Developing Neuronal KCNQ Channel Modulators for Mood Disorders
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SPONSOR: JAMES W. MURROUGH, MD

INSTITUTION: ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI (ISMMS)

Clinical Trial Protocol

DEVELOPING NEURONAL KCNQ CHANNEL MODULATORS FOR MOOD DISORDERS

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Investigational Product:	Ezogabine (Potiga, GlaxoSmithKline)
IND Number:	Exempt
Development Phase:	Phase IIa
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Approval:

PI or Sponsor Signature (Name and Title) *Date*

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing the Sponsor (Dr. James Murrough, MD) with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: EZOR61-01

Protocol Title: **DEVELOPING NEURONAL KCNQ CHANNEL MODULATORS FOR MOOD DISORDERS**

Protocol Date: 9/28/2018

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Investigator Signature *Date*

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Site # _____

Site Name _____

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List of Abbreviations

ACC	Anterior cingulate cortex
AE	Adverse Event
CFR	Code of Federal Regulations
CTOBB	Clinical Trials Operations and Biostatistics Branch
CNS	Central Nervous System
CRF	Case Report Form
CRP	C-reactive protein
DMC	Data Monitoring Committee
DS	Dorsal Striatum
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
fMRI	functional Magnetic Resonance Imaging
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IFT	Incentive Flanker Task
IRB	Institutional Review Board
MDD	Major Depressive Disorder
MID	Monetary Incentive Delay
mPFC	Medial Prefrontal Cortex
MRI	Magnetic Resonance Imaging
NAc	Nucleus Accumbens
NIH	National Institutes of Health
NIMH	National Institute of Mental Health
PI	Principal Investigator
PK	Pharmacokinetic
SAE	Serious Adverse Event
VS	Ventral Striatum
WHODAS	WHO Disability Assessment Schedule
WHO	World Health Organization

PROTOCOL SYNOPSIS

TITLE	**Developing Neuronal KCNQ Channel Modulators for Mood Disorders**
SPONSOR	James W. Murrough, MD
FUNDING ORGANIZATION	National Institutes of Health (NIH) National Institute of Mental Health (NIMH)
NUMBER OF SITES	2
RATIONALE	Depressive disorders are among the most disabling medical conditions worldwide and currently available treatments fall short of addressing this large public health burden. Dysfunction within the brain reward system is emerging as a core feature of depressive disorders, in particular related to deficits in motivation, interest, and response to pleasure (e.g., anhedonia: markedly diminished response to pleasure). Evidences from a series of preclinical studies highlighted the KCNQ subtype of neuronal potassium (K+) channel as a novel target for the treatment of depressive disorders and data from human pilot study showed a reduction in anhedonia and related symptoms, with an increased brain response to reward (as measured by functional magnetic resonance imaging [fMRI]) following treatment with ezogabine, a KCNQ channel opener. Building on these data, the current project will assess reward circuit activity following treatment with ezogabine in depressed patients with anhedonia, and will examine the relationship between change in reward circuit activity and clinically relevant symptom and behavior outcomes.
STUDY DESIGN	This is a phase IIa, randomized, parallel arm, placebo-controlled clinical trial.
PRIMARY OBJECTIVE	To establish target engagement of a KCNQ channel opener (ezogabine) in patients with depressive disorder and anhedonia
SECONDARY OBJECTIVES	To determine if treatment with ezogabine is associated with a reduction in clinical symptoms of depression and anhedonia, and modulation of behavioral responses to reward.
NUMBER OF SUBJECTS	48 randomized across both sites (n=24 in each treatment group)
SUBJECT SELECTION CRITERIA (ABBREVIATED)	Inclusion Criteria: Participants will be aged 18-65 years, with clinically significant anhedonia (Snaith-Hamilton Pleasure Scale [SHAPS] ≥ 20) and a current diagnosis of depressive disorder (Major depressive disorder [MDD], persistent depressive disorder, other specified depressive disorder) according to DSM-5 and at least moderate current illness severity at least moderate (Clinical Global Impression–Severity [CGI-S] Scale ≥ 4). Exclusion Criteria: A primary psychiatric diagnosis other than a depressive disorder as defined by DSM-5, neurocognitive disorder, substance use disorder within the past 6 months, contraindication to

	MRI, history of retinal abnormalities, and any unstable medication condition or any other condition that is contraindicated for treatment with ezogabine according to the FDA.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Ezogabine at 900 mg daily. Product will be administered orally according to FDA recommended titration and taper schedule.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	The placebo product used in this study will be manufactured according to GMP and will contain inert substances. Product will be administered orally.
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study for up to 14 weeks **Screening:** up to 6 weeks **Titration and Treatment Period:** 5 weeks **Taper Period:** 3 weeks The total duration of the study is expected to be 24 months.
CONCOMMITANT MEDICATIONS	See Table 1 for protocol prohibited medications
EFFICACY EVALUATIONS	
PRIMARY ENDPOINT	**Change in activation within the reward circuit (VS/mPFC)** from Visit 0 (baseline) to Study Visit 5, as measured by functional magnetic resonance imaging (fMRI) and a validated reward task (a variant of the monetary incentive delay task [MID]).
SECONDARY ENDPOINTS	**Change in behavioral and clinical symptoms of anhedonia** and on **depressive symptoms** assessed through a behavioral task and self- and clinician administered ratings scales
OTHER EVALUATIONS	**Pharmacokinetics evaluation**: plasmatic ezogabine concentrations will be determined at specific time point and summarized by study visit.
SAFETY EVALUATIONS	**Frequency and severity of observed adverse events** over the ezogabine titration, treatment and tapering periods. Suicidal ideation and behavior will be monitored throughout the study. According to FDA recommendations, a complete ophthalmological exam at Visit -1 (screening) and at Visit 6 (Study Exit) will be performed.
PLANNED INTERIM ANALYSES	None / Not applicable
STATISTICS **Primary Analysis Plan**	The primary endpoint is the baseline-corrected fMRI activation in an a priori bilateral ventral striatum (VS) area mask during the gain cue contrasted with neutral cue of the Incentive Flanker Task (IFT) . A linear mixed effects model will be used to assess the change in activation of the bilateral VS by treatment group.

Rationale for Number of Subjects	Based on our pilot data, the within-subject effect of time (ezogabine 900 mg daily) on the neural reward circuit was Cohen's d = 0.95. Assuming a placebo effect on the brain of Cohen's d = 0.15, we estimate a between-group effect of 0.8 standard deviations. A sample size of 48 provides 85% power to detect a difference in means between groups of 0.8 standard deviations assuming a correlation of 0.6 between a pair of measurements made on the same subject using a two-sided 0.05 significance level test.

1 BACKGROUND

Ezogabine (Potiga®, GlaxoSmithKline) is a first-in-class KCNQ-selective potassium (K+) channel opener approved by the U.S. Food and Drug Administration (FDA) for the adjunctive treatment of partial-onset seizures. Ezogabine selectively binds to and activates KCNQ transmembrane K+ ion channels enhancing trans-membrane potassium currents mediated by the KCNQ (Kv7.2 to 7.5) family of ion channels. By activating KCNQ channels, ezogabine is thought to stabilize the resting membrane potential and reduce brain excitability. In vitro studies suggest that ezogabine may also exert therapeutic effects through augmentation of GABA-mediated currents.
For more detail refer to the Package Insert.

1.1 Overview of Non-Clinical Studies

Recent work demonstrates a causal role for the KCNQ-type K+ channel in reversing depressive phenotypes following social defeat stress (Chaudhury et al., 2013; Friedman et al., 2014). Mice manifesting the depressive/anhedonic phenotype (compared to resilient mice) show a failure to increase KCNQ channel activity within the ventral tegmental area (VTA) – Ventral Striatum (VS) reward circuit. This susceptible phenotype can be reversed through (a) overexpression of KCNQ channels, (b) direct VTA injection of KCNQ channel openers, or (c) peripheral injection of KCNQ channel openers. Remarkably, repeated peripheral daily administration the KCNQ channel opener ezogabine completely reversed the depressive/anhedonic phenotype in susceptible mice. These results provide a direct validation of KCNQ channel openers as a completely novel class of antidepressants. The proposed project takes advantage of the availability of ezogabine for human use to test this hypothesis in patients with depression. This “re-purposing” approach is hoped to speed novel drug discovery since crucial knowledge concerning pharmacokinetics (PK), toxicology, brain exposure and safety has already been established for ezogabine, thereby considerably de-risking its development.

1.2 Overview of Clinical Studies

The efficacy of ezogabine as adjunctive therapy in partial-onset seizures was established in 3 multicenter, randomized, double-blind, placebo-controlled studies in 1,239 adult patients (Ezogabine product information). The most common adverse effects of ezogabine are dizziness (23%), drowsiness (22%) and fatigue (15%). For more detail refer to the FDA Package Insert.

Based on the data mentioned above and the knowledge that ezogabine readily crosses the blood brain barrier (BBB) (Orhan et al., 2012; Large et al., 2012), our group examined the effects of ezogabine on reward circuit responses in patients with depression using fMRI and a variant of the Monetary Incentive Delay (MID) task developed by our group called the Incentive Flanker Task (IFT) (Stern et al., 2011). N=9 patients with Major Depressive Disorder (MDD) and anhedonia (defined by Snaith-Hamilton Pleasure Scale [SHAPS] ≥ 20)

underwent functional Magnetic Resonance Imaging (fMRI) and completed clinical and behavioral assessments at baseline and following treatment with ezogabine titrated to 900 mg daily. Ezogabine was well tolerated, no subjects discontinued the treatment protocol and no serious adverse events (SAEs) occurred and no unexpected adverse events (AEs) occurred. Baseline and outcome scans were available for n=6 patients. Based on our mechanistic model, we hypothesized that ezogabine would lead to an increase in brain responses to both reward expectancy (e.g., anticipation) and reward responsiveness (e.g., consumption) within the cortico-striatal reward circuit (Der-Avakian & Markou, 2012; Grabenhorst & Rolls, 2011; Levy & Glimcher, 2012; Delgado et al., 2000; Elliott, et al., 2000; Knutson et al., 2001; Knutson et al., 2005; Haber & Knutson, 2010). Following treatment with ezogabine compared to baseline (Time 2>Time 1), brain responses to both reward expectancy and reward responsiveness were enhanced. Specifically, activation during anticipation was increased within the caudate, putamen, and medial prefrontal cortex (mPFC); activation during feedback was increased within the VS/nucleus accumbens (NAc). Clinically, ezogabine led to a reduction in depression severity with a comparatively larger improvement in symptoms specifically linked to an altered reward processing, namely anhedonia, loss of interest, and reduced motivation [$t(8)=4.4$, $p=0.002$]. Overall, our preliminary data are consistent with the ability of ezogabine to modulate reward circuit activity in humans and the plausibility of KCNQ potentiation as a novel treatment approach for reward alterations in depression.

2 STUDY RATIONALE

Depressive disorders – chief among them MDD and persistent depressive disorder (previously ‘dysthymia’) – are among the most disabling medical conditions worldwide (Collins, 2011; Whiteford et al., 2013). Depression ranks first in disability in both high and low income countries among all brain-based disorders, including all psychiatric, neurological and substance disorders (Collins et al., 2011). In the United States, the total economic cost of depression in 2012 was estimated to be $188 billion (Mrazek et al., 2014). Contributing to the problem, a substantial proportion of patients with depression fail to achieve a clinically meaningful improvement in illness severity despite numerous antidepressant trials and augmentation strategies (Rush et al., 2006; Trivedi et al., 2006).

Currently available psychotropic medications for the treatment of depression largely share the same basic pharmacology and mechanism of action based on discoveries made decades ago (Berton & Nestler, 2006). This lack of mechanistic diversity is an important cause of the observed limitations in the efficacy of these treatments, and leaves little opportunity for personalized medicine. In contrast, rational drug discovery based on a mechanistic understanding of disease pathology promises to deliver more effective, targeted therapies (Berton & Nestler, 2006; Hasler et al., 2004; Agid et al., 2007; Anacker, 2014; Millan et al., 2015). The current project represents a set of translational experiments stemming from fundamental research into the molecular basis of depression (Chaudhury et al., 2013; Friedman et al., 2014; Nestler & Carlezon, 2006; Berton et al., 2006; Krishnan et al., 2007;

Krishnan & Nestler, 2008; Cao et al., 2010; Tye et al., 2013; Russo & Nestler, 2013; Walsh et al., 2014). Our unique investigational team is poised to translate these recent discoveries into urgently needed, mechanistically novel treatments for patients with depression.

This study represents the first part of the R61/R33 National Institutes of Mental Health (NIMH) funded project. A clear increase in reward circuit activation in the ezogabine treatment group compared to placebo, given acceptable tolerability, will constitute a "go" and the project will move to the next phase (R33) (see paragraph "Primary Outcome Measure: Neuroimaging Methods", section 9 "Study procedures and guidelines" and section 16.3 "Analysis of Primary Endpoint" for details); the R33 will examine the relationship between treatment, reward circuit activity, and behavioral and clinical outcomes in subjects suffering from depression and anhedonia.

2.1 Risk / Benefit Assessment

The most common adverse effects of ezogabine are dizziness (23%), drowsiness (22%) and fatigue (15%). No contraindications to treatment were reported in the manufacturer's label. Since QT prolongation has been observed, ECG will be monitored at screening and study exit. Skin discoloration around the lips or nail bed, either blue or gray, has been reported and is estimated to affect up to 10% of patients treated for more than two years. Below we summarize the risks of the study drug and strategies to mitigate risk. In addition, please see section 5.3 "Safety Evaluation" for additional description of study safety evaluations.

QT Prolongation:
QT prolongation has been observed in healthy subjects during a randomized, double-blind study where the maximum mean increase of QT interval of 7.7 msec was observed at 3 hours after 22 days of treatment with ezogabine 1,200 mg daily. No effects on heart rate, PR, or QRS intervals were noted. In our trial, the dosage of 1200 mg daily will not be reached and participants will be at the highest dose of 900 mg per day, respectively, for one week. Moreover, since patients who are prescribed ezogabine with medicines known to increase QT interval or who have known prolonged QT interval, congestive heart failure, ventricular hypertrophy, hypokalemia, or hypomagnesemia should be observed closely, to ensure participants' safety we will exclude from the trial subjects with:
- unstable medical illness including ischemic heart disease or other cardiovascular disease;
- history of QT prolongation or congenital QT syndrome
- prolonged QT interval at screening (QTc > 480 msec)
- clinically significant abnormalities of laboratory tests and EKG

To reduce risks for participants in this trial, an EKG will be performed at the screening, at visit 4 (to monitor any QT effects that have emerged prior to escalating to the highest dose; see Section 10.3 "Double Blind Treatment Period" for additional details) and at study exit visits.

Skin Discoloration:

Skin discoloration around the lips or nail bed, either blue or gray, has been reported and is estimated to affect up to 10% of patients treated for more than two years. Given the limited exposure to the study drug in the current study, we anticipate the risk of skin discoloration to study participants will be very small.

Suicidality:
Antiepileptic drugs (AEDs), including ezogabine, have been found to increase the risk of suicidal thoughts or behavior in pooled analyses. Pooled analyses of 199 placebo-controlled clinical trials (mono- and adjunctive-therapy) of 11 different AEDs showed that patients randomized to one of the AEDs had approximately twice the risk (adjusted relative risk 1.8, 95% confidence interval [CI]: 1.2, 2.7) of suicidal thinking or behavior compared to patients randomized to placebo. In these trials, which had a median treatment duration of 12 weeks, the estimated incidence of suicidal behavior or ideation among 27,863 AED-treated patients was 0.43% compared to 0.24% among 16,029 placebo-treated patients, representing an increase of approximately 1 case of suicidal thinking or behavior for every 530 patients treated. There were 4 suicides in drug-treated patients in the trials and none in placebo-treated patients, but the number is too small to allow any conclusion about drug effect on suicide. Suicide ideation and behavior will be monitored during the length of the study through clinical assessments at screening and exit visits, and weekly during the treatment period. The Columbia Suicide Severity Rating Scale (C-SSRS) will also be conducted at each study visit to monitor suicidal ideation and behavior. If, at any point during the clinical trial, a participant will be assessed with a suicidal ideation with any intent or plan, as measured by a C-SSRS score of greater than 3 during the past week and/or with a Global Clinical Impression – Severity Scale (GCI-S) score ≥6 (indicating extreme illness severity) or a Global Clinical Impression – Improvement Scale (CGI-I) score ≥ 6 (indicating an extreme worseness of the symptoms), a safety review will be performed by the site PI, or his designee, to determine the appropriate course of action including whether acute intervention is needed and whether it is in the best interests of the subject to continue in the study. For more information, refer to paragraphs 5.3 “Safety evaluations” and section 9 “Study Procedures and Guidelines”.

Other Adverse Effects:
Other side effects include urinary retention, neuropsychiatric symptoms (confusional state, psychotic symptoms, and hallucinations) and withdrawal seizures. To protect participants from these possible side effects the following actions will be completed:
- urologic symptoms will be carefully monitored and a comprehensive evaluation of urologic symptoms prior to and during treatment with ezogabine will be completed;
- neuropsychiatric symptoms will be investigated at screening and ezogabine will be titrated on a 5-week period according to the FDA package insert in order to reduce the risk of psychosis and hallucinations; to monitor the impact of dizziness and somnolence, that usually occur during the titration phase, subjects will be evaluated on a weekly based by a study physician;
- withdrawal symptoms will be prevented by a gradual withdrawn over a period of at least 3 weeks, unless safety concerns require abrupt withdrawal.

Retinal Changes:
The FDA had issued a black box warning regarding the potential for retinal abnormalities following treatment with ezogabine, with an unknown relationship to vision loss. In June 2015, the FDA issued a Safety Alert stating that there was no evidence for treatment associated vision loss following a review. In keeping with FDA recommendations, study participants undergo full ophthalmological exams at screening and study exit. For more information please refer to sections 5.3 “Safety evaluations” and 9 “Study procedures and guidelines.

Potential for Unforeseen Risks:
In addition to these risks there may be other risks associated with ezogabine, which are currently unknown as the study drug has never been used on a sample of anhedonic subjects with mood and anxiety disorders. Data from our pilot study show that ezogabine was well tolerated, no subjects discontinued the treatment protocol, no SAEs and no unexpected AEs occurred.

Potential for Harms Unrelated to Study Drug:
The following additional sources of potential discomfort or harm could also occur:

- Psychological Screening: Subjects that meet exclusion criteria (e.g. positive pregnancy test) may become distressed when informed. All information will be shared in a private office with the door closed and a trained study team member will be available to offer information, support, and referral for any services indicated.
- Medical Screening: Participants may experience minor discomfort or pain when a needle is used to draw blood. Bruising, infection, and dizziness are also common side effects. Risks of venipuncture are minimized by having experienced nursing personnel who will perform the procedure. Infection is avoided by adequate cleansing of the skin prior to intravascular line insertion.
- fMRI Scans: The fMRI procedure is very safe and exposes the study subjects to minimal risk or discomfort. The MRI scanning involves the subject placing their head in a tube and some subjects may experience a feeling of claustrophobia while in the scanner. However, a study investigator will be present for all scanning sessions in case of any unexpected complications. In addition, the participant will remain in verbal contact with a study investigator (via intercom) throughout the procedure so that they may communicate immediately any problems, or express a desire to terminate the scan at any time.
- Questionnaires: Some participants may experience psychological distress when asked to answer personal questions about their emotions. In this case, experimenters will remind subjects that they can refuse to answer any upsetting questions. Clinicians will also be available to meet with any distraught individuals.
- Privacy risks: There always exists the potential for loss of private information; however, there are procedures in place to minimize this risk. Please refer to page 40 section “Data Collection, Retention and Monitoring” for more details.

To protect subjects from such unknown risks we will not allow subjects to participate in the study if they have: 1) any clinically significant abnormality on the screening tests; 2) any clinically significant history of medical, psychiatric, or substance use-related disorder other than a mood and anxiety spectrum disorder; 3) excessive alcohol intake or unwillingness to stop alcohol consumption for the duration of the study; 4) taken any prescribed or not prescribed medication prohibited by the protocol within 30 days of the first study visit, or need to continue any of the abovementioned medications during the study; 5) QT prolongation, electrolyte abnormalities, concomitant medications that may augment QT prolongation, or any underlying cardiac abnormality which may potentiate risk; 6) any alteration emerging at the ophthalmological exam performed at screening that in the opinion of the ophthalmologist or the Principal Investigator (PI), may increase risk for potential retinal abnormalities for the subject. For more details on study eligibility, see section 6 “Subject Selection”.

Subjects may not receive any direct benefit from participating in this study. There is a small possibility that we may detect an unrelated medical condition during the procedures conducted in this study that could have health benefits for some individuals. Some individuals may experience improvement in their symptoms through participation in the study, though some are likely to remain the same or get worse. The information obtained from this study may help improve the treatment of future patients with MDD or other mood disorders. Since the available treatments for patients with MDD have significant limitations (Trivedi et al., 2006), this study has the potential to take steps to address these limitations by: 1) establishing that ezogabine is a safe and efficacious treatment for MDD; 2) exploring the impact of the drug on a discrete brain circuit for reward 3) validating a new class of treatments for an important medical problem, with significant impact on patients and on society; 4) exploring the impact of ezogabine on behavioral and clinical measures of anhedonia and depression. As mentioned before, this study represents the first part of the R61/R33 NIH funded project. A clear increase in reward circuit activation (in terms of both significance and effect size) in the ezogabine treatment group compared to placebo, given acceptable tolerability, will constitute a “go” and the project will move to the phase (R33).

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective of the current study is to assess evidence of target engagement by the study drug as measured by change in the cortico-striatal circuit response to reward in a sample of subjects with a current depressive disorder (MDD, persistent depressive disorder, other specified depressive disorder) and anhedonia (indexed by a score of ≥ 20 on the Snaith-Hamilton Pleasure Scale [SHAPS]). Two treatment conditions in a parallel arm design will be: (1) ezogabine arm (ezogabine 900 mg/day), or (2) placebo. The effect of ezogabine relative to placebo on the reward circuit will be assessed studying the brain response during the IFT at baseline and after 5 weeks of treatment. Please, refer to section

9 “Study Procedures and Guidelines” for a description of the procedures and specific instrument that will be used.

Go/No-Go Decision: A clear increase in reward circuit activation in the ezogabine treatment group compared to placebo, given acceptable tolerability, will constitute a “go” and the project will move to the R33 phase.

3.2 Secondary Objectives

Secondary efficacy objective: the study will examine the effects of ezogabine on clinical and behavioral measure of anhedonia, depressive symptoms, and suicide ideation in an exploratory manner. In addition, general functioning will be measured with the WHO Disability Assessment Schedule 2.0 (WHODAS 2.0; World Health Organization [WHO], 2012). Please, refer to sections 9 “Study procedures and guidelines” for more details.

Secondary safety objective: to examine the safety and tolerability of ezogabine in patients with depressive disorder.

4 STUDY DESIGN

4.1 Study Overview

This is a multi-center, phase IIa, parallel arm, double-blind, randomized, placebo-controlled, clinical trial. Forty-eight treatment-seeking patients with a primary diagnosis of depressive disorder according to Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition (DSM-5; MDD, persistent depressive disorder, other specified depressive disorder), clinically significant anhedonia (defined SHAPS ≥ 20) and at least moderate current illness severity (measured as Clinical Global Impression–Severity [CGI-S] Scale ≥ 4) will be enrolled. Each subject will be randomly assigned to one of the treatment arms (ezogabine, placebo) in a 1:1 fashion under double-blind conditions; randomization will be under the direction of the coordinating center and stratified by center. Screening data will be reviewed by the site PI to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study. Study participants will undergo assessments at baseline then following study drug/placebo weekly for five weeks (titration period and primary outcome visit) and then after three weeks (taper period and exit visit). During the taper period participants will undergo weekly phone calls from a member of the study team to assess compliance and side effects. Please, refer to **Table 2** “Schedule of Assessments, Outcome Measures, Labs, and Procedures.”

Following screening and randomization, study participants undergo a 5-week medication titration schedule according to the FDA package insert. For more information about randomization, treatment and taper schedules please refer to section 8 “Study Treatments”.

The following treatment regimens will be used:

- Ezogabine group: ezogabine at 900 mg daily
- Placebo group: inactive compound daily.

Total duration of subject participation will be up to 14 weeks. Total duration of the study is expected to be 24 months.

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

Our primary efficacy endpoint is change in activation within the bilateral VS from baseline (Study Visit 0) to week 5 (Study Visit 5) as measured by fMRI during the IFT. Patients with depression show abnormal responses to reward expectancy and reward responsiveness within the cortico-striatal reward circuit (VS/mPFC/Anterior cingulate cortex [ACC]) and data from our pilot study on ezogabine showed that following treatment with ezogabine, brain responses to reward expectancy and reward responsiveness were enhanced. The VS is considered to be the principal reward related region and therefore functions as the primary region of interest (ROI). The right and left VS regions will be combined (averaged) into a single bilateral ROI as there are no laterality hypotheses regarding effect of treatment on the VS.

5.2 Secondary Efficacy Endpoints

Similarly to the primary efficacy endpoint, change in activation within other regions of the reward cortico-striatal circuit (for example, mPFC and ACC) from baseline to week 5 will be measured by fMRI during the IFT.

Clinical outcome

Change in clinical symptoms of anhedonia (as measured by the SHAPS, Temporal Experience of Pleasure Scale [TEPS], Specific Loss of Interest and Pleasure Scale [SLIPS], Anticipatory and Consummatory Interpersonal Pleasure Scale [ACIPS] and on depressive symptoms assessed through clinical ratings scales (Montgomery-Asberg Depression Rating Scale [MADRS]). Global illness improvement and severity will be evaluated through changes in CGI-I and CGI-S scores. The level of disability and functioning will also be investigated via the World Health Organization Disability Assessment Schedule (WHODAS 2.0). For more details on the clinician and self-report measure that will be administered, please refer to section 9 “Study procedures and guidelines”, paragraph 9.1.6 “Other clinical procedures”.

Behavioral outcome

Change in behavioral symptoms of anhedonia will be captured through a computer-based task, the Probabilistic Reward Task (PRT). In this task, a signal detection theory approach is used, whereby subjects must discriminate between two ambiguous stimuli, displayed

rapidly on a computer screen, in order to receive a monetary reward. Unbeknownst to the subjects, correct identification of one stimulus is reinforced three times more frequently than the other stimulus. Under these experimental circumstances, healthy subjects reliably develop a response bias (that is, preference) for the stimulus that is reinforced more frequently, regardless of which stimulus was actually presented. Thus, reward responsiveness assessed in this task reflects the rapid shaping of future behavioral choices based on prior reinforcement experiences. Subjects with MDD and healthy subjects with elevated depressive symptoms fail to develop this biased response for the more frequently reinforced stimulus, and tend to respond similarly to both stimuli, reflecting decreased responsiveness to rewards. Moreover, response bias was found to predict current and future anhedonic symptoms in both nonclinical and clinical samples. For more details on PRT, please refer to section 9 “Study procedures and guidelines”, paragraph 9.1.6 “Other clinical procedures”.

5.3 Safety Evaluations

Safety will be monitored by the evaluation of potential adverse events, clinical laboratory test results, vital signs measurements, physical examination, ECG and ophthalmological findings. Our safety evaluations include frequency and severity of observed adverse events over the study treatment period. The frequency and severity of adverse events will be compared between the treatment conditions. For a complete discuss of risks associated with the study drug, please see section 2.1 “Risk/Benefit Assessment”. For a complete discussion of study procedures, please see section 9 “Study Procedures and Guidelines”. Below, we outline specific safety evaluations conducted as part of the study protocol.

Monitoring for Worsening Depression and Suicidal Ideations or Behaviors
Participants will meet with a study clinician weekly during the treatment period. The interval medical history will be obtained and adverse events, changes to concomitant medications and dosing compliance will be recorded. Suicidal ideation and behavior will be specifically assessed, including a review of the Columbia Suicide Severity Rating Scale (C-SSRS) and the overall severity of symptoms will be monitored by the clinical global impression scale (CGI-S). If, at any point during the clinical trial, a participant will be assessed with a suicidal ideation with any intent or plan, as measured by a C-SSRS score of greater than 3 during the past week and/or with a GCI-S score ≥6 (indicating extreme illness severity) or a CGI-I score ≥ 6 (indicating an extreme worseness of the symptoms), the site PI, or his designee, will conduct a safety review to determine the appropriate course of action including whether acute intervention is needed and whether it is in the best interests of the subject to continue in the study.

Safety review: The safety review will consist of a separate review of safety and well-being of the participant by the site PI or his designee. This review will be documented by a Safety Review Form (SRF) that will be filed in the subject binder and that will consist of a brief description of the case and of the event that triggered the review. Outcome of the review will be the decision whether to continue or discontinue the subject from the protocol (if

this is the case, appropriate clinical care will be provided). In the eventuality the event satisfies the definition of an adverse event (AE) or of a serious adverse event (SAE), it will be reported accordingly. For more detail about adverse event monitoring and reporting, please refer to section 11 “Adverse experience reporting and documentation”.

Ophthalmological exams

All the participants will also undergo a complete ophthalmological exam at screening and at study end by a study ophthalmologist, to include the following procedures: Visual acuity, Color plates, Amsler grid, Visual field test, Dilated fundus exam, Fundus photography, and Ocular coherence tomography (OCT) of the macula. Should patient exhibit any changes in fundus exam and/or OCT from screening, they will undergo a fluorescein angiogram and possibly electroretinogram depending on the discretion of the Ophthalmologist. After cessation of medication the regularity of baseline dilated eye exams will be determined by the presence or absence of retinal pathology at the discretion of the Ophthalmologist.

Additional Safety Monitoring

In addition to the procedures described above, the following procures are performed as part of the study safety evaluations. Medical history, current and past physical and psychiatric history, venous blood sampling for clinical laboratory tests (comprehensive metabolic panel, CBC, TSH), vital signs, urine toxicology, pregnancy test (if applicable), and ECG will be performed by qualified staff. For more information refer to sections 2.1 “Risk/Benefit Assessment” and 9 “Study Procedures and Guidelines”.

Provision of study follow-up for treatment emergent adverse events at study exit: in case of any adverse event (including, but not limited to suicidal ideation or behavior) present at the study exit visit, subject will be referred to follow-up care until the event is resolved and/or a stable care plan is in place. The site PI will follow up with the subject until the abnormal parameter or symptom has resolved or stabilized.

5.4 Other evaluations

Pharmacokinetic (PK) Procedures: Venous blood samples will be collected for determination of plasmatic ezogabine concentrations at the primary outcome visit (V5). Drug concentrations will be used to determine protocol adherence, inclusion in the planned per-protocol analysis and to examine PK – pharmacodynamic (PD) relationships. Plasmatic ezogabine levels will be used to determine drug concentration.

6 SUBJECT SELECTION

6.1 Study Population

Subjects who will meet DSM-5 criteria for depressive disorder (MDD, persistent depressive disorder, other specified depressive disorder), clinically significant anhedonia (SHAPS ≥ 20)

and at least moderate current illness severity (Clinical Global Impression–Severity [CGI-S] Scale ≥ 4) at the time of screening meeting all study inclusion and exclusion criteria will be eligible for participation in this study.

Recruitment feasibility: The research plan calls for the randomization of n=48 patients ages 18-65 with a primary diagnosis of current depressive disorder and significant anhedonia across the two performance sites (ISMMS and BCM) over a 20-month recruitment period, which is 1.2 patients randomized per month per site. This is a feasible goal based on current patient flow at the two sites, and our demonstrated track record of enrollment at similar rates in prior studies recruiting from similar populations.

6.2 Inclusion Criteria

1. Written informed consent obtained from subject and ability for subject to comply with the requirements of the study;
2. Men and women, age 18-65;
3. Participants must meet DSM-5 criteria for current depressive disorder (major depressive disorder [MDD], persistent depressive disorder, other specified depressive disorder) as determined by a study psychiatrist and confirmed using the Structured Clinical Interview for DSM-5 Research Version (SCID-5-RV);
4. Clinically significant anhedonia as determined by a SHAPS score ≥ 20 at screening;
5. Current illness severity is at least moderate, defined as a score of ≥4 on the Clinical Global Impression–Severity (CGI-S) Scale;
6. If female of childbearing potential, must agree to use of a medically accepted form of contraception, or else agree to abstinence.

6.3 Exclusion Criteria

1. A primary psychiatric diagnosis other than a depressive disorder as defined by DSM-5; [co-morbid anxiety disorders (including agoraphobia, generalized anxiety disorder, social anxiety disorder and panic disorder) and Posttraumatic Stress Disorder (PTSD) are allowed];
2. Diagnosis of a major cognitive disorder or evidence of cognitive impairment as assessed by a score of the Mini Mental Status Exam (MMSE) of <24;
3. Meets criteria for a substance or alcohol use disorder in the past 6 months;
4. Suicidal ideation with any intent or plan as measured by a Columbia Suicide Severity Rating Scale [C-SSRS] score of greater than 3 during the past month at the time of screening;
5. Female participants who are pregnant, breastfeeding, or may become pregnant, or unwilling to practice birth control during participation in the study;
6. Positive urine toxicology screen for drugs of abuse at the time of screening*;
7. Inability to swallow capsules;

8. Any unstable medical illnesses including hepatic, renal, gastroenterologic, respiratory, cardiovascular (including ischemic heart disease), endocrinologic, neurologic, immunologic, or hematologic disease;
9. Clinically significant abnormalities of laboratory tests, physical examination, or ECG;
10. Positive Hepatitis B surface antigen (HBsAg) and/or Hepatitis C Anti-Hepatitis C virus (HCV) antibody†
11. Moderate to severe renal insufficiency at the time of screening or at any point throughout study participation;
12. Prolonged QT Interval at screening, operationalized as a QTc of > 480 ms;
13. History of abnormal QT prolongation or congenital QT syndrome;
14. Concomitant use of a medication with a known significant risk of QT prolongation;
15. A history of retinal abnormalities (i.e., pigment changes, retinal dystrophy) or findings of retinal pathology on ophthalmological exam at baseline;
16. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data;
17. Use of any dis-allowed medication according to the study protocol**;
18. Serious and imminent risk of self-harm or violence as determined by the PI;
19. Extreme illness severity as defined by a GCI-S score >6;
20. Any contraindication to MRI including claustrophobia, any trauma or surgery which may have left magnetic material in the body, magnetic implants or pacemakers, and inability to lie still for 1 hour or more;
21. History of non-response to electroconvulsive therapy in the current depressive episode

** Exceptions:*
a) Subjects with a positive urine drug screen for cannabinoids, barbiturates, opiates, amphetamines, or benzodiazepines may be allowed in the study provided that the drug was used for a documented, legitimate medical purpose and/or the use of such products may be discontinued (documented by a negative repeat test) prior to randomization; Subjects with a positive urine drug screen for drugs of abuse at any study visit following the randomization visit (e.g. V5 or V8) will not be discontinued from the study.
b) Medically appropriate episodic use (up to 3 days) of narcotic analgesics for acute medical indications is allowed (Discussion with PI required)

** *Potential participants will not be discontinued from medication for the purposes of this study. If a patient is taking a protocol dis-allowed medication at the time of screening, the patient may discontinue the medication under the supervision of their treating physician in the case that the patient is not benefiting from the medication or otherwise wishes to discontinue the medication. In no case will a dis-allowed medication be discontinued for the purpose of study participation if the patient is receiving clinical benefit from the medication.*

† *Subjects who will test positive for Hepatitis B or C at the time of the screening will not allowed to participate in the study. If a subject tests positive for Hepatitis B or C, the study physician will inform the participant and provide a referral for medical evaluation and treatment as appropriate.*

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies. Refer to Table 1 for list of prohibited medications during the study.

7.1 Allowed Medications and Treatments

Study subjects are required to be free of antidepressant and other dis-allowed medication with CNS activity for a duration equivalent to 5 half-lives of the medication at the time of randomization. As needed (e.g., "PRN) use of non-benzodiazepine hypnotic agents (including zolpidem, zaleplon, eszopliclone, diphenhydramine, suvorexant, and ramelteon) or benzodiazepines not in excess of the equivalent of 2 mg of lorazepam daily will be allowed. Subjects will be prohibited from taking as needed benzodiazepines or other as needed therapy with CNS activity on the day of study assessments. Subjects receiving psychotherapy may continue to receive psychotherapy provided this therapy has been stable in terms of frequency for at least 2 months prior to screening and is expected to remain unchanged during the study treatment period.

7.2 Prohibited Medications and Treatments

A list of medications prohibited during the study is provided in Table 1 ("List of Prohibited Medications"). The administration of the cited drugs will be considered a protocol violation. In general, the following classes of medication are prohibited: (1) antidepressants, (2) antipsychotics, (3) anticonvulsants/mood stabilizers, (4) lithium, and (5) stimulants. In addition, concomitant use of a medication with a known significant risk of QT prolongation is prohibited.

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

Central randomization will be implemented in this study by the coordinating site. Forty-eight eligible patients will be randomly assigned to ezogabine (900 mg/day; n=24) or placebo treatment (n=24) groups in a 1:1 ratio using a computer-generated randomization scheme developed by the coordinating site. The randomization will be balanced by using randomly permuted blocks. The interactive web response system (IWRS) will assign a unique treatment code, which will dictate the treatment assignment and matching study drug kit for the subject.

8.2 Blinding

Patients who meet all entry criteria will be randomly assigned to one of the treatment arms, and assigned randomization numbers in consecutive order. The randomization list will be a computer-generated random-number list generated by the research pharmacist (who has no patient contact) at the coordinating site using a permuted block procedure. The randomization list will be provided by the research pharmacist at the coordinating site to the research pharmacist at the collaboration site. Due to the objective of the study, the identity of test and control treatments will not be known to investigators, research staff, or patients.

The following study procedures will be in place to ensure double-blind administration of study treatments:

- Access to the randomization code will be strictly controlled;
- Packaging and labeling of test and control treatments will be identical to maintain the blind;
- Investigators, study coordinators and all study personnel with study patient contact will remain blind to the treatment assignment.

The study blind will be broken on completion of the clinical study and after the study database has been locked. During the study, the blind may be broken by the research pharmacist **only** in emergencies when knowledge of the patient's treatment group is necessary for further patient management.

8.3 Test and Control Formulation

8.3.1 Formulation of Test Product

Ezogabine (Potiga®, GlaxoSmithKline) is approved by the FDA for the adjunctive treatment of partial-onset seizures in patients aged 18 years and older. Ezogabine is produced by GlaxoSmithKline and supplied as film-coated immediate-release tablets for oral administration: 50 mg, purple, round, film-coated tablets debossed with "RTG 50" on one side; 200 mg, yellow, oblong, film-coated tablets debossed with "RTG-200" on one side; 300 mg, green, oblong, film-coated tablets debossed with "RTG-300" on one side; and 400 mg, purple, oblong, film-coated tablets debossed with "RTG-400" on one side. The tablets should be swallowed whole. The study drug will be formulated and manufactured according to currently established commercial production standards.

8.3.2 Formulation of Control Product

A placebo tablet for oral administration will be provided by the Investigational Drug Service at each site ready for administration in a drug case labeled with the required FDA warning statement, the protocol number, a treatment number, the name of the Sponsor, and directions for patient use and storage. The placebo product used in this study will be manufactured according to GMP and will contain inert substances. The research pharmacist at ISMMS will centrally randomize participants at all performance sites.

8.3.3 Packaging and Labeling

Packaging: Study drug will be supplied in a drug case at the research pharmacy at each site in the dosage prescribed by the PI or his designee (another study M.D. approved by the PI and the Institutional Review Board [IRB] for this role).
Labeling: Each drug case of study drug will be labeled with the required FDA warning statement, the protocol number, a treatment number, the name of the Sponsor, and directions for patient use and storage

8.4 Supply of Study Medication at the Site

Ezogabine will be purchased by the research pharmacy at each site by an appropriate wholesale drug company. The Investigational Drug Service at ISMMS purchases ezogabine for research purposes from Amerisouce-Bergen Corporation, based in Chesterbrook, Pennsylvania. The initial study drug shipment will be shipped after site activation. Subsequent study drug shipments will be made after site request for resupply.

8.4.1 Dosage/Dosage Regimen

Titration Period. Study drug titration will follow the guidelines contained within the FDA package insert and will not exceed the maximum approved dose for partial-onset seizures (1,200 mg daily). For this study, a daily dose of 900 mg has been selected, along with placebo.

For the ezogabine arm, participants start at 100 mg 3 times daily (300 mg per day) and increase at weekly intervals by 150 mg (50 mg 3 times per day) up to a stable dosage of 900 mg daily. A 150 mg increase each week is the maximum increase recommended by the FDA. Those assigned to receive placebo will receive capsules containing an inactive compound at a frequency comparable to those in active treatment (i.e., 1 capsule, 3 times per day). Dose titration will be conducted based on subject tolerability.
Subjects who are unable to tolerate a protocol-specified dose increase will be retained in the study at the highest tolerated dose for that subject through to the Primary Outcome Visit (V5), before beginning to taper off of the medication. In the event that a subject experiences a potential dose-dependent adverse medication effect in the opinion of the investigator, the subject will be maintained at the subject's highest previously tolerated dose. One dose reduction for the purpose of resolving suspected medication-related adverse effects will be allowed per subject. Subjects who continue to experience tolerability issues despite the dose reduction will initiate the taper phase and will be exited early from the study protocol. The taper schedule will follow procedures reported in section 10.5 "Taper Period" and section 10.6 "Study Exit Visit" (Visit 6) and early withdrawal". All available data for each subject will be included in the statistical analyses, including from subjects who discontinue early for any reason. Study subjects who discontinue early for any reason, however, will not be replaced. During the trial, the following dosages of ezogabine will be used: 50 mg, 200 mg and 300 mg. To assure the blindness of the trial, the research pharmacist at each site will encapsulate every pill so that all study pills regardless of dosage will look identical.

8.4.2 Dispensing

The drug will be dispensed by the pharmacist of the research pharmacies of both study sites where it will be collected by study personnel. The Research pharmacy at each site will encapsulate study drug and placebo so that all study pills will look identical. Only the research pharmacist at each site will know the true identify of the study pills for each participant.

8.4.3 Administration Instructions

Following FDA packaging information, ezogabine will be given orally according to the titration and taper schedule described above. Participants will be instructed to swallow the whole tablet, with or without food. At every study visits the subject will receive the study drug in a drug case labeled with the required FDA warning statement, the protocol number, a treatment number, the name of the Sponsor, and directions for patient use and storage. The dosage will be prescribed by the PI or the Study Doctor, according to the period of the study (titration, treatment, and taper period), tolerability showed by the participant and length from the subsequent visits.

8.4.4 Storage

Study drug should be stored by the study site at controlled room temperature, 15 to 30ºC (59 to 86ºF). If the temperature of study drug storage in the clinic/pharmacy exceeds or falls below this range, this should be reported to the PI or designee and captured as a deviation. Subjects will be instructed to store the medication in original packaging (protected from light) at room temperature.

8.5 Study Medication Accountability

An accurate and current accounting of the dispensing of study drug for each subject will be maintained on an ongoing basis by a member of the study site staff. The number of study drug dispensed will be recorded on the Investigational Drug Accountability Record.

8.6 Measures of Treatment Compliance

Subsequent to randomization, study participants are provided with study drug or placebo in identical packaging and sufficient medication to reach the next visit is dispensed (plus a few extra days supply to account for scheduling and related issues). Adherence to study medication will be measured throughout the study using pill count, patient diary, subject report, and drug plasmatic levels. Subjects will be asked to keep a patient diary noting the date and time they take the study drug and any adverse effects. They will be asked to bring their patient diary to each study visit along with all used and unused study drug containers. Compliance monitoring by pill counting will occur at each study visit: pills will be returned for pill counting in the study drug containers. A study team member will count the number of returned pills and record the number on the “Pill tracking log”. Venous blood samples will be collected for determination of plasmatic ezogabine concentrations at the time point

specified and will be summarized by visit. Drug concentrations will be used to determine protocol adherence, inclusion in the planned per-protocol analysis and to examine PK and PD relationships.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is summarized in Table 2.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at screening (V-1) and at each study visit, and at early termination when possible. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured using the Concomitant Medication Log case report form.

9.1.2 Demographics

Demographic information (date of birth, gender, race) will be recorded at screening (V-1). The case report forms used to capture this information include the Demographics Form and the Edinburg Handedness Inventory.

9.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent history, and information regarding underlying diseases will be recorded at screening (V-1). Case report forms used to record the medical history include the Medical History Form, Family Psychiatric History Form, and the Anti-Depressant Treatment History Form (ATHF).

9.1.4 Physical Examination

A complete physical examination will be performed by qualified staff (MD, NP, RN, or PA) at screening (V-1) and at study end (V6). New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit. According to FDA recommendation, all study participants will undergo a complete ophthalmological exam at screening and at study end by a study ophthalmologist, to include the following procedures: Visual acuity, Color plates, Amsler grid, Visual field test, Dilated fundus exam, Fundus photography, and Ocular coherence tomography (OCT) of the macula. For more information about the measures to minimize participants risks related to the study drug and participation in the clinical trial please refer to sections 2.1 “Risk/Benefit

Assessment" and 5.3 "Safety evaluation". This is documented on the Physical Exam case report form.

9.1.5 Vital Signs

Body temperature, blood pressure, pulse and respirations will be performed after resting for 5 minutes at screening (V-1) and at study end (V6). This is documented on the Vitals case report form.

9.1.6 Other clinical procedures

Screening and baseline procedures

Structured Clinical Interview for DSM-5 (SCID-5). The Structured Clinical Interview for DSM-5 (First et al., 2015) is the most recent version of the SCID and is the first update since the SCID-IV-TR (First et al., 2002). The SCID-5 is a semi-structured interview guide for making DSM-5 diagnoses. It includes an overview to obtain information about demographics, work history, chief complaint, history of present illness, past history, treatment history, and current functioning. The main body of SCID-5 includes 9 modules that are designed to diagnose 51 mental illnesses in all. During the study screening period, the SCID-5 will be administered by a trained study team member who has completed SCID-5-RV training and who is familiar with the DSM-5 classification and diagnostic criteria. The current study will utilize the most comprehensive version of the SCID-5, the SCID-5-Research Version (RV), which contains more disorders than the Clinician Version and includes all of the relevant subtypes, severity, and course specifiers. An important feature of the SCID-5-RV is its customizability, allowing the instrument to be tailored to meet the requirements of a particular study. The SCID-5-RV comes in a standard "core" configuration that includes the disorders most researchers are likely to assess routinely for most studies, as well as in an "enhanced" configuration that includes a number of optional disorders, in addition to the disorders from the "core" configuration.

Snaith-Hamilton Pleasure Scale (SHAPS): The SHAPS (Snaith et al., 1995) is a well-validated 14-item self-report questionnaire commonly used to assess anhedonia. Each item on the SHAPS is worded so that higher scores indicate greater pleasure capacity. A total score can be derived by summing the responses to each item. Items answered with "strongly agree" are coded as "1", while a "strongly disagree" response was assigned a score of "4." Total scores on the SHAPS can range from 14 to 56, with higher scores corresponding to higher levels of anhedonia. This scale provides our primary measure of anhedonia in the study.

Clinical Global Impression - Severity (CGI-S): this is a widely administered clinician rated global measure of subject overall illness severity (Guy, 1976). Subjects are rated on a 1-7 scale where 1 corresponds to "Normal, Not at All Ill", 2 is "Borderline Mentally Ill", the anchor for 3 is "Mildly Ill", the anchor for 4 is "Moderately Ill", 5 is "Markedly Ill", 6 is "Severely Ill", and 7 is "Among the Most Extremely Ill Patients". It will be administered at screening and at all study visits.

World Health Organization Disability Assessment Schedule 2.0 (WHODAS 2.0): the WHODAS 2.0 (World Health Organization, 2012) is a 12-item generic assessment instrument that measures investigating the level of functioning in six domains: Cognition, Mobility, Self-care, Getting along, Life activities, and Participation. For all six domains, WHODAS 2.0 provides a profile and a summary measure of functioning and disability that is reliable and applicable across cultures, in all adult populations. WHODAS 2.0 has proven useful for assessing health and disability levels in the general population and in specific groups (e.g. people with a range of different mental and physical conditions).

Mini–Mental State Examination (MMSE): The MMSE (Folstein et al., 1975) is a 30-point questionnaire that is used extensively in clinical and research settings to measure cognitive impairment. It is commonly used to screen for dementia, to estimate the severity and progression of cognitive impairment and to follow the course of cognitive changes in an individual over time. Administration of the test takes between 5 and 10 minutes and examines functions including registration, attention and calculation, recall, language, ability to follow simple commands and orientation. The MMSE will be used in the current study in order to exclude for cognitive impairment at screening.

Primary Outcome Measure: Neuroimaging Methods

fMRI Task Description. The current proposal will implement a variation of the monetary incentive delay (MID) task, the incentive flanker task (IFT). The IFT incorporates a longer mean cue time, jittered cue time, and catch trials, all features that facilitate differentiation between reward expectancy and reward responsiveness (Stern et al., 2011), though data should be comparable to that obtained from the MID for purposes of comparison (thereby facilitating the NIH common data elements initiative). We have demonstrated in our laboratory that the IFT robustly engages the cortico-striatal reward circuit. During the flanker conflict portion of the task, participants are instructed to press the left response button for target letters S/K and the right response button for letters H/C (letter assignment is counterbalanced across subjects). Target letters are flanked by letters representing same or different button presses. Cues presented prior to letter stimuli (2-6 s) designate the monetary value for each trial: (1) “gain” cues indicate that subjects earn 50 cents with a correct response (and fail to gain with an error); (2) “loss” cues indicate that subjects can avoid a loss of 50 cents with a correct response (and lose money with an error); (3) “neutral” cues indicate that no money is at stake. Two-thirds of all cues will be followed by the letter stimuli. Immediately after the response, outcome feedback is presented for 2 s, followed by a blank inter-trial interval (ITI) for 2–6s before the next trial begins with a new cue. One-third of cues will be followed by a blank screen for 2s (“catch” trials), which will break co-linearity between the cue and feedback, allowing differentiation of brain activity related to expectancy and feedback response despite the slow hemodynamic response of the Blood-Oxygen-Level-Dependent (BOLD) signal. Even though BOLD signals to the button press response and outcome are not distinguishable in the current paradigm, comparisons between gain, loss, and null outcomes will subtract out brain activity related to motor responses occurring in each condition.

fMRI Task Analysis. Prior to analyzing imaging data, task behavioral data including reaction time (RT) and accuracy in response to flanker stimuli will be analyzed using ANCOVAs (including site, and any potential confounds) to examine main effects and interactions of treatment condition and cue type on reaction time and accuracy. fMRI data will undergo preprocessing using a combination of AFNI, FSL, and Advanced Normalization Tools (ANTs), including despiking, motion correction, alignment to anatomical image (using boundary-based registration), intensity normalization, smoothing (FWHM = 5mm), and warping to standard space. Motion and intensity outliers will be regressed out the data.

Following preprocessing, we will first conduct subject-level (also called 'first-level') analyses. As is the norm in the field, subject-level analyses conduct multiple linear regressions for each subject whereby the BOLD signal is predicted from a series of categorical regressors specifying the presence or absence of the conditions of interest across the full timeseries. As an example, in a trial where a cue is shown for 4 seconds followed by an outcome for two seconds, followed by another cue for 4 seconds, the cue regressor would be coded "1" (present) for the first and last 4 timepoints (in seconds) and "0" (absent) for the 2 middle timepoints where the outcome was shown. For each subject, regressions are performed within a standardized neuroimaging analysis software (such as Statistical Parametric Mapping, FSL, or AFNI), producing a single beta weight for each regressor reflecting the relationship between the presence/absence of condition of interest and BOLD signal at each voxel in the brain. For all analyses, the predicted BOLD signal is convolved with a standard hemodynamic response function (hrf) to model the slow rise and fall of the hemodynamic response in the brain. For each subject, the beta weights are then linearly combined to create contrasts (gain > neutral cue contrast, the primary contrast of interest for this study, is the beta weight for gain cue minus beta weight for neutral cue). This contrast represents the mental construct of reward anticipation.

Group level analysis is described in Section 16, Statistical Methods and Consideration.

Resting State fMRI (R-fMRI). Approximately 10 minutes worth of resting state fMRI data will be collected at both sites, pre- and post-treatment. Participants will be instructed to rest, eyes open and fixed on a central fixation cross, and to try to remain as still as possible. R-fMRI data will be examined primarily as an exploratory aim and additionally contributes to the common data element initiative. As there are a number of serious, potential confounds to the analysis of R-fMRI, advanced processing and analytic methods will be employed, including the use of robust artifact detection and removal (Pruim et al., 2015). We will conduct targeted analysis to explore whether treatment alters connectivity and network patterns with known abnormalities and that are specifically impacted by our treatment (e.g., fronto-striatal connectivity implicated in reward; see Heller et al., 2013).

Please see **MRI Procedures Appendix** for details regarding image acquisition, data transfer, processing, and multi-site data quality assurance.

Secondary Outcomes and Safety Measures

Clinical Assessments

Temporal Experience of Pleasure Scale (TEPS): The TEPS is an 18-item self-report measurement of anticipatory (10 items) and consummatory (eight items) components of anhedonia which consists of a series of statements that must be rated according to how accurate they are for the individual (Gard et al. 2006). The scale differentiates the role of anticipatory pleasure ('wanting') from consummatory pleasure ('liking'). This scale provides a measure of anhedonia and will be used in exploratory analyses.

Anticipatory and Consummatory Interpersonal Pleasure Scale (ACIPS): The ACIPS (Gooding & Pflum, 2014) is a measure specifically designed to assess hedonic capacity for social and interpersonal pleasure. Given the importance of social rewards for normal human experience, and for depressive psychopathology, we include the recently developed ACIPS in our current study as an exploratory measure. The ACIPS was designed to assess one's ability to experience pleasure in the interpersonal domain. It is a 17-item self-report measure that consists of seven anticipatory and 10 consummatory items. The ACIPS is scored on a Likert scale, ranging from 1 (very false for me) to 6 (very true for me). The format is therefore quite similar to that of the TEPS. Validation studies show good internal consistency, convergent and discriminant validity, and good test-retest stability.

Specific Loss of Interest and Pleasure Scale (SLIPS): The SLIPS (Winer et al., 2014) is a recently developed and validated measure of anhedonia that is tailored to detect recent changes in anhedonia. Many scales, including the SHAPS and the TEPS, appear tailored more towards assaying trait-like features related to anhedonia. The scale consists of 23 items; items range from 0-3 to indicate the degree of change in interest or pleasure from a specific experience, relative to the past. Validation studies show that the SLIPS is internally consistent, converged with existing state measures of anhedonia and depression, and diverged from general measures of affect and trait anticipatory and consummatory pleasure. The SLIPS predicted recent changes in anhedonia independent of existing measures. Moreover, in respondents reporting moderate or severe symptoms of depression, only the SLIPS was associated with recent changes in anhedonia.

The Quick Inventory of Depressive Symptomatology, Self-Report (QIDS-SR): The QIDS (Rush et al. 2003) is a 16-item self-rated instrument designed to assess the severity of depressive symptoms. The 16 items cover the nine symptom domains of major depression, and are rated on a scale of 0-3. Total score ranges from 0 to 27, with ranges of 0-5 (normal), 6-10 (mild), 11-15 (moderate), 16-20 (moderate to severe), and 21+ (severe).

Montgomery-Asberg Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979): this is a 10-item instrument used for the evaluation of depressive symptoms in adults and for the assessment of any changes to those symptoms. Each of the 10 items is rated on a scale of 0 to 6, with differing descriptors for each item. These individual item scores are added

together to form a total score, which can range between 0 and 60 points. The MADRS provides a measure of the overall level of depression.

The Mood and Anxiety Symptoms Questionnaire (MASQ) (Wardenaar et al., 2010): This is a 30-item self-report questionnaire, designed to measure dimensions of depression and anxiety and has been validated in both clinical and non-clinical samples. The MASQ provides three factors with good construct validity: negative affect, positive affect and somatic arousal. The scale will be used in the current study to explore changes in the dimensions of depression and anxiety.

Clinical Global Impression - Improvement (CGI-I): this is a widely administered clinician rated global measure of the degree of improvement from the initial assessment in subject overall illness severity. Subjects are rated on a 1-7 scale where 1 corresponds to "Very Much Improved", 2 is "Much Improved", the anchor for 3 is "Minimally Improved", the anchor for 4 is "No Change", 5 is "Minimally Worse", 6 is "Much Worse", and 7 is "Very Much Worse". It will be administered at all subject visits. During the course of the trial subjects for whom the CGI-I is greater than 5 will be removed from the study and appropriate care given, for safety purposes.

Columbia-Suicide Severity Rating Scale (C-SSRS): The Columbia-Suicide Severity Rating Scale (C-SSRS) (Posner et al., 2007) is a comprehensive, semi-structured interview that uniquely measures the full spectrum of suicidality including passive and active suicidal ideation, suicidal intent as well as suicidal behaviors. Subjects will be assessed at every visit with the CSSRS by a qualified rater. Study participants will meet with a study physician at each study visit, which will include a review of the C-SSRS. Any subject with an increase in suicidality and all those subjects found to have a plan or intent will undergo thorough assessment by the study psychiatrist. If, at any point during the clinical trial, a participants will be assessed by the study physician with a suicidal ideation with any intent or plan, as measured by a C-SSRS score of greater than 3 during the past week and with either a GCI-S score ≥6 (indicating extreme illness severity) or a CGI-I score ≥ 6 (indicating an extreme worseness of the symptoms), the site PI, or his designee, will conduct a safety review to determine the appropriate course of action including whether acute intervention is needed and whether it is in the best interests of the subject to continue in the study. For more details regarding the safety review procedures, please refer to section 5.3 "Safety evaluation".

Behavioral assessment

Probabilistic Reward Task (PRT): This 25-min task is presented on a 17" PC monitor using E-Prime (version 1.1; Psychology Software Tools, Inc, Pittsburgh, PA). Stimuli consist of simple cartoon faces presented in the center of the monitor. At the beginning of the trial, the face has no mouth. After a given delay, either a straight mouth of 11.5 mm ("short mouth") or 13 mm ("long mouth") is presented for 100 ms. On each trial, subjects are instructed to press an appropriate button to decide whether a long or small mouth had been presented. Unbeknownst to subjects, correct identification of one stimulus (the "rich stimulus") is

rewarded three times more frequently ("Correct! You won 20 cents") than the other ("lean") stimulus. In healthy controls, this reinforcement schedule leads to a response bias (i.e., a preference for the more frequently rewarded stimulus). The task consists of three 100-trial blocks, and the degree of response bias toward the more frequently reinforced stimulus is used for operationalizing sensitivity to reward. An index of reward learning across the task is then computed by subtracting the total response bias in the first block from the total response bias in the last block. Stimulus exposure (100 ms) and the difference between mouth sizes (11.5 vs. 13 mm) are identical to those in prior studies using this paradigm (Pizzagalli et al., 2005, Pizzagalli et al., 2008, Vrieze et al., 2013) and were selected to achieve optimal psychometric properties (e.g., overall hit rates of 75-85%).

9.1.7 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, expectedness, outcome, treatment and relation to study drug will be recorded on the case report form (CRF). Please refer to section 11 "Adverse experience reporting and documentation" for more details.

We will further protect subjects from unanticipated risks by monitoring for adverse effects at every visit during treatment by carefully checking for any adverse reactions and by assessing suicidality with the CSSRS (Posner et al., 2007). The careful monitoring of suicidal ideation and behavior is a critical component of any study conducted in patients with depressive disorder and treatment emergent suicidal ideation has been associated with the use of antidepressants and other drugs with CNS penetration. A study clinician assesses SI, suicidal behavior and risk at each study visit and a CSSRS is completed.

If, at any point during the clinical trial, a participant will be assessed by the study physician with a suicidal ideation with any intent or plan, as measured by a C-SSRS score of greater than 3 during the past week and with either a GCI-S score ≥6 (indicating extreme illness severity) or a CGI-I score ≥ 6 (indicating an extreme worseness of the symptoms), the site PI, or his designee, will conduct a safety review to determine the appropriate course of action including whether acute intervention is needed and whether it is in the best interests of the subject to continue in the study. For more details regarding the safety review procedures, please refer to section 5.3 "Safety evaluation".

9.2 Clinical Laboratory Measurements

9.2.1 Hematology and Blood Chemistry Profile

Blood will be obtained and sent to each site's clinical hematology lab for a complete blood count with differential, Hemoglobin, Hematocrit, Electrolytes, Metabolic Panel, Lipid Panel, Thyroid Function Tests, Hepatic and Renal Function Tests, Serology (only at screening) and plasmatic drug concentrations. Blood samples for determination of plasmatic ezogabine concentrations will be collected at the Primary outcome visit (V5), stored at each site (at MSSM blood sample will be stored at Russo Lab, Mount Sinai School of Medicine, 1425

Madison Avenue, ICAHN 10 – 26, New York, NY 10029) and will be processed at a specialized lab during the course of the study. These samples will be handled, processed, and mailed in accordance with applicable state, federal, and international regulations.

Please, refer to table 2 "Schedule of Assessments, Outcome Measures, Labs, and Procedures."

9.2.2 Pregnancy Test

A urine pregnancy test will be obtained from female subjects who are of childbearing age prior to their participation in the study (screening visit, V-1), at baseline (V 0), at the primary outcome visit (V5), and at study end (V6).

9.2.3 Urinalysis

Urine will be obtained and each site's clinical laboratory for determination of color, urine special gravity, osmolality, cellules, proteins and bacteria prior to their participation in the study (screening visit; V-1) and at study exit (V6). A urine toxicology screen for drugs of abuse will be performed at screening (V-1), baseline (V0), primary outcome visit (V5) and study exit (V6).

9.3 Pharmacokinetics Measurements

Venous blood samples will be collected for determination of plasmatic ezogabine concentrations at the time point specified in Table 2 and will be summarized by visit. Blood samples will be stored at each site and will be processed at a specialized lab throughout the course of the study. Results will be sent to the study data manager (unblinded member of the Mount Sinai study team) via the lab website's secure online account portal.

10 EVALUATIONS BY VISIT

Study visits may occur within 3 days before or after the scheduled visit, except for V5 (Primary outcome visit), which may occur at any point after participants have been on their highest tolerated treatment dose from at least one week (7 days) up to a maximum of two weeks (14 days), and V8 which may take place within six weeks of the Primary Outcome Visit (V5).Please refer to Table 2 "Schedule of Assessments, Outcome Measures, Labs, and Procedures."

10.1 Screening Visit (Visit -1)

1. Review the study with the subject and obtain written informed consent and HIPAA authorization.
2. Review inclusion and exclusion criteria.
3. Assign the subject a unique screening number.
4. Record demographics data.
    - Demographics Form
    - Edinburg Handedness Inventory

5. Perform a clinical interview.
6. Record medical, psychiatric, medication and treatment history, diagnosis date, and prior treatments.
    - Medical History Form
    - Family Psychiatric History Form
    - Anti-Depressant Treatment History Form
7. Record concomitant medications and tapering of prohibited medications (if applicable).
8. Perform a complete physical examination.
9. Perform and record vital signs and EKG.
10. Perform an ophthalmological exam.
11. Collect blood for clinical laboratory tests (complete blood count with differential, Hemoglobin, Hematocrit, Electrolytes, Metabolic Panel, Lipid Panel, Thyroid Function Tests, Hepatic and Renal Function Tests, and serology) and urine for urinalysis, urine toxicology and pregnancy test (female subjects who are of childbearing age).
12. Perform self and clinician administered ratings scale:
    - SCID-5-RV
    - SHAPS
    - CGI-S
    - CSSRS
    - MMSE
13. Schedule subject for Visit 0 within 6 weeks.

<u>Note:</u> The screening visit may occur on up to three separate days. The whole procedures will be completed before the randomization day (baseline visit - visit 0) and within six weeks of the first screening visit. Screening measures may be completed under a separate screening protocol at each site. As long as the assessments are completed within four weeks of the signing of consent for this protocol, the screening measures will not be repeated.

10.2 Baseline (Visit 0)

1. Obtain interval medical history.
2. Record any AEs.
3. Record changes to concomitant medications.
4. Collect urine for toxicology and pregnancy test (female subjects who are of childbearing age)
5. Randomization of the subject.
6. Illustrate drug administration and patient diary compilation.
7. Perform self and clinician administered ratings scale:
    - SHAPS
    - TEPS
    - ACIPS
    - SLIPS

- QIDS-SR
- MASQ
- MADRS
- CSSRS
- CGI-S
- CGI-I
- WHODAS
- PRT
- Structural MRI, fMRI during IFT, resting state connectivity.

Note: The Baseline/Randomization Visit should take place within six-weeks of the date that the subject signed consent for this study.

10.3 Double Blind Treatment Period (Visits 1 – 4)

1. Obtain interval medical history.
2. Record any AEs and review subject diary for adverse experiences and dosing compliance.
3. Record changes to concomitant medications.
4. EKG*
5. Perform self and clinician administered ratings scale:
   - SHAPS
   - TEPS
   - ACIPS
   - SLIPS
   - QIDS-SR
   - MADRS
   - CSSRS
   - CGI-S
   - CGI-I

Note: Study Visits 1, 2, 3, and 4 are due to take place 1, 2, 3, and 4 weeks after the Baseline/Randomization Visit (Visit 0) respectively. To allow for scheduling feasibility, Visits 1-4 can take place within 3 days before or after the scheduled visit.

** During the Double Blind Treatment Period (Visits 1 – 4), EKG will occur only on visit 4, when participants reach drug exposure of 750 mg daily over the prior week and are poised to escalate to the highest dose tested in the study of 900 mg daily. The additional EKG screening at visit 4 will ensure that no adverse QT effects have emerged prior to escalating to the highest dose. If a subject will show a QT intervals over 500 milliseconds or a 60-milliseconds increase in the QT interval from baseline, the dose will not be escalated and the subject will be tapered down from study medication over a three-week period. The study participant will be asked to return to study site to complete the primary outcome*

assessment after one week and will be exited from study upon completion of the taper period. Please, refer to section 10.6 Study exit Visit (Visit) and early withdrawal.

10.4 Primary outcome visit (Visit 5)

1. Obtain interval medical history.
2. Record any AEs and changes in concomitant medications and review subject diary for adverse experiences and dosing compliance.
3. Collect urine for toxicology and pregnancy test (female subjects who are of childbearing age)
4. Illustrate drug tapering schedule.
5. Perform self and clinician administered ratings scale:
   - SHAPS
   - TEPS
   - ACIPS
   - SLIPS
   - QIDS-SR
   - MASQ
   - MADRS
   - CSSRS
   - CGI-S
   - CGI-I
   - WHODAS
6. PRT
7. Structural MRI, fMRI during Incentive Flanker Task (IFT), resting state connectivity.
8. Beginning of the tapering period, according to FDA guidelines.

<u>Note:</u> The Primary Outcome (Visit 5) study visit will be performed when participants are on the highest tolerated treatment dose from at least one week (7 days) up to a maximum of two weeks (14 days). During this time, subjects will remain on the same dose of study medication (i.e. the highest tolerate dose) and the taper will not be initiated until the Primary Outcome Visit has been completed.

At the discretion of the investigator, the study team can repeat any and all primary outcome procedures on an alternate date (within the given V5 study window of + 7-14 days) if the visit is unable to completed as originally scheduled (e.g., due to fMRI scanner malfunction, due to subject and/or study team availability, etc.) given that the procedures are not clinically contraindicated.

10.5 Taper Period (Phone calls* 1-2)

1. Perform a brief interview and history interval.
2. Complete the phone check-in form.

3. Record any new medical problems and general tolerability.
4. Record changes to concomitant medications and review dosing compliance.
5. Provide information on the next dose of the drug, according to the tapering schedule.

The taper period will occur over a three-week period according to the following scheme:

Study drug (ezogabine)
750 mg orally daily for 1 week
600 mg orally daily for 1 week
300 mg orally daily for 1 week
Discontinue

Subjects who are not able to tolerate the 900mg daily dose will follow one of the below taper schedules depending on the maximally tolerable dose.

Case 1. Patient is taking 750 mg/daily at primary outcome visit: 2-week taper

Study drug (ezogabine) – 750 mg
600 mg orally daily for 1 week
300 mg orally daily for 1 week
Discontinue

In Case 1, subjects will be contacted by a research coordinator for Visits 6 and 7 during the first and second weeks of the taper period, respectively.

Case 2. Patient is taking 600 mg/daily at primary outcome visit: 1-week taper

Study drug (ezogabine) – 600 mg
300 mg orally daily for 1 week
Discontinue

In Case 2, subjects will be contacted by a research coordinator for Visit 6 during the first week of the taper period. The Visit 7 telephone call will not take place as the Study Exit Visit will be scheduled after the subject completes their first week of the medication taper.

Case 3. Patient is taking 450 mg/daily at primary outcome visit: 1-week taper

Study drug (ezogabine) – 450 mg
300 mg orally daily for 1 week
Discontinue

In Case 3, subjects will be contacted by a research coordinator for Visit 6 during the first week of the taper period. The Visit 7 telephone call will not take place as the Study Exit Visit will be scheduled after the subject completes their first week of the medication taper

Case 4. Patient is taking 300 mg/daily at primary outcome visit: No additional taper needed

In Case 4, subjects who are taking a maximum daily dose of 300mg will not require further taper before ending study drug intake. In these cases, subjects will not undergo a taper period and/or complete a Visit 6 or Visit 7 telephone call. The study exit visit will be scheduled after completion of the the Primary Outcome Visit (V5).

** The weekly phone calls during taper period (i.e. Visit 6 and Visit 7) will be conducted by a study research coordinator. The weekly phone calls will be scheduled one and two weeks after the Primary outcome visit (Visit 5), respectively. However, to ensure feasibility of the study and participants' availability, weekly phone calls may occur within 3 days before or after the scheduled one. If patients follow a one- or two-week taper period, one or two weekly phone calls will be scheduled respectively. An explicit request by the study participants or any safety concern (e.g., new and unexpected adverse effects, increased suicidality risk) will trigger a follow up phone call assessment by a study physician. At physician's discretion, an in-person visit will be scheduled.*

10.6 Study exit Visit (Visit 6) and early withdrawal

1. Perform a clinical interview and history interval
2. Record any AEs.
3. Record changes to concomitant medications and review subject diary for adverse experiences and dosing compliance.
4. Perform a physical examination with recording of vital signs
5. Perform EKG.
6. Perform an ophthalmological exam.
7. Collect blood for clinical laboratory tests
8. Collect urine for urinalysis, urine toxicology and pregnancy test (applies to women of child-bearing potential only).
9. Perform self and clinician administered ratings scale:
   - SHAPS
   - TEPS

- ACIPS
- SLIPS
- QIDS-SR
- MADRS
- CSSRS
- CGI-S
- CGI-I

10. PRT*.
11. Structural MRI, fMRI during IFT, resting state connectivity*

Note: The exit visit may occur on up to three separate days, within 6 weeks of the Primary Outcome Visit (Visit 5). The subject will be considered exited from the study when all the procedures listed above will be completed.

** This procedure will be performed ONLY for early withdrawal visits that take place before completion of the primary outcome visit. Capturing outcome data for patients who do not complete the full study protocol will allow a more precise estimate of the treatment effect.*

Provision of study follow-up for treatment emergent adverse events at study exit: in case of any adverse event (including, but not limited to suicidal ideation or behavior) present at the study exit visit, subject will be referred to follow-up care until the event is resolved and/or a stable care plan is in place. The site PI will follow up with the subject until the abnormal parameter or symptom has resolved or stabilized.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Event

An Adverse Event (AE) is any untoward medical occurrence in a study subject administered an investigational product and that does not necessarily have a causal relationship with this treatment.

An AE therefore can be any unfavorable and unintended sign (including laboratory finding), symptom or disease temporally associated with participation in an investigational study, whether or not considered drug-related. In addition to new events, any increase in the severity or frequency of a pre-existing condition that occurs after the subject signs a consent form for participation is considered an AE. This includes any side effect, injury, toxicity, or sensitivity reaction.

Any condition, laboratory abnormality, or physical finding with an onset date prior to the subject signing consent for study participation is considered to be pre-existing in nature and part of the subject's medical history and will not be recorded as AEs.

AE Expectedness

An unexpected AE is any adverse drug event, the specificity or severity of which is not consistent with the current IB or prescribing information for a marketed compound. Also, reports which add significant information on specificity or severity of a known, already documented AE constitute unexpected AEs. For example, an event more specific or more severe than described in the IB would be considered "unexpected".

AE Severity

Adverse events will be summarized by the Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred terms. Separate tabulations and listings will be produced for related adverse events, serious adverse events, discontinuation due to adverse events and events of at least grade 3 severity.

List criteria for AEs according to the Medical Dictionary for Regulatory Activities (MedDRA) Version 19.0 (March 2016) should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. If the experience is not covered in the abovementioned criteria, the guidelines shown in Table 3 below should be used to grade severity. Notably, the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 3. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 4.

Table 4. AE Relationship to Study Drug

Relationship to Drug	Comment

Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.2 Serious Adverse Events (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- Death
- Life threatening experience defined as any adverse experience that places the subject, in the view of the treating physician, at immediate risk of death at the time of occurrence; i.e, it does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires inpatient hospitalization or prolongation of an existing hospitalization (except scheduled hospitalizations for non-acute, unrelated cause such as an elective surgery)
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect in the offspring of an exposed subject
- Important medical events that may not result in death, be life threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, it jeopardizes the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
- Any death occurring within 30 days of the subject receiving study drug, regardless of the subject having discontinued from the protocol, must be reported as an SAE.

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

11.2.1 AE/SAE Reporting Procedures

To local PPHS (IRB) and Sponsor (e.g., Study PI, Murrough)

AEs are reportable to the IRB and to the Study PI (Dr. Murrough) within 5 business days *when they meet the following definition*:

> Any harm experienced by a subject or other individual that in the opinion of the investigator is *unexpected* AND *at least probably related* to the research
>
> A harm is at *least probably related* to the research if in the opinion of the investigator the research procedures more likely than not caused the harm

All AE/SAEs with an onset date after the subject signs consent for study participation must be reported to the IRB at the time of annual renewal. Details of the event must include severity, relationship to study drug, duration, action taken, and outcome.

All AE/SAEs that are considered related to study drug must be followed to resolution or stabilization if improvement is not expected. AE/SAEs that completely resolve and then recur should be recorded as a new AE/SAE. AE/SAEs that are considered related to study drug and continuing at 30 days post-last dose should have a comment in the source documents by the site PI that the event has stabilized or is not expected to improve.

To NIMH DSMB

All AEs and SAEs will be reported to the NIMH DSMB in data reports prepared three times annually. Details of the event must include the AE, severity, expectedness, relationship to study drug, duration (start/ stop date), action taken, and outcome. In addition, SAEs that meet the following definition are considered immediately reportable SAEs and will be reported to the NIH DSMB within **3 business days** of the site PI awareness of the event:

- All deaths and immediately life-threatening events, whether related or unrelated;
- SAEs that are unexpected and at least probably related;
- SAEs that are expected or unrelated, and are not deaths or immediately life threatening (i.e., suicide attempts), will be reported to the DSMB/NIMH in the tri-annual report.

All AE/SAEs that are considered related to study drug must be followed to resolution or stabilization, if improvement is not expected. AE/SAEs that completely resolve and then recur should be recorded as a new AE/SAE. AE/SAEs that are considered related to study drug and continuing at 30 days post-last dose should have a comment in the source documents by the PI that the event has stabilized or is not expected to improve. Other supporting documentation of the event may be requested by the DSMB/NIMH and should be provided as soon as possible.

To FDA

A report on the MedWatch 3500A form must be sent to the FDA when the event is:

(1) serious, unexpected suspected reaction (the investigator judges there is evidence to suggest a causal relationship);
(2) findings from other clinical, animal, or in-vitro studies that suggest significant human risk and
(3) a clinically important increase in the rate of a serious suspected adverse reaction no later than 15 days after determining that the information qualifies for reporting.

Unexpected fatal or life-threatening suspected SAEs should be reported no later than 7 calendar days after initial receipt of the information.

Pregnancy
Subjects will be asked to immediately inform the study team and her doctor if any of the following occur:

- She becomes pregnant while taking the study drug
- She misses her menstrual period, or experiences unusual menstrual bleeding
- She stops using birth control
- She thinks, FOR ANY REASON, that she may be pregnant

Pregnancies occurring while the subject is participating in the study or within 30 days after the subject's administration of study drug are considered expedited reportable events. Ezogabine is to be discontinued immediately and the subject instructed to return any drug to the Investigator. The pregnancy will be considered an immediately reportable event and will be reported to the DSMB and the study PI/Sponsor within 3 business days of the site PI becoming aware of the event. The event will also reported to the site local IRB within 5 business days or following the local IRB regulations, whichever is more conservative.

According to the ezogabine packaging information, physicians are advised to recommend that pregnant patients who took ezogabine enroll in the North American Antiepileptic Drug (NAAED) Pregnancy Registry. This can be done by calling the toll-free number 1-888-233-2334, and must be done by patients themselves. Information on the registry can also be found at the website www.aedpregnancyregistry.org.

Suicidality
The careful monitoring of suicidal ideation (SI) and suicidal behavior is a critical component of any study conducted in patients with MDD as treatment emergent suicidal ideation has been associated with the use of antidepressants and other drugs with CNS penetration. A study clinician assesses SI, suicidal behavior, and risk at each study visit and a CSSRS is completed.

If, at any point during the clinical trial, a participant will be assessed with a suicidal ideation with any intent or plan, as measured by a C-SSRS score of greater than 3 during the past week and/or with a GCI-S score ≥6 (indicating extreme illness severity) or a CGI-I score ≥ 6 (indicating an extreme worseness of the symptoms), the site PI, or his designee, will

conduct a safety review to determine the appropriate course of action including whether acute intervention is needed and whether it is in the best interests of the subject to continue in the study. For more information refer to section 2.1 "Risk/Benefit Assessment" and 5.3 "Safety evaluations."

11.3 Unanticipated Problems (UP)

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

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This study will use the OHRP definition of UP.

11.3.1 Unanticipated Problem Reporting

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. It is the site investigator's responsibility to report UPs to their IRB and to the DCC/study sponsor. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported to the IRB, NIMH DSMB, and to the DCC/study sponsor within 3 business days of the investigator becoming aware of the event.

- Any other UP will be reported to the IRB, the NIMH DSMB, and to the DCC/study sponsor within 2 weeks of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and OHRP within one month of the IRB's receipt of the report of the problem from the investigator.

11.4 Medical Monitoring

Dr. Dan Iosifescu, MD is the medical monitor for the study. Dr. Iosifescu is a Board-certified psychiatric and clinical investigator with over a decade of experience in clinical trials of psychopharmacological agents in depression and other mood disorders. The medical monitor for this study will act as a safety officer who is separate from the study team and will assist the study Sponsor/PI in ensuring the quality of the medical monitoring during the study. The medical monitor will perform the following specific activities:

- Review all SAEs
- Review all UPs
- Review all major protocol deviations (PDs)
- Review all DSMB and IRB annual safety reports
- In addition, the medical monitor will be available as a resource to the site PIs and the Sponsor/overall project PI in order to assist with review of individual subject eligibility

All SAEs, UPs, and major (serious) protocol deviations will be sent to Dr. Iosifescu for review within three business days of site awareness. Dr. Iosifescu will provide feedback as to whether any action should be taken in regards to the subject's participation in the trial.

Medical Monitor Contact Information:

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12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Discontinuation of Study Drug

A subject may be discontinued from study treatment at any time if the subject, the investigator feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent
- Subject is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Lost to follow-up
- Need for early termination of study
- Positive pregnancy test (females)

If a subject is withdrawn from treatment due to an adverse event, the subject will be discontinued from the study. The site PI will follow up with the subject until the abnormal parameter or symptom has resolved or stabilized. All subjects who discontinue study treatment should come in for an early discontinuation visit as soon as possible. All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. Refer to section 10.6 "Study exit Visit (Visit 6 – Day 57) and early withdrawal" for early termination procedures.

12.2 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject or the investigator, feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the Investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. As noted above, subjects who discontinue study treatment early (i.e., they withdraw prior to Visit 6; Day 57) will have an early discontinuation visit. Please, refer to section 10.6 "Study exit Visit (Visit 6 – Day 57) and early withdrawal" for early termination procedures.

At the time of study discontinuation, the study team will discuss with the participants an appropriate venue of care. If appropriate and requested by the subject, reasonable efforts will be made by the study team to provide the participant information for consultation and appropriate transfer of care after study discontinuation.

12.3 Replacement of Subjects

Subjects randomized who withdraw or are discontinued from the study will not be replaced.

13 PROTOCOL DEVIATIONS

A Protocol Deviation (PD) is defined as any change, divergence, or departure from the IRB-approved study procedures in a research protocol.

In the present study, protocol deviations will be classified as either minor (non- serious) deviations or major (serious) deviations according to the definitions listed below:

Minor Protocol Deviations:

A minor protocol deviation occurs when accidental or unintentional changes are made to the research protocol (or non-compliance with the protocol) that does not increase risk or decrease benefit to subjects, or does not have a significant effect on the subject's rights, safety or welfare; and/or on the integrity of the primary outcome data. Deviations may result from the action of the subject, researcher, or research staff.

A minor protocol deviation may be due to the research subject's non-adherence, or an unintentional change to or non-compliance with the research protocol on the part of a researcher. Examples of a minor protocol deviation include:

- A rescheduled study visit or a study visit held out of window
- Failure to collect an ancillary self-report questionnaire

Major Protocol Deviations:

A major protocol deviation occurs when accidental or unintentional changes are made to (or non-compliance occurs with) the IRB approved protocol without prior sponsor and IRB approval that increases risk or decreases benefit to subjects, affects the subject's rights, eligibility, safety, or welfare, or the integrity of the primary outcome data. Examples of major protocol deviations include (but are not limited to):

- Failure to obtain valid informed consent
- Loss of laptop computer that contained identifiable, private information about subjects
- Accidental distribution of incorrect study medication or dose

Randomizing a subject who does not meet inclusion/exclusion criteria

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a major protocol deviation. The PI will determine if a major protocol deviation will result in withdrawal of a subject.

When a protocol deviation occurs, it will be discussed with the Site PI and a Protocol Deviation Form detailing the deviation will be generated. This form will be signed by a the PI or delegated co-investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files. A major protocol deviation that has the potential to impact participant safety will be reported to the DSMB as an immediately reportable event, within 3 business days of site awareness. Please refer to section 11.2.1 "AE/SAE Reporting Procedures" for more information.

14 DATA SAFETY MONITORING PLAN

14.1 Role of PIs in Safety Monitoring

The PIs will regularly monitor potential risks and procedures for protecting risk at each site. The PIs will have monthly teleconference meetings during which any serious adverse events (SAEs) or unexpected problems (UPs) will be discussed. The PIs will also meet in-person at a minimum of twice yearly to review all aspects of study progress and safety. Each PI is ultimately responsible for monitoring the data and safety at that site and will provide continuous, close monitoring of adverse events. The investigators and study coordinators will evaluate the progress of the study, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the various sites, and other factors that can affect study outcome. They will also consider factors external to the study, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study.

The overall study PI/Sponsor is responsible for AE reporting to the IRB, Data and Safety Monitoring Board (DSMB) (see below), and any other pertinent entities.

14.2 Data and Safety Monitoring Board (DSMB)

A Data and Safety Monitoring Board (DSMB) will be created by the NIMH as an independent body charged with ensuring that the safety of study subjects is protected and that the scientific goals of the study are being met. The DSMB conducts a review of all adverse events at least three times yearly, and reports their findings to the study PIs, who will inform their respective IRBs regarding the findings. The DSMB will be organized in compliance with the NIMH Charter, to ensure the safety of study participants and the integrity of the data collected in the projects. To achieve this goal, the DSMB will perform an ongoing evaluation of the overall clinical outcomes and the safety of patients participating in the clinical trial. The DSMB will also review any proposed amendments to the study protocol, perform expedited monitoring of immediately reportable serious adverse events, perform ongoing monitoring of all other AEs and SAEs, as well as drop-outs, determine whether study procedures should be changed or the study should be halted for reasons related to the safety of study subjects, and perform periodic review of the completeness and validity of data to be used for analysis of safety and efficacy. The DSMB will also ensure subject privacy and research data confidentiality. The DSMB will remain extrinsic to the study's infrastructure.

Please see Protocol Appendix for NIMH **DSMB Charter** for additional details.

15 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s). See separate **clinical monitoring plan** (CMP) in **APPENDIX** for full details. The main features are below.

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

16 STATISTICAL METHODS AND CONSIDERATIONS

The Statistical Analysis Plan (SAP) will contain any modification to the analysis plan described below, and in the case of a discrepancy between the protocol and the SAP, all analyses will be performed according to the SAP. Any modifications will be made prior to the unblinding of study data.

16.1 Data set analyzed

Data will be analyzed on an intent-to-treat basis, i.e., patients will be analyzed according to the treatment assigned by randomization regardless of the treatment actually received. Patients missing the follow up fMRI will be excluded from the primary outcome analysis. As it is impossible to test the missingness mechanism and data may not be missing completely at random, a secondary sensitivity analysis will be conducted using multiple imputation for the primary endpoint to investigate possible violations of the missing at random assumption. Extreme case imputation, whereby the lowest and highest overall observed values for the primary endpoint are imputed for any missing value, will also be used to assess the robustness of the primary endpoint analysis.

16.2 Demographic and Baseline Characteristics

Descriptive and exploratory analyses will be conducted for all measures to characterize the sample. While randomization should protect against baseline group differences, the treatment groups will be compared with respect to key baseline assessments and demographic variables including race, gender, age, and BMI. Additionally, the patients who complete the study will be compared with those who initiate but subsequently drop out of the study. All comparisons will be conducted using univariate tests as appropriate (i.e. Chi-squared test, t-test, or Wilcoxon test).

16.3 Analysis of Primary Endpoint

The primary endpoint is the change in activation of the bilateral VS, a region of the reward circuit in the brain, at 5 weeks from randomization. Activation will be measured by fMRI, and beta weights for each subject will be calculated to create a contrast between gain and neutral cues to model reward anticipation as described in the Neuroimaging Methods in Section 9. To calculate the beta weight for the bilateral VS, the beta weights for the right and left VS will be averaged.

A linear mixed effects model with a single random intercept term will be used to assess the change in activation of the bilateral VS by treatment group.

Although randomization is stratified by site, the primary endpoint analysis will be of the pooled dataset; secondary analyses will provide site-specific estimates.

16.4 Analysis of Secondary Endpoint

Change in activation of other regions of the cortico-striatal rewards circuit in the brain, namely the bilateral dorsal striatum (DS), mPFC, and ACC, will be analyzed in the same manner as the primary endpoint.

The performance of the PRT and the clinical and self-reports will be examined by using descriptive statistics such as the mean, standard deviation, median and interquartile range for continuous measures, and by frequency distributions for categorical variables.

Safety and tolerability data will be summarized by treatment group. Adverse event rates will be coded by body system and MedDra classification term. Adverse events will be tabulated by treatment group and will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study drug. Incidence of adverse events will be compared between treatment groups using Poisson regression.

16.5 Interim Analysis

None

16.6 Sample Size and Randomization

Based on our pilot data, the within-subject effect of time (corresponding to treatment with high-dose ezogabine 900 mg daily) was Cohen's d = 0.95. As noted above, we utilized mean values extracted from a bilateral anatomical mask in order to minimize effect size inflation. Assuming a placebo effect on the brain of Cohen's d = 0.15 (see Price et al., 2008; Fournier et al., 2010), we estimate a between-group effect of 0.8 (Morris, 2008). A sample size of 48 provides 85% power to detect a difference in means between groups of 0.8 standard deviations assuming a correlation of 0.6 between a pair of measurements made on the same subject using a two-sided 0.05 significance level test.

17 DATA COLLECTION, RETENTION AND MONITORING

17.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (CRF) when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by the site number, subject number and initials. If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

An Electronic Data Capture (EDC) system that includes several components, including a Screening Database and a Subject Tracking Database will be employed. Following completion of informed consent, a unique screening ID number will be assigned and basic identifying information will be collected on all individuals who undergo evaluation for study eligibility. This data, linked to the Screening ID, will be entered into the Screening Database and at a minimum will include subject name and date of birth. Once an individual meets all eligibility criteria and proceeds to the Randomization phase of the study, a unique Study ID number will be assigned and the subject will be entered into the Subject Tracking Database. A subject record in the Patient Tracking Database will be initiated with entries (variables) for each visit, and for each form included in the protocol for each visit. As patients complete visits the date of the visit will be entered into the database. Missed visits will be coded for reason (such as illness, scheduling problem, inability to contact, etc.). Within each visit, a field will be filled in for each form expected for that evaluation, containing either an indicator that the form was completed or a code indicating why it was not completed. If

patients terminate early, the date and reason will be coded in the Patient Tracking Database.

The PI is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator or his/her designee.

17.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed. All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

17.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. In EDC system queries are entered, tracked, and resolved through the EDC system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

17.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

17.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the medical monitor, other authorized representatives of the PI, IRB, NIMH, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing of the investigational product or for two years after study end.

17.6 Subject Confidentiality

In order to maintain subject confidentiality, only a site number, subject number and subject initials will identify all study subjects on CRFs and other documentation submitted to the Sponsor.

18 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number for the site and the subject number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the NIMH or FDA. The Investigator must also comply with all applicable privacy regulations (Health Insurance Portability and Accountability Act of 1996).

18.1 Protocol Amendments

Any amendment to the protocol will be written by the PI. Protocol amendments cannot be implemented without prior written IRB and DSMB approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs and the DSMB are notified within five working days.

18.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning

patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.
The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

18.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA), and local regulations.

The Investigator will prepare the informed consent form and HIPAA authorization and provide the documents to the IRB for approval. The study DSMB will also review the protocol and informed consent form (ICF) prior to study initiation. The ICF generated by the Investigator must be approved by the IRB and the DSMB. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects must be given ample opportunity to inquire about details of the study.. A copy of the signed ICF will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

18.4 Publications and Data Sharing

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined to be appropriate by the study PI and co-Investigators and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

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

The ICMJE policy, and the Section 801 of the Food and Drug Administration Amendments Act of 2007, requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine.

FDAAA mandates that a "responsible party" (i.e., the sponsor or designated principal investigator) register and report results of certain "applicable clinical trials"; for trials of drugs and biologics, this entails controlled, clinical investigations, other than Phase 1 studies, of a product subject to FDA regulation.

NIH grantees must take specific steps to ensure compliance with NIH implementation of FDAAA.

All the data from this trial will be recorded in the National Database for Clinical Trials Related to Mental Illness (NDCT), an informatics platform for the sharing of human subjects' data from all clinical trials funded by the National Institute of Mental Health (NIMH).

The data repository is accessible only to qualified investigators and all subject data will be de-identified and not linkable directly to the study participant. None of the personally identifiable information (PII) will be exposed and each subject will be identified with a Global Unique Identifier (GUID). All participants will be assigned a GUID by the Data Management Center (DMC) for this study. The following information will be collected and entered into the study database to generate a GUID: First name, Last name, Middle name (if applicable), Month of birth, Day of birth, Year of birth, Physical sex at birth, and Name of city/municipality of birth. Once the GUID is generated, all personal information will be deleted from the study database.

During and after study conclusion, qualified study team members will send de-identified information of study participants to the NDCT. The de-identified study data will be accessible for research purposes to other researchers nationwide under the supervision of the NIMH that will regulate and supervise the access to study data in order to minimize the risks to participants' privacy. NDCT data could also be used by NIMH to report to Congress and on its web site. For more information on NDCT, please refer to http://ndct.nimh.gov.

18.5 Conflict of Interest

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

18.6 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the PI/Sponsor (or designee), except when to protect the safety, rights or welfare of subjects
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the PI/Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the PI/Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the PI/Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others.
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

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20 APPENDIX

Table 1. List of Prohibited Medications*

Antidepressants					
Generic	**Trade name**				
Clomipramine	Anafranil (SR)	Placil			
Sertraline	Zoloft	Lustral			
Citalopram	Celexa				
Escitalopram	Lexapro	Cipralex			
Fluoxetine	Prozac				
Fluvoxamine	Luvox				
Duloxetine	Cymbalta				
Mirtazapine	Remeron	Remeron SolTab			
Paroxetine	Paxil	Seroxat			
Venlafaxine	Effexor				
Desvenlafaxine	Pristiq				
Levomilnacipran	Fetzima				
Tofenacin	Elamol	Tofacine			
Doxepine	Sinequan				
Desipramine	Norpramin	Pertofran			
Imipramine	Apo-Imipramine	Norfranil	Janimine	Novopramine	Impril
Mianserine	Tolvon				
Nefazodone	Dutonin	Serzone			
Protriptyline	Triptil	Vivactil	Concordin		
Reboxetine	Edronax				
Isocarboxazid	Marplan				
Phenelzine	Nardil				
Tranylcypromine	Parnate				
Selegiline	Deprenyl	Eldepryl	Emsam		
Centrally active sedating antihistamines					
Generic	**Trade name**				
Azelastine HCl	Astelin	Optivar			
Dimenhydrinate	Dramamine				
Hydroxyzine HCl	Atarax	Vistaril			
Levocabastine	Livostin				
Methdilazine					
Promethazine	Phenergan	Anergan	Pentazine	Phenazine	
	Phencen	Phenerzine	Phenoject	Pro-50	
	Promacot	Pro-Med 50	Promet	Prorex	
	Prothazine	Shogan	V-Gan-50		
Trimeprazine					
Antipsychotics					
Generic	**Trade name**				
Aripiprazole	Abilify				
Chlorpromazine	Chlorpromanyl	Largactil	Novo-Chlorpromazine	Thorazine	
Clozapine	Clozaril				
Fluphenazine decanoate	Modecate	Prolixin Decanoate			
Fluphenazine enanthate	Moditen Enanthate	Prolixin	Enanthate		
Fluphenazine HCl	Anatensol				
Haloperidol	Haldol	Dozic	Peridol	Serenace	
Lurasidone	Latuda				
Loxapine	Loxapac				
Mesoridazine Besylate	Serentil				
Molindone	Moban				
Olanzapine	Zyprexa				
Prochloperazine	Compazine				
Quetiapine Fumarate	Seroquel				
Risperidone	Risperdal				
Thioridazine HCl	Phenothiazines	Serentil	Prolixin	Thorazine	
	Mellaril	Trilaton	Vesprin		

Thiothixene	Navane			
Ziprasidone	Geodon			
Psychotropics miscellaneous				
Generic	**Trade name**			
Lithium	Camcolit	Carbolith	Duralith	Eskalith
	Lituane	Lithicarb	Lithizine	Lithobid
	Lithonate	Lithotabs	Priadel	Cibalith-S
Modafinil	Provigil			
R-modafinil	Nuvigil	Armodafinil		
Phenelzine sulfate	Nardil			
Sodium oxybate	Xyrem			
Stimulants				
Dextroamphetamine	Dextrostat	Ferndex		
Amphetamine/dextroamphetamine	Adderall	Benzedrine		
Methamphetamine	Methedrone	Desoxyn	Gradumet	
Methylphenidate	Ritalin	Metadate	Methylin	Concerta
Dopaminergic agents (e.g. levodopa, pramipexole,)				
Generic	Trade name			
Levodopa	Sinemet			
Pramipexole	Mirapex			
Ropinorole	Requip			
Rotigotine	Neupro			
Anti-Seizure Medications				
Generic	Trade name			
Gabapentin	Neurontin			
Valproic Acid	Depakote			
Carbemazepine	Tegretol			
Lamotrigine	Lamictal			
Pregabalin	Lyrica			
Opioids				
Generic	Trade name			
Buprenorphine	Suboxone			
Dihydrocodeinone	Vicodin			
Methadone	Methadone			
Morphine	Morphine			
Oxycodone	Oxycontin			
Tramadol	Ultram			
(Dextro)propoxyphene	Darvon			
Meperidine	Demerol			

* In addition to specific medications listed, the following classes of medication are prohibited: (1) antidepressant medication, (2) antipsychotic medication, (3) anticonvulsant/mood stabilizer medications, (4) lithium, (5) stimulant medication. hypnotic medications used PRN are allowed (including zolpidem, zaleplon, eszopliclone, diphenhydramine, suvorexant, and ramelteon) except within 24 hours of the baseline and primary outcome fMRI and behavioral assessments. Benzodiazepine medications used PRN (not to exceed 2 mg of lorazepam equivalents daily) except within 24 hours of the baseline and primary outcome fMRI and behavioral assessments.

Table 2: Schedule of Assessments, Outcome Measures, Labs, and Procedure

Study Phase	**Screening**	**Double-Blind Treatment Period (Weekly Study Visits)**					**Primary outcome**	**Taper Period (Weekly Phone Call)**		**Taper + Study exit**
Study Visit	V-1	V0 [e]	V1	V2	V3	V4	V5	PC1 [f]	PC2 [f]	V6
Screening										
Informed Consent	x									
Inclusion/Exclusion Criteria	x									
Demographics	x									
Medical History	x									
Family Psychiatric History	x									
Anti-Depressant Treatment History Form	x									
Edinburg Handedness Inventory	x									
SCID-5-RV	x									
Study drug/Placebo administration										
Randomization		x								
Ezogabine/Placebo Treatment			x	x	x	x	x			
Ezogabine/Placebo Taper								x	x	x
Safety assessments, concomitant medication and compliance monitoring										
Physical Exam	x									x
Vital Signs	x									x
Laboratory Tests [a]	x									x
ECG	x					x				x
U-Toxicology	x	x					x			x
Pregnancy Test	x	x					x			x
Ophthalmological Exam	x									x
Adverse Events	x	x	x	x	x	x	x	x	x	x
C-SSRS	x	x	x	x	x	x	x			x
Medication Compliance [b]		x	x	x	x	x	x	x	x	x
MMSE	x									
Clinical efficacy assessments										
SHAPS [c]	x	x	x	x	x	x	x			x
CGI-S [c]; CGI-I	x	x	x	x	x	x	x			x
MADRS		x	x	x	x	x	x			x
SLIPS		x	x	x	x	x	x			x
ACIPS		x	x	x	x	x	x			x
TEPS		x	x	x	x	x	x			x
QIDS-SR		x	x	x	x	x	x			x
MASQ		x					x			
WHODAS		x					x			
Behavioral efficacy assessments										
PRT		x					x			
Pharmacokinetics										
Ezogabine serum level							x			
Biomarkers										
Neuroimaging [d]		x					x			

* Study visits may occur 3 days before or after the scheduled visit. For V5 (Primary outcome), subjects will be on the highest tolerated treatment dose for at least one week and up to two weeks from V4 (during this time the subject will remain on the same dose of medication and taper will not be started until V5 is completed). Visits 6 and 7 will occur as dictated by Section 10.5 Taper Period (Phone calls* 1-2) of the protocol, in accordance with the corresponding taper schedule that is followed. Visit 8 will occur within 6 weeks of Visit 5 (Primary Outcome).[a] Clinical laboratory tests include chemistry, complete

blood count, liver function tests, serology (only at screening) and thyroid-stimulating hormone levels (see protocol for details); [b] Compliance will be assessed by subject report, pill count and serum drug levels; [c] SHAPS score ≥ 20 and CGI-S score ≥ 4 required for inclusion; [d] Neuroimaging acquisition includes a high resolution T1 anatomical scan and BOLD acquisitions for resting state functional connectivity (rs-fMRI) and task-activation fMRI during the incentive flanker task (IFT); [e] Baseline assessment; [f] Weekly phone call during taper period will be conducted by a study research coordinator and may occur within 3 days before or after the scheduled phone call. An explicit request by the study participants or any safety concern will trigger a follow up phone call assessment by a study physician. At physician's discretion, an eventual in person visit will be scheduled.

Abbreviations: ACIPS, Anticipatory and Consummatory Interpersonal Pleasure Scale; C-SSRS, Columbia-Suicide Severity Rating Scale; CGI-I, Clinical Global Impression-Improvement; CGI-S, Clinical Global Impression-Severity; ECG, Electrocardiogram; fMRI, functional Magnetic Resonance Imaging; MADRS, Montgomery-Asberg Depression Rating Scale; MASQ, Mood and Anxiety Symptom Questionnaire; MMSE, Mini Mental State Examination, PC, Phone Call; PRT, Probabilistic Reward Task; QIDS-SR, Quick Inventory of Depressive Symptomatology-Self-Report; SHAPS, Snaith-Hamilton Pleasure Scale; SLPIS, Specific Loss of Interest and Pleasure Scale; TEPS, Temporal Experience of Pleasure Scale; World Health Oraganization Disability Assessment Schedule 2.0 (WHODAS 2.0).

Magnetic Resonance Imaging (MRI) Procedures Appendix

MRI data will consist of the following for each subject, at each timepoint:

- MP2RAGE 0.1mm^3 Anatomical Image
- Multi-Echo (4) Multiband (5) MEMB 3.0mm^3 Field Maps
- 1 x MEMB 3.0mm^3 Resting State fMRI data (10 min)
- 4 x MEMB 3.0mm^3 Task fMRI data (5m50s each)
    - Accompanying EDat file containing behavioral response data

MRI data will be centrally stored and preprocessed at the MSSM site, using dedicated servers that will be accessible to all sites via secured on-site and off-site networking channels. Storage, management, and access of data will be managed using the software XNAT, a package developed by the Neuroinformatics Research Group at Washington University at St. Louis. The XNAT system is optimized for the aforementioned functions, using validated safeguards to safeguard Protected Health Information. Key features of the XNAT system include an easy-to-use web interface, a Representation State Transfer (REST) Application Program Interface (API) for programmer-level access to data, and easy connection to DICOM application entities. The XNAT database is organized by subjects, scan sessions, and individual MRI runs. Our XNAT system will also be configured to support other subject and scan-specific resources, such as clinical data at the subject level, and at the experiment-level, as well as timing files (i.e., EDat files) from fMRI tasks performed by subjects, to be used later at analysis stages. The MSSM site has three years of hands-on experience in installing and administering XNAT and keeping installations current with security and performance updates. The XNAT system will be managed by one or more postdoctoral fellows with expertise in neuroinformatics, supervised by Dr. Prantik Kundu, with additional support provided by existing technical personnel at the Translational and Molecular Imaging Institute (TMII) at MSSM.

Prior to the first scan of the study, the MRI scanners at both sites will be configured to directly access the XNAT system, via a built-in DICOM catcher. This setup will enable seamless uploads of DICOM files during the study directly from site scanners. A Standard Operating Protocol (SOP) will be distributed to scan sites that will describe appropriate local and global system configuration. In the case of issues during initial configuration or ongoing use, the team at MSSM will provide technical support via phone or video-conference. If severe or time-sensitive issues arise, a member from the MSSM team will travel to the Baylor College of Medicine (BCM) site to correct problems. Each imaging session will be labeled according to the anonymized subject code, then input to scanners by MRI technicians, and transferred to XNAT as part of the session label. Subject names will not be stored on the XNAT system, and de-anonymization of data for analysis will be done at analysis sites, not in the framework of the XNAT system.

The XNAT system will be installed on a large-capacity storage and analysis server at MSSM. The system will be physically racked at the university's datacenter, which has a high-capacity AC system and an uninterruptable power supply (UPS) with generator back-up. The server will be hosted on the university network, with security by a firewall configured by MSSM system administrators to allow secure incoming connections from remote sites. The server will be configured with a long-term-support Linux operating system (OS) and the ZFS file system. The ZFS will be configured to provide both disk and file-level redundancies so that even multiple disk-related corruptions or total disk failures will be transparently recovered from. In any such case, which as a matter of record and by design is rare, the MSSM team will be informed to allow necessary remediation. The ZFS will also be configured with disk-level encryption to enable further security. The ability of ZFS to make fast snapshots will be used for protecting system and data files regularly and especially for software or database updates, so errors can be recovered with minimal effort and downtime. For backup at BCM, the system files, database, and MRI data will be sent to cloud storage via snapshots created with encryption to protect PHI. This encrypted backup data will only be accessed in the case of catastrophic failure as by an "act of God."

For each upload of MRI session data from a site scanner to the XNAT system (e.g. DICOM files), pipelines will be automatically launched to do quality assurance (QA) and preprocessing. Recognizing that MB-fMRI is among the most taxing sequences for MRI, the QA metrics for resting state data will be tracked to detect major changes in scanner performance. Since head motion in rs-fMRI data tends to indicate the overall motion seen over an MRI session, rs-fMRI motion diagnostics will be tracked for trends in subject behavior across sites. Feedback on these trends will be regularly sent to sites and be posted as interactive report pages in XNAT under the listing for each MRI session. The QA metrics planned are: absolute and relative root-mean-square (RMS) head motion, in millimeter and percent, full-width-half-max image smoothness, signal time series standard deviation, temporal signal-to-noise ration, and DVARS 1st and second moments. Moreover, montages of the acquired images will be available directly from the XNAT web interface to make detection of major issues easy for users across levels of expertise. QA metrics will also be available for later analysis as covariates to account for otherwise unmitigated variance in data quality.

QA values will be checked by algorithm to flag if a scan is usable or requires further attention. Accepted data will then be preprocessed automatically, using the XNAT pipeline engine. First, DICOM files will converted to NIFTI files, which removes PHI, and NIFTI files for the different scans will be saved as session-specific resources in XNAT. In essence, this makes the raw data available to users via the PHI-stripped NIFTI format. Then, NIFTI files will be routed to the appropriate HCP-based preprocessing scripts, using MRI parameters from DICOM headers to assure proper matching. The XNAT server will be able to fully preprocess around all data at a rate comparable to the scanning schedule. Final output files from HCP-style preprocessing will then be added to the XNAT session as NIFTI resources. These NIFTI files would also later be migrated to data repositories for open data sharing, in accordance with NIH mandates.