The treatment has been selected with consideration of safety in mind. Additionally, exposure to the intervention will be brief and carefully monitored. The risks of participation in the study are therefore judged to be small, and adequate protections are in place to monitor the medical wellbeing of participants. In contrast, the morbidity and mortality associated with TRD risks are well known to be very high, given the long-term disability associated with MDD.

Because of the tremendous public health impact of depression, if these treatments are shown to be effective for TRD, they could reduce the burden of depression-related morbidity and mortality.

Risks to subjects in relation to the importance of the knowledge that reasonably may be expected to result: The aforementioned data suggest that risks to subjects are minimal. The benefit to society from the development of efficacious interventions with rapid onset for TRD would be a substantial public health benefit.

L. DATA HANDLING AND RECORD KEEPING

L.1. COMPLETION OF eCRF

An Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated with the investigational product or entered as a control in the investigation.

L.2. PROCEDURE FOR CORRECTIONS

An EDC system will be built for the study. The EDC system will include eCRFs designed to capture study information, which are completed by trained site staff. Specific details of the procedures for Data Management are found in the Data Management Plan and details regarding the EDC system training process can be found in the Study MOP.

L.3. RECORD RETENTION

The Investigator must retain investigational product disposition records, case report forms, and source documents for the maximum period required by applicable regulations and guidelines, or Institution procedures, and at least for 10 years.

If the Investigator withdraws from the study (e.g., relocation, retirement) the records shall be transferred to a mutually agreed upon designee (e.g., another Investigator, IRB).

L.4. Data Confidentiality

Potential risks to data confidentiality will be mitigated by requirements for the de-identification of all study data and by security protocols for all data capture systems. All users of the EDC system will be tracked and provided access in a secure fashion following established SOPs for this process.

As with all research data, information gathered by the study will be used only for aggregate analysis; it will not be released with any information that identifies research participants. The data managers, statistician, Investigator, and Sponsor do not have access to the identities of patients. That information is retained only at the clinical centers. Uses and risks related to data collection will be outlined in the informed consent and reviewed with the subjects.

M. Monitoring and Oversight

M.1. Evaluation of Study Sites

Potential study sites have been evaluated by MGH CTNI to determine suitability for the proposed study. Information reviewed included, but was not limited to, facility details and site capabilities, past performance in similar studies, investigator, and staff experience, ongoing studies at the site, projected enrollment in this study, and FDA or other agency audit findings. Study sites may be asked to complete a study-specific Site Selection Questionnaire and other documents for consideration for participation and MGH CTNI or clinical study monitors may make a Site Qualification Visit prior to completing the evaluation process.

M.2. Initiation of Study Sites

Prior to subject enrollment, a study initiation visit will be completed at each investigational site to ensure the following: IRB approval has been obtained and documented prior to subject screening, the Investigators and study personnel are appropriately trained and clearly understand the study, the Investigators and study personnel accept the obligations incurred in undertaking this clinical investigation.

M.3. Periodic Monitoring Visits

Qualified MGH clinical monitors or qualified contract monitors representing MGH CTNI will conduct investigational site monitoring visits to ensure that all Investigators conduct the study in compliance with the protocol and applicable regulations. The site will receive notification prior to each monitoring visit during the course of the study. It is expected that the Investigator and/or sub-Investigator and other appropriately trained study staff are available on the day of the visit in case any questions might arise.

Periodic monitoring visits will be made in accordance with the approved monitoring plan at all active study sites throughout the clinical study to assure that the Investigator obligations are fulfilled and all applicable regulations and guidelines are being followed. These visits will assure that the facilities are still acceptable, the protocol and investigational plan are being followed, the IRB has been notified of approved protocol changes as required, complete records are being maintained, appropriate and timely reports have been made to the Sponsor and the IRB, and the Investigator is executing all agreed activities.

MGH CTNI retains the right to remove either the investigator or the investigational site from the study for issues of non-compliance with the protocol or regulatory requirements.

On one or more occasions, the study site may be inspected or audited by the MGH CTNI representative or a third party. The Investigator will be informed in advance of this visit.

M.4. Study Closeout Visit

Upon completion of the clinical study (when all subjects at the site have completed follow up visits, all data has been entered in the EDC system and cleaned, all queries resolved, and final electronic signatures have been obtained), a study closeout visit will be performed. Any unused study materials and equipment will be collected and returned to MGH CTNI. The Monitor will ensure that the Investigator's regulatory files are up to date and complete and that any outstanding issues from previous visits have been resolved. Other issues which will be reviewed at this visit include: discussing retention of study files, possibility of site audits, publication policy, and ensuring that the Investigator will notify the IRB regarding study closeout.

M.5. Direct Access to Source Data/Documents

Subcontracts with study sites will specify that MGH CTNI or its representatives will have direct access to source data and documents for study monitoring. Additionally, NIMH, and the site IRBs may review source data following appropriate guidelines for this process.

M.6. Interim Analysis

No interim analysis is planned for this study.

M.7. Medical Monitoring

The medical monitor for this trial will be an M.D. designated by MGH CTNI. Throughout the course of the study the MGH CTNI Medical Monitor will review all adverse events, review issues related to subject eligibility, and assess the benefits and risks of protocols on an ongoing basis.

In addition, the Medical Monitor will be available for site questions. The Medical Monitor will be available to sites for questions regarding inclusion/exclusion criteria, protocol conduct, and safety.

Trained and qualified MGH CTNI staff physicians will be available to provide coverage during times when the Medical Monitor is unavailable. Sites will be provided with the Medical Monitor's cell phone number for emergency situations. Otherwise, sites are instructed to contact the Medical Monitor through email. All communications with sites will be documented by the Medical Monitor and kept in a file managed by the MGH CTNI Research Assistant, and reviewed periodically by the MGH CTNI Program Manager.

Each month the Medical Monitor will receive a listing of protocol violations for review and identification of possible trends.

Serious adverse events and clinically significant suicidal ideation or behavior will be reported by the site within 24 business hours of their knowledge of the event. For reportable (unexpected and/or related) serious adverse events and clinically significant suicidal ideation or behavior, the Medical Monitor will contact the Sponsor-Investigator within 48 business hours of the notice by the site. In addition, the Medical Monitor may request individual patient records, including laboratory data, clinical records, and other study related data, to evaluate these events against the known safety profile of the study treatment and the disease. Any records sent to the Medical Monitor by the site will be redacted for any identifying information before transmission. The Medical Monitor may recommend actions including partial or complete unblinding, and/or modifying or terminating the study. In addition to safety monitoring, the Medical Monitor may review enrollment data, demographic information, retention status, and other reports prepared by the study statistician that describe study performance and progress. The Medical Monitor will provide a report to the sSponsor-Investigator that describes study safety, progress and performance and provide recommendations regarding safe continuation or early termination of the trial. After review and evaluation of the specified periodic reports prepared by the statistician, the Medical Monitor will prepare a summary cover letter, according to pre-specified criteria, for submission to the Sponsor-Investigator. The letter will provide comments on the report, discuss any concerns or suggestions for change, and recommend continuation or cessation of the trial.

Urgent Clinical Situations: During the course of the study, a patient will be reassessed for continued participation based on clinical judgment of the investigators. All subjects will be offered access to 24/7 coverage for medical emergencies through the clinical staff at each site. Treatment options and their risks and benefits, including continued study participation and other treatment options, will be sensitively explained. All investigators have extensive clinical experience in the treatment of MDD and TRD, and can make the decision (while blinded) as to removal of subjects from the trial, particularly in the event of clinical deterioration or emergence/worsening of suicidal ideation. When a patient is removed from the trial based on clinical judgment, investigators will implement an appropriate treatment plan and arrange for follow-up for the patient.

N. DATA ANALYSIS

To analyze the primary outcome data, we will use a linear mixed model for the observations taken at baseline, and subsequent visits, with terms for visit, and visit*treatment for visits other than baseline, the primary contrast of interest being the difference between the two weeks (Day 14) value for active treatment and placebo. To address missing data, we will use all available data and use a restricted maximum likelihood estimation procedure, as implemented by SAS Proc MIXED. The model will include a site, a site*time effect and a site*time*treatment interaction. The latter will be removed if not significant. Number of comorbid medical and psychiatric disorders and duration of illness, as measured prior to randomization, will be included as covariates. For the continuous secondary efficacy variables, the same approach as for the primary efficacy variable will be used for the analysis.

Power analysis: the sample size was chosen to ensure 80% power to detect an effect size of 0.8 on the SDQ between brexpiprazole plus intranasal ketamine treatment and placebo at a two-sided 0.05 significance level. This assumes the randomization of 52 subjects (26 subjects per arm).

O. PROTOCOL SIGNATURE PAGE

I have read this protocol and agree to adhere to the requirements. I will provide copies of this protocol and all pertinent information to the study personnel under my supervision. I will discuss this material with them and ensure they are fully informed regarding the conduct of the study according to 21 CFR parts 50, 54, 56 and 812, 45 CFR 46, to GCP as described in ICH guideline E6 and to hospital Institutional Review Boards.

_____

Clinical Site

_____ _____

Principal Investigator Signature Date

_____

Principal Investigator

Printed Name

APPENDIX 1 CONCOMITANT MEDICATIONS

Medications Allowed (Y) and Not Allowed (N) as Concomitant Medications

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Analgesics	Y	Y	Nonnarcotic analgesics are allowed. Medically appropriate episodic use of narcotic analgesics is allowed for acute medical indications but is limited to no more than 3 days for each episode, and may not be taken the day of the ketamine administration starting at midnight. Chronic NSAID use is exclusionary; tramadol is also not allowed.
Anesthetics, general	Y (except for ketamine, which is excluded)	—	If procedures requiring general anesthesia are to occur/have occurred, please contact MGH CTNI to report the medical condition(s).
Anesthetics, local	Y	N	—
Anorexics	N	N	—
Antacids	Y	Y	—
Antiacne	Y	Y	Topical agents only, including topical antibiotics. Isotretinoin (Accutane) is not allowed.
Antianginal agents	N	N	—
Antiarrhythmics	N	N	Amiodarone is excluded
Antiasthma agents	Y	Y	—
Antibiotics	Y	Y	Chronic use of topical antibiotics for acne is allowed, with the exception of the MAOI linezolid (Zyvox) and isoniazid, which are not allowed. Erythromycin, clarithromycin, rifampin are excluded.
Anticoagulants	N	N	Warfarin (Coumadin) is not allowed. Antiplatelet agents are allowed (see "Antiplatelets").

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Anticholinergics	Y	Y	Except for scopolamine.
Anticonvulsants	N	Y	Gabapentin, and pregabalin are allowed. Other anticonvulsants are not allowed, including lamotrigine and carbamazepine. Stable in dosing at least four weeks prior to randomization is required.
Antidepressants	N	Y	Stable (for at least 4 weeks prior to screening), ongoing antidepressant therapy is required during the course of the study. No dose changes are allowed during the study. Monoamine oxidase inhibitors (which may have unknown drug-drug interactions) are excluded. Concomitant use of trazodone (up to 200mg daily) is allowed. Nefazodone is excluded.
Antidiarrheal preparations	Y	N	Only loperamide HCl (Imodium), bismuth subsalicylate (Pepto-Bismol), and kaolin preparations are allowed.
Antifungals, systemic	N	Y	—
Antifungals, topical	Y	Y	Ketoconazole and itraconzole are excluded
Antihistamines	Y	Y	The use of combinations containing pseudoephedrine or phenylephrine is not allowed. Combination products containing the word nighttime or are specifically marketed for before sleep routinely include an antihistamine and are not allowed. Combination products ending in “-D” routinely contain a stimulant such as phenylephrine, and the appropriate limits above apply to them. (See “Cough and Cold Preparations” for combination products.)

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Antihypertensives	N	Y	Diltiazem, verapamil are excluded
Anti-impotence medications	Y	Y	—
Anti-inflammatory drugs	Y	Y[a]	Indomethacin (Indocin) and systemic corticosteroids are not allowed. Chronic NSAID use is exclusionary.
Antifungal	Y	Y	Itraconazole is excluded
Antimigraine	N	N	Triptans are not allowed
Antinauseants/Antiemetics	Y	N	Phosphoric acid preparations (Emetrol, Emecheck), bismuth subsalicylate (Pepto-Bismol), cola syrup, 5-HT_3 receptor antagonists (e.g., ondansetron), and prokinetic agents (metoclopramide) are allowed. Scopolamine is not allowed (see section on antihistamines).
Antineoplastics/ Immunosuppressant agents	N	Y[c]	Interferons, methotrexate, and other immunosuppressant agents are not allowed. Call MGH CTNI for approval for certain cases in cancer remission maintaining therapy.

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Antiobesity/Appetite suppressants	N	N	OTC Alli (Xenical), sibutramine (Meridia), and phentermine (Adipex-P and others) are not allowed.
Antiplatelet agents	N	Y[b]	Aspirin (maximum 325 mg/day) and clopidogrel (Plavix) are allowed. Note that use of an SSRI or of a triple uptake inhibitor may increase bleeding times and possibly prothrombin times.
Antipsoriatic treatments	Y	Y	Only topical agents are allowed. Acitretin (Soriatane) is not allowed.
Antipsychotics	N	N	
Antismoking medications	N	Y[c]	Varenicline (Chantix) is not allowed. Chronic nicotine replacement may be allowed in certain cases after review with MGH CTNI.
Antiviral agents	Y	Y	Only oral or topical agents are allowed. Only acyclovir, famciclovir, valacyclovir, penciclovir, docosanal, trifluridine, and vidarabine are allowed. Amantadine,rimantadine, indinavir, nelfinavir, ritonavir, saquinavir are not allowed. Tamiflu (oseltamivir phosphate), and Relenza (zanamivir) inhalants are permitted for influenza prophylaxis but use is limited to a 7- to 14-day course in accordance with prescribing information. Interferons are not allowed.
Anxiolytics	N	Y	Chronic, stable treatment with benzodiazepines is allowed. Stable in dosing at least four weeks prior to randomization is required. Benzodiazepines should not be taken within 2 hours of ketamine administration.
Barbiturates	N	N	Barbiturates are not allowed.

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Benign prostatic hyperplasia treatments	N	Y[b]	Male patients who have symptoms of obstructed voiding should not be included in the study. Surgically or medically treated patients must be asymptomatic and receiving a stable dosage of allowed medications (α-1 blockers, finasteride, or dutasteride) for 1 month before screening.
Buspirone	N	Y	Stable in dosing at least four weeks prior to randomization is allowed.
Cough/cold preparations	Y	N	Use of cough and cold preparations containing pseudoephedrine or dextromethorphan is not allowed, as are those containing phenylephrineCombination products ending in "-D" routinely contain a stimulant such as phenylephrine, and the appropriate limits apply to them. (See "Antihistamines".)
Diuretics	Y	Y[b]	Episodic use of diuretics is restricted to treatment of premenstrual symptoms. For chronic use, medication and dosage should be stable for 1 month before screening.
Dopaminergics	N	Y	Dopamine agonists for restless leg syndrome are allowed.
Gastrointestinal: • H_2-blockers/ • proton pump inhibitors/ • prokinetic agents	Y	Y	Cimetidine (Tagamet) is not allowed. Metoclopramide is not allowed.
Hormonal (noncontraceptive) therapies	N	Y	See below.
Hormone suppressants	N	Y[b]	Only finasteride (Proscar) and dutasteride (Avodart) are allowed.

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Hormones: reproductive	N	Y	Systemic hormonal contraceptives (oral contraceptives of estrogen and progestin combinations, depot injections such as Depo-Provera, the contraceptive implant Implanon, or transdermally delivered contraceptives such as Ortho Evra) are allowed
Hormones: thyroid	N	Y	Thyroid hormone replacement is allowed (dosage of thyroid medication should be stable for 3 months before screening). Therapeutic use for psychiatric disorders (e.g., T3 augmentation) is not allowed
Hypoglycemic agents	N	Y	Oral hypoglycemic agents are allowed. Insulin is not allowed
Hypolipidemics	N	Y[b]	Ezetimibe (Zetia) is allowed
Hypolipidemics: bile acid sequestrants	N	N	—
Hypolipidemics: fibrates	N	Y[b]	Gemfibrozil and fenofibrate are allowed
Hypolipidemics: niacin	N	N	Niacin and niacinamide are not allowed
Hypolipidemics: statins	N	Y[b]	Lovastatin, simvastatin, pravastatin, atorvastatin, fluvastatin, and rosuvastatin are allowed
Laxatives	Y	Y[a]	Only fiber-based products and docusate sodium (Colace) are allowed
Lithium	N	Y	Stable in dosing at least four weeks prior to randomization is allowed.
Medications that are primarily metabolized by CYP2C8 (e.g., cerivastatin, paclitaxel, repaglinide, sorafenib, and torsemide)	N	N	—
Muscle relaxants	N	N	—
NMDA receptor antagonist	N	N	Memantine is excluded.

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Opioid agonists/analgesics (e.g., codeine, hydrocodone, methadone, morphine, maperidine, propoxyphene) and antagonists (e.g., naltrexone, naloxone, nalmefene)	N*	N*	See section on analgesics for exceptions
Proton pump inhibitors or H2 receptor blockers	Y	Y	Cimetidine
Sedatives/hypnotics	N	Y	Ongoing, stable hypnotic therapy (e.g., zolpidem, zaleplon, benzodiazepine hypnotics, and low-dose trazodone 50-200mg) will be allowed during the course of the study. Eszopiclone is not allowed. Patients should not take benzodiazepines, within 2 hours of ketamine administration.
Steroids/systemic	Y	N	Systemic steroid treatment will be allowed only for medical emergencies, such as severe allergic reactions
Steroids/topical and inhalant	Y	Y	—
Steroids/intra-articular	Y	NA	—
Stimulants	N	N	Oral or transdermal methylphenidate, amphetamine products or prodrugs, pseudoephedrine, modafinil (Provigil), and other medications of same category are notallowed
Vaccines	Y	NA	—

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments

a If being taken prior to enrolling in the study.

b If being taken for at least 3 months prior to enrolling in the study and the dose has been stable for at least 1 month.

c If approved by MGH CTNI Medical Monitor$5\text{-}HT_3$ = 5-hydroxytryptomine receptor type 3; 5-HTP = 5-hydroxytryptophan; ACE = angiotensin-converting enzyme; CR = controlled release; DHEA = dehydroepiandrosterone; N = no; NA = not applicable; OTC = over the counter; PRN = as needed (prorenataT3prorenataT3=triiodothyronine; Y = yes.

APPENDIX 2 REFERENCES

Anderson IM.Meta-analytical studies on new antidepressants. Br Med Bull 2001. 57:161-78.

Anderson IM. Selective serotonin reuptake inhibitors versus tricyclic antidepressants: a meta-analysis of efficacy and tolerability. J Affect Disord 2000. 58(1):19-36.

Abdel-Rahman MS, Ismail EE. Teratogenic effect of ketamine and cocaine in CF-1 mice. Teratology. 2000 Apr;61(4):291-6.

Adhvaryu SG, Trivedi AH, Jani KH, Vyas RC, Dave BJ. Genotoxic effects of ketamine on CHO cells. Arch Toxicol. 1986 Jul;59(2):124-5.

Bajbouj M, Merkl A, Schlaepfer TE, Frick C, Zobel A, Maier W, O'Keane V, Corcoran C, Adolfsson R, Trimble M, Rau H, Hoff HJ, Padberg F, Müller-Siecheneder F, Audenaert K, van den Abbeele D, Matthews K, Christmas D, Eljamel S, and Heuser I. Two-year outcome of vagus nerve stimulation in treatment-resistant depression. J Clin Psychopharmacol 2010. 30(3):273-81.

Bakish D. New standard of depression treatment: Remission and full recovery. J Clin Psychiatry 2001. 62(Suppl 26):5–9.

Bauer M, El-Khalili N, Datto C, Szamosi J, and Eriksson H.A pooled analysis of two randomised, placebo-controlled studies of extended release quetiapinefumarate adjunctive to antidepressant therapy in patients with major depressive disorder. J Affect Disord 2010. 127(1-3):19-30.

Bech P, Allerup P, Gram LF, Reisby N, Rosenberg R, Jacobsen O, and Nagy A.The Hamilton depression scale. Evaluation of objectivity using logistic models. Acta Psychiatr Scand 1981. 63(3):290-9.

Bech P, Wilson P, Wessel T, Lunde M, Fava M. A validation analysis of two self-reported HAM-D6 versions. Acta Psychiatr Scand 2009: 119: 298–303.

Bech P. Fifty Years with the Hamilton Scales for Anxiety and Depression: A Tribute to Max Hamilton. Psychother Psychosom 2009; 78:202–211.

Bech P, Boyer P, Germain JM, Padmanabhan K, Haudiquet V, Pitrosky B, Tourian KA. HAM-D17 and HAM-D6 sensitivity to change in relation to desvenlafaxine dose and baseline depression severity in major depressive disorder.Pharmacopsychiatry 2010. 43(7):271-6.

Bech, P, Kastrup M, and Rafaelsen OJ. Mini-compendium of rating scales for states of anxiety depression mania schizophrenia with corresponding DSM-III syndromes. Acta Psychiatr Scand 1986. 73(suppl. 326):1-37.

Bremner JD, Krystal JH, Putnam FW, Southwick SM, Marmar C, Charney DS, Mazure CM. J Trauma Stress.1998 Jan;11(1):125-36.

Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, Krystal JH: Antidepressant effects of ketamine in depressed patients. Biol Psychiatry 2000; 47(4):351-4.

Chandler GM, Iosifescu DV, Pollack MH, Targum SD, and Fava M. Validation of the Massachusetts General Hospital Antidepressant Treatment History Questionnaire (ATRQ). CNS NeurosciTher 2010. 16(5):322-5.

Committee for Medicinal Products for Veterinary Use (CVMP). Summary Report 2007.

Correll GE, Futter GE: Two case studies of patients with major depressive disorder given low-dose (subanesthetic) ketamine infusions. Pain Med 2006; 7(1):92-5.

Cuijpers P, van Straten A, Bohlmeijer E, Hollon SD, Andersson G. The effects of psychotherapy for adult depression are overestimated: a meta-analysis of study quality and effect size. Psychol Med 2010. 40(2):211-23.

Cuijpers P, van Straten A, Warmerdam L, and Andersson G. Psychotherapy versus the combination of psychotherapy and pharmacotherapy in the treatment of depression: a meta-analysis. Depress Anxiety 2009. 26(3):279-88.

Deckersbach T, Hoelzel, BK, Eisner LR, Stange JP, Peckham AD, Dougherty DD, Rauch SL, Lazar S, Nierenberg AA. Mindfulness-based cognitive therapy for non-remitted patients with bipolar disorder. CNS Neurosci Ther 2011. 17:410-419.

Denninger JW, Papakostas GI, Mahal Y, Merens W, Alpert JE, Nierenberg AA, Yeung A, and Fava M. Somatic symptoms in outpatients with major depressive disorder treated with fluoxetine. Psychosomatics 2006. 47(4):348-52.

Dunnett, CW "A multiple comparison procedure for comparing several treatments with a control", Journal of the American Statistical Association 1955. 50:1096–1121.

Entsuah AR, Huang H, and Thase ME. Response and remission rates in different subpopulations with major depressive disorder administered venlafaxine, selective serotonin reuptake inhibitors, or placebo. J Clin Psychiatry 2001. 62(11):869-877.

Faries D, Herrera J, Rayamajhi J, DeBrota D, Demitrack M, and Potter WZ. The responsiveness of the Hamilton Depression Rating Scale. J Psychiatr Res 2000. 34(1):3-10.

Fava M. Diagnosis and definition of treatment-resistant depression. Biol Psychiatry 2003. 53:649–659.

Fava M and Davidson KG.Definition and epidemiology of treatment-resistant depression. Psychiatr Clin North Am 1996. 19:179–200.

Fava M, Mischoulon D, Iosifescu D, Witte J, Pencina M, Flynn M, Harper L, Levy M, Rickels K, Pollack M. A double-blind, placebo-controlled study of aripiprazole adjunctive to antidepressant therapy among depressed outpatients with inadequate response to prior antidepressant therapy (ADAPT-A Study). Psychother Psychosom. 2012; 81(2):87-97.

Fava M, Shelton R, Zajecka J, Rickels K, Clain A, Baer L, Schoenfeld D, Nelson E, Barbee J, Lydiard B, Mischoulon D, Alpert J, Zisook S, Papakostas G. L-Methylfolate Augmentation of Selective Serotonin Reuptake Inhibitors (SSRIs) for SSRI-Resistant Major Depressive Disorder: Results of Two Randomized, Double-Blind Trials. Poster presented at the 49th Annual ACNP Meeting, Miami, FL: 2010.

Fava M, Ménard F, Davidsen CK, Baker RA. Adjunctive Brexpiprazole in Patients With Major Depressive Disorder and Irritability: An Exploratory Study. J Clin Psychiatry. 2016 Jul 5. doi: 10.4088/JCP.15m10470. [Epub ahead of print].

Fawcett J, Epstein P, Fiester SJ, Elkin I, and Autry JH. Clinical Management-Imipramine/Placebo Administration Manual: NIMH Treatment of Depression Collaborative Research Program. Psychopharm Bull 1987. 23:309-324.

Fekadu A, Wooderson SC, Markopoulo K, Donaldson C, Papadopoulos A, and Cleare AJ. What happens to patients with treatment-resistant depression? A systematic review of medium to long term outcome studies. J Affect Disord 2009. 116(1-2):4-11.

Fergusson DM, Horwood LJ, and Lynskey MT. Maternal depressive symptoms and depressive symptoms in adolescents.J Child Psychol Psychiatry 1995.36(7):1161-1178.First BM, Spitzer RL, Gibbon M, Williams JBW. Structured Clinical Interview for DSM-IV Axis I Disorders - Patient Edition (SCID I/P). Biometrics Research Department, New York State Psychiatric Institute, 1995.

Frank E, Prien RF, Jarrett RB, Keller MB, Kupfer DJ, Lavori PW, Rush AJ, and Weissman MM. Conceptualization and rationale for consensus definitions of terms in major depressive disorder. Remission, recovery, relapse, and recurrence. Arch Gen Psychiatry 1991.48:851–855.

Freeman MP, Walker R, Laughren T, Miller KK, Fava M. Female Reproductive Hormones and Lifecycle Status: Methodology to Improve Clinical Trials. Journal of Clinical Psychiatry 2013 Oct., 74(10) 1018-21..

Furey ML and Drevets WC. Antidepressant efficacy of the antimuscarinic drug scopolamine: a randomized, placebo-controlled clinical trial. Arch Gen Psychiatry 2006. 63(10):1121-9.

Gram LF; Danish University Antidepressant Group (DUAG). Acute and continuation therapy in unipolar depression: observations from the run-in phase of a maintenance trial. Acta Psychiatr Scand 2008. 118(2):123-9.

Greenberg PE, Kessler RC, Birnbaum HG, Leong SA, Lowe SW, Berglund PA, and Corey-Lisle PK. The economic burden of depression in the United States: how did it change between 1990 and 2000? J Clin Psychiatry 2003. 64(12):1465-1475.

Guy W. Clinical Global Impression (CGI) ECDEU Assessment manual for Psychopharmacology. Rockville, MD: U.S. Dept Health Education and Welfare 1976.

Hamilton M.A rating scale for depression. J Neurol Neurosurg Psychiatry 1960. 23:56-62.

Hamilton M. Development of a rating scale for primary depressive illness. Br J Social Clin Psychology 1967. 6:278-296.

Ingrande J. and Lemmens H.J.M. Dose adjustment of anaesthetics in the morbidly obese. Br J Anaesth. (2010). 105 (suppl 1): i16-i23.

Insel TR and Wang PS. The STAR*D trial: revealing the need for better treatments. Psychiatr Serv 2009. 60(11):1466-7.

Institute of Medicine (US) Forum on Neuroscience and Nervous System Disorders. CNS Clinical Trials: Suicidality and Data Collection, Workshop Summary. Washington (DC): National Academies Press (US); 2010.

Jevtovic-Todorovic, Vesna, and Lisa B. Carter. "The anesthetics nitrous oxide and ketamine are more neurotoxic to old than to young rat brain." Neurobiology of aging 26.6 (2005): 947-956.

Jevtovic-Todorovic, Vesna, et al. "A comparative evaluation of the neurotoxic properties of ketamine and nitrous oxide." Brain research 895. 1 (2001): 264-267.

Jevtovic-Todorovic, Vesna, Nicholas Benshoff, and John W. Olney. "Ketamine potentiates cerebrocortical damage induced by the common anaesthetic agent nitrous oxide in adult rats." Br J Pharmacology 130.7 (2000): 1692-1698.

Judd LL, Akiskal HS, Maser JD, Zeller PJ, Endicott J, Coryell W, Paulus MP, Kunovac JL, Leon AC, Mueller TI, Rice JA, and Keller MB. Major depressive disorder: A prospective study of residual subthreshold depressive symptoms as predictor of rapid relapse. J Affect Disord 1998. 50:97–108.

Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, Rush AJ, Walters EE, Wang PS; National Comorbidity Survey Replication. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA 2003. 289(23):3095-3105.

Kochhar MM, Aykac I, Davidson PP, Fraley ED. Teratologic effects of d,1-2-(o-chlorophenyl)-2-(methylamino) cyclohexanone hydrochloride (ketamine hydrochloride) in rats. Res Commun Chem Pathol Pharmacol 1986 Dec;54(3):413-6.

Kudoh A, Takahira Y, Katagai H, Takazawa T: Small-dose ketamine improves the postoperative state of depressed patients. Anesth Analg 2002; 95(1):114-8.

Lapidus KA, Levitch CF, Perez AM, Brallier JW, Parides MK, Soleimani L, Feder A, Iosifescu DV, Charney DS, Murrough JW. A randomized controlled trial of intranasal ketamine in major depressive disorder. Biol Psychiatry. 2014 Dec 15;76(12):970-6.

Levine J and Schooler NR. SAFTEE: a technique for the systematic assessment of side effects in clinical trials. Psychopharmacol Bull 1986. 22(2):343-81.

Machado-Vieira R, Salvadore G, Diazgranados N, and Zarate CA Jr. Ketamine and the next generation of antidepressants with a rapid onset of action. Pharmacol Ther 2009. 123(2):143-50. PMC ID: PMC2726824.

Majewski-Tiedeken CR1, Rabin CR, Siegel SJ. Ketamine exposure in adult mice leads to increased cell death in C3H, DBA2 and FVB inbred mouse strains. Drug Alcohol Depend. 2008 Jan 1;92(1-3):217-27. Epub 2007 Oct 24.
Montgomery SA and Asberg M. A new depression rating scale designed to be sensitive to change. Br J Psychiatry 1979. 134:382–389.

McIntyre RS, Weiller E, Zhang P, Weiss C. Brexpiprazole as adjunctive treatment of major depressive disorder with anxious distress: Results from a post-hoc analysis of two randomised controlled trials. J Affect Disord. 2016 Sep 1;201:116-23. doi: 10.1016/j.jad.2016.05.013. Epub 2016 May 12.

McKeage K. Adjunctive Brexpiprazole: A Review in Major Depressive Disorder. CNS Drugs. 2016 Feb;30(2):91-9. doi: 10.1007/s40263-016-0320-0.

Nelson JC. The STAR*D study: a four-course meal that leaves us wanting more. Am J Psychiatry 2006. 163(11):1864-6.

Nelson JC and Papakostas GI. Atypical antipsychotic augmentation in major depressive disorder: a meta-analysis of placebo-controlled randomized trials. Am J Psychiatry 2009. 166(9):980-91.

Nierenberg AA, Katz J, and Fava M.A critical overview of the pharmacologic management of treatment-resistant depression. Psychiatr Clin North Am 2007. 30(1):13-29.

Nierenberg AA, Keck P, Samson J, Rothschild AJ, and Schatzberg AF. Methodological considerations for the study of treatment-resistant depression. In: Amsterdam JD, editor. Refractory Depression. New York: Raven Press 1991. 1–12.

Olney, John W., Joann Labruyere, and Madelon T. Price. Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 244.4910 (1989):1360-1362.

O'Reardon JP, Solvason HB, Janicak PG, Sampson S, Isenberg KE, Nahas Z, McDonald WM, Avery D, Fitzgerald PB, Loo C, Demitrack MA, George MS, and Sackeim HA. Efficacy and safety of transcranial magnetic stimulation in the acute treatment of major depression: a multisite randomized controlled trial. Biol Psychiatry 2007. 62(11):1208-16.

O'Sullivan RL, Fava M, Agustin C, Baer L, and Rosenbaum JF.Sensitivity of the six-item Hamilton Depression Rating Scale.ActaPsychiatrScand 1997. 95(5):379-84.Papakostas GI, Shelton RC, Smith J, and Fava M. Augmentation of antidepressants with atypical antipsychotic medications for treatment-resistant major depressive disorder: a meta-analysis. J Clin Psychiatry 2007. 68(6):826-31.

Papakostas GI, Charles D, Fava M. Are typical starting doses of the selective serotonin reuptake inhibitors sub-optimal? A meta-analysis of randomized, double-blind, placebo-controlled, dose-finding studies in major depressive disorder. *World J Biol Psychiatry.* 2007 Jul 13:1-8.

Paykel ES.Achieving gains beyond response. Acta Psychiatr Scand 2002. 415(suppl):12–17.

Paykel ES.Social functioning and the depressed patient. Int J Psychiatry Clin Pract 1999. 3:S9-S11.

Pedrelli P, Blais MA, Alpert JE, Shelton RC, Walker RS, Fava M. Reliability and validity of the Symptoms of Depression Questionnaire (SDQ). CNS Spectr. 2014 Dec;19(6):535-46.

Perry EB, Cramer J, Cho H-S, Petrakis I, Karper L, Genovese A, O'Donnell E, Krystal J and D'Souza C. Psychiatric Safety of Ketamine in psychopharmacology research. Psychopharmacology (2007) 192:253-260.

Phelps LE, Brutsche N, Moral JR, Luckenbaugh DA, Manji HK, Zarate CA, Jr.: Family history of alcohol dependence and initial antidepressant response to an N-methyl-D-aspartate antagonist. Biol Psychiatry 2009; 65(2):181-4.

Posner K, Oquendo MA, Gould M, Stanley B, and Davies M. Columbia Classification Algorithm of Suicide Assessment (C-CASA): classification of suicidal events in the FDA's pediatric suicidal risk analysis of antidepressants. Am J Psychiatry 2007. 164:1035-1043.

Price RB, Nock MK, Charney DS, Mathew SJ: Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. Biol Psychiatry 2009; 66(5):522-6.

Puddu PE., Jouve R., Marlotti S., et al. Evaluation of 10 QT prediction formulas in 881 middle-aged men from the Seven Countries study: emphasis on the cubic root Fridericia's equation. J Electrocardiol 1988;2:219–229.

aan het Rot M, Collins KA, Murrough JW, Perez AM, Reich DL, Charney DS, Mathew SJ: Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. Biol Psychiatry 2010; 67(2):139-45

Rush, AJ. Kraemer HC, Sackeim HA, Fava M, Trivedi MH, Frank E, Ninan PT, Thase ME, Gelenberg AJ, Regier DA, Rosenbaum JF, Ray O, and Schatzberg AF. Report by the ACNP Task Force on Response and Remission in Major Depressive Disorder. Neuropsychopharmacology 2006. 31:1841-1853.

Russell JM, Hawkins K, Ozminkowski RJ, Orsini L, Crown WH, Kennedy S, Finkelstein S, Berndt E, and Rush AJ. The cost consequences of treatment-resistant depression. J Clin Psychiatry 2004. 65(3):341-7.

Sackeim HA.The definition and meaning of treatment resistant depression. J Clin Psychiatry 2001. 62(suppl 16):10–17.

Simon GE. Social and economic burden of mood disorders. Biol Psychiatry 2003. 54(3):208-215.

Simon GE, VonKorff M, and Barlow W. Health care costs of primary care patients with recognized depression. Arch Gen Psychiatry 1995. 52:850-856.

Snaith RP, Hamilton M, Morley S, Humayan A, Hargreaves D, Trigwell P. A scale for the assessment of hedonic tone the Snaith-Hamilton Pleasure Scale. Br J Psychiatry 1995 Jul;167(1):99-103.

Steffens DC, Krishnan KR, and Helms MJ. Are SSRIs better than TCAs? Comparison of SSRIs and TCAs: a meta-analysis. Depress Anxiety 1997. 6(1):10-8.

Stewart WF, Ricci JA, Chee E, Hahn SR, and Morganstein D. Cost of lost productive work time among US workers with depression. JAMA 2003. 289(23):3135-3144.

Tohen M, Case M, Trivedi MH, Thase ME, Burke SJ, and Durell TM. Olanzapine/fluoxetine combination in patients with treatment-resistant depression: rapid onset of therapeutic response and its predictive value for subsequent overall response in a pooled analysis of 5 studies. J Clin Psychiatry 2010. 71(4):451-62.

Toyama Y, Shimizu H, Suzuki Y, Miyakoshi Y, Yoshioka H..Genotoxic effects of N-nitrosoketamine and ketamine as assessed by in vitro micronucleus test in Chinese hamster lung fibroblast cell line. Environ Health Prev Med 2006 May;11(3):120-7. doi: 10.1265/ehpm.11.120.

Trevino K, McClintock SM, and Husain MM. A review of continuation electroconvulsive therapy: application, safety, and efficacy. J ECT 2010. 26(3):186-95.

Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, Norquist G, Howland RH, Lebowitz B, McGrath PJ, Shores-Wilson K, Biggs MM, Balasubramani GK, and Fava M; STAR*D Study Team. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. Am J Psychiatry 2006. 163(1):28-40.

Trivedi MH, Wisniewski SR, Morris DW, Fava M, Gollan JK, Warden D, Nierenberg AA, Gaynes BN, Husain MM, Luther JF, Zisook S, Rush AJ. Concise Health Risk Tracking scale: a brief self-report and clinician rating of suicidal risk. J Clin Psychiatry 2011 Jun;72(6):757-64.

Trivedi MH, Wisniewski SR, Morris DW, Fava M, Kurian B, Gollan JK. Nierenberg AA, Warden D, Gaynes BN, Luther JF, Rush AJ. Concise Associated Symptoms Tracking (CAST) Scale: A Brief Self and Clinician Rating of Symptoms Associated with Suicidality. J Clin Psychiatry 2011 Jun;72(6):765-74.

Unützer J, Patrick DL, Simon G, Grembowski D, Walker E, Rutter C, and Katon W. Depressive symptoms and the cost of health services in HMO patients aged 65 years and older. A 4-year prospective study. JAMA 1997. 277:1618-1623.

Van Londen L, Molenaar RP, Goekoop JG, Zwinderman AH, and Rooijmans HG. Three- to 5-year prospective follow-up of outcome in major depression. Psychol Med 1998.28:731–735.

Vickers A and deCraen A. Why use placebos in clinical trials? A narrative review of the methodological literature. J ClinEpidemiol 2000. 53:157-61.

Wiersma D. Measuring social disabilities in mental health. Social Psychiatry and Psychiatric Epidemiology 1996. 31(3-4):101-108.

Xu Y, Hackett M, Carter G, Loo C, Gálvez V, Glozier N, Glue P, Lapidus K, McGirr A, Somogyi AA, Mitchell PB, Rodgers A. Effects of Low-Dose and Very Low-Dose Ketamine among Patients with Major Depression: a Systematic Review and Meta-Analysis. Int J Neuropsychopharmacol. 2016 Apr 20;19(4). pii: pyv124. doi: 10.1093/ijnp/pyv124. Print 2016 Apr.

Zarate CA, Jr., Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, Charney DS, Manji HK: A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch Gen Psychiatry 2006; 63(8):856-64

Zuo DY, Wu YL, Yao WX, Cao Y, Wu CF, Tana.ka M: Effect of MK-801 and ketamine on hydroxyl radical generation in the posterior cingulate and retrosplenial cortex of free-moving mice, as determined by in vivo microdialysis. Pharmacology Biochemistry and Behavior 2007 Jan;86(1):1-7. Epub 2006 Jun 27