**Study Title:**

A Phase 2a Study to Evaluate the Kappa Opioid Receptor As a Target for the Treatment of Mood and Anxiety Spectrum Disorders by Evaluation of Whether CERC-501 Engages Key Neural Circuitry Related to the Hedonic Response

**Version Date:**

October 06, 2016

NCT02218736

# CLINICAL STUDY PROTOCOL

**Study Title:** **A Phase 2a Study to Evaluate the Kappa Opioid Receptor As a Target for the Treatment of Mood and Anxiety Spectrum Disorders by Evaluation of Whether CERC-501 Engages Key Neural Circuitry Related to the Hedonic Response**

**Abbreviated Title:** FAST-MAS KOR Phase 2a

**Protocol Version:** 6.0
**Version Date:** October 06, 2016

**Contract Title:** New Experimental Medicine Studies: Fast-Fail Trials in Mood and Anxiety Spectrum Disorders (FAST-MAS)
**Contract #:** HHSN271201200006I
**Contractor:** Duke University

**Contract PI:** Andrew D. Krystal, M.D., M.S.
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**Task Order #:** HHSN27100004
**Overall Study PI:** Andrew D. Krystal, M.D., M.S.

**Supported by:** National Institute of Mental Health (NIMH)

Abbreviated Study Title: FAST-MAS KOR Phase 2a

Contract #: HHSN2712012000061
Task Order #: HHSN27100004

Coordinating Center [CC]: Duke Clinical Research Institute (DCRI)
Data center [DCC]: Duke Clinical Research Institute (DCRI)

Phase of Study: Phase 2a
Target enrollment: 90 Patients

FDA Approval(s)
IND ☐ No ☑ Yes

Drug: CERC-501
IND #: 121225 held by Dr. Andrew Krystal
Drug supplied by: Cerecor

IRB Approvals:
Multi-Institutional Project ☐ No ☑Yes
NIMH Data and Safety Monitoring Board ☐ No ☑ Yes

Third Party Agreement(s):
Technology Transfer Agreement ☐ No ☑ Yes
Agreement type and number: Clinical Trial Research Support Agreement
Effective Date: 10/1/13
Expiration Date: End of study

Samples are being stored ☐ No ☑ Yes

## STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, International Conference on Harmonisation (ICH) guideline E6: Good Clinical Practice: Consolidated Guideline and the applicable regulatory requirements from Title 45 of the Code of Federal Regulations (CFR) Part 46 and 21 CFR parts 50 (informed consent) and 56 (institutional review board (IRB) and 312 (Investigational New Drug Application IND).

All individuals responsible for the design and conduct of this study have completed Human Participants Protection Training and are qualified to be conducting this research prior to the enrollment of any participants.

## INVESTIGATOR STATEMENT

I have read the protocol and agree to conduct the study as outlined and in accordance with all applicable local, state, and federal regulation.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

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Signature of Principal Investigator Date

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Name of Principal Investigator (printed or typed)

# PRÉCIS (Protocol Synopsis)

| | |
|---|---|
| **Protocol Title** | "A Phase 2a Study to Evaluate the Kappa Opioid Receptor as a Target for the Treatment of Mood and Anxiety Spectrum Disorders by Evaluation of Whether CERC-501 Engages Key Neural Circuitry Related to the Hedonic Response" Trial |
| **Product** | CERC-501, previous research name LY2456302 |
| **Objectives** | Primary<br>• Evaluate the impact of CERC-501 10 mg relative to PBO on reward-related neural circuitry in terms of ventral striatal fMRI activation during anticipation of reward during the Monetary Incentive Delay (MID) Task.<br>Secondary<br>• Clinical Anhedonia Measure: To determine if 10 mg of CERC-501 is superior to PBO in improving a clinical self-report measure of anhedonia the Snaith Hamilton Pleasure Scale (SHAPS)<br>• Behavioral Anhedonia Measure: To evaluate the impact of CERC-501 relative to PBO on a behavioral measure of anhedonia the Probabilistic Reward Task (PRT) |
| **Exploratory Objectives** | To assess the effects of CERC-501 relative to placebo on:<br>• Ventral striatal fMRI activation during anticipation of loss during the MID Task<br>• Resting state delta EEG current density in the rostral anterior cingulate.<br>• Resting state fMRI connectivity<br>• Self-rated affective responses to cues and feedback during the MID Task<br>• The Effort-Expenditure for Rewards Task (EEfRT)<br>• The Visual Analogue Scale for Anhedonia (VAS)<br>• The Temporal Experience of Pleasure Scale (TEPS)<br>• The Hamilton Depression Rating Scale (HAM-D)<br>• The Hamilton Anxiety Scale (HAM-A)<br>• The Cognitive and Physical Functioning Questionnaire (CPFQ)<br>To assess the safety and tolerability of CERC-501 on systematically collected and spontaneously reported adverse events. |
| **Study Design** | Double-Blind, Placebo-Controlled Multi-Site Trial |
| **Study Population** | Patients with mood and anxiety disorders and anhedonia; age 21 – 65 years inclusive |
| **Number of Participants** | 90 |
| **Duration of Participant Participation** | A total of up to 16 weeks<br>Up to 30 days for screening, 8 weeks of treatment and 4 weeks for a follow-up visit |
| **Dose Schedule** | 10 mg CERC-501 daily for 8 weeks |
| **Estimated Start** | May, 2015 |
| **Estimated End** | April 2017 |

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| AE | Adverse Event |
| CGI-I | Clinical Global Impression-Improvement |
| CGI-S | Clinical Global Impression-Severity |
| CPFQ | The Cognitive and Physical Functioning Questionnaire |
| CSSRS | Columbia Suicide Severity Rating Scale |
| DCRI | Duke Clinical Research Institute |
| DSMB | Data Safety Monitoring Board |
| EDC | Electronic Data Capture |
| EEfRT | Effort Expenditure for Rewards Task |
| EEG | Electroencephalogram |
| FDA | United States Food and Drug Administration |
| fMRI | Functional Magnetic Resonance Imaging |
| HAM-A | Hamilton Rating Scale for Anxiety |
| HAM-D | Hamilton Rating Scale for Depression |
| HIPPA | Health Insurance Portability and Accountability Act |
| HSP | Human Subjects Protection |
| KOR | Kappa Opioid Receptor |
| CERC-501 | Investigational Kappa Opioid Receptor Antagonist |
| MID | Monetary Incentive Delay Task |
| MINI | Mini International Neuropsychiatric Interview (Mental State Examination) |
| MRI | Magnetic Resonance Imaging |
| NIMH | National Institute of Mental Health |
| PBO | Placebo |
| PHI | Protected Health Information |
| PK | Pharmacokinetic |
| POC | Proof of Concept |
| PRISE | Patient Reported Inventory of Side-Effects |
| PRT | Probabilistic Reward Task |
| RDoC | Research Domain Criteria Project |
| SAE | Serious Adverse Event |
| SHAPS | Snaith-Hamilton Pleasure Scale |
| TEPS | Temporal Experience of Pleasure Scale |
| VAS | Visual Analogue Scale |

# 1. BACKGROUND AND SIGNIFICANCE

## 1.1. Overview

This Phase 2A study is a double-blind, placebo-controlled, randomized, parallel-group, 6-site, trial of 8 weeks of daily dosing with 10 mg of CERC-501 (LY2456302) that is intended to test whether we can establish Proof of Concept (POC) that kappa opioid receptor (KOR) antagonists engage neural circuits involved in mediating the reward response. This will be specifically determined by testing whether CERC-501, a relatively selective KOR antagonist, increases ventral striatal activation in response to reward-predicting cues. We are attempting to establish POC in this study in order to determine whether there is a sufficient basis for pursuing future work evaluating whether KOR antagonism has therapeutic effects on clinical and behavioral measures of reward-related functioning, a domain of function that is impaired in many patients with Mood and Anxiety Spectrum Disorders.

The primary outcome measure which is being used to establish POC is neural activation within the ventral striatum during the Monetary Incentive Delay (MID) Task, as assessed with fMRI (Wacker et al., 2009; Pizzagalli et al., 2004, 2008a, 2009; Treadway et al., 2012; Stoy et al., 2012; Ossewaarde et al., 2011; Knutson et al., 2008). This study also includes a number of secondary and exploratory measures to assess various domains of reward-related function including: the Snaith-Hamilton Pleasure Scale (SHAPS), a self-report assessment of global reward function (Gilbert et al., 2002; Snaith et al., 1995); the Probabilistic Reward Task (PRT), an objective behavioral assessment of the capacity to learn from rewards (Barr et al., 2008; Bogdan and Pizzagalli, 2006, 2009; Pizzagalli et al., 2005, 2007, 2008a; Vrieze et al., 2013); the Effort-Expenditure for Rewards Task (EERT), a behavioral measure of the extent to which an individual is motivated by reward (Treadway et al., 2009, 2012a,b); the Temporal Experience of Pleasure Scale (TEPS), a self-report measure that assesses anticipatory pleasure (‘wanting’) and consummatory pleasure (‘liking’) (Gard et al., 2006; Stanford et al., 2011); a visual analogue scale (VAS) rating of hedonic respone; an additional measure of the engagement of neural circuits mediating the hedonic response, resting qEEG current density measures of delta power (Wacker et al., 2009; Pizzagalli et al., 2004); and self-ratings of the reward response to stimuli in terms of ratings of affective responses to cues and feedback during the Monetary Incentive Delay Task (Pizzagalli et al., 2009). All of these are exploratory measures except the SHAPS and the PRT, which have previously been shown to be modulated by dopamine, affected by stress, associated with striatal function, predictive of clinical outcome and anhedonic symptoms, and improved with antidepressant therapy (Bogdan et al., 2011; Pizzagalli et al., 2005, 2008a,b, 2009; Santesso et al., 2008; Vrieze et al., 2013a,b; Di Giannantonio and Martinotti, 2012; Martinotti et al., 2011; Wacker et al., 2009). Other measures included in this study are: assessment for adverse effects, the Cognitive and Physical Functioning Questionnaire (CPFQ), and traditional anxiety (Hamilton Rating Scale for Anxiety [HAM-A]) and depression (Hamilton Rating Scale for Depression [HAM-D] measures, which are being evaluated in exploratory analyses to provide information regarding changes in depression and anxiety severity with treatment (Hamilton 1960, 1967; Fava et al., 2009).

## 1.2. Background

The available treatments for patients with mood and anxiety disorders have significant limitations (Rush, 2007; Denys and de Geus, 2005). There is a need to develop new treatments for people with these disorders. Many research studies carried out in animals and a few preliminary studies carried out in humans suggest that medications which block kappa opioid receptors (KOR) have potential for being effective new treatments for patients with mood and anxiety spectrum disorders (see below). These medications have shown particular promise for improving one important type of difficulty experienced by many patients who suffer from mood and anxiety spectrum disorders referred to as anhedonia, which is an impairment in reward-related function. In this study we will test the hypothesis that KOR antagonism is a promising means of improving anhedonia in patients with mood and anxiety spectrum disorders. We will do so by evaluating whether we can establish Proof of Concept (POC) that a relatively selective KOR antagonist, CERC-501 (LY2456302; see Investigator Brochure), engages neural circuits involved in mediating reward-related function in patients with mood and anxiety spectrum disorders with anhedonia. We are attempting to establish POC in this study in order to determine whether there is a sufficient basis for pursuing future work evaluating whether KOR antagonism has therapeutic effects on clinical and behavioral measures of reward-related functioning.

In addition to being a relatively selective KOR antagonist, CERC-501 is also well-suited for this study based on its pharmacologic and safety profiles (see Investigator Brochure). The 10 mg dosage of CERC-501 was chosen for evaluation because of preclinical studies, single and multiple ascending dose studies in humans, a single-dose PET study of KOR occupancy (Zheng et al., 2013), and pupillometry data obtained following administration of the mu agonist fentanyl (see Investigator Brochure). The following sections include details of the rationale for this study.

### 1.2.1. KOR Antagonism Promising Target for Treating Mood and Anxiety Disorders

There is an extensive set of pre-clinical studies suggesting that KOR antagonists are likely to have therapeutic effects in those with mood and anxiety spectrum disorders. This includes studies indicating a role of the kappa opiate system in mediating both anxiety and depression symptoms and studies suggesting that KOR antagonists have effects on animal models of both major depression and anxiety.

A number of studies indicate that the kappa opiate system is critical for mediating the adverse effects of stress. An important aspect of stress-related pharmacology is the dynorphins, a group of opioid peptides that exert their effects primarily through binding to KOR (Bruchas et al., 2009). Evidence suggests that stress leads to anxiety by CRF1 receptor activation of dynorphins in the basolateral amygdala which then bind to KOR (Bruchas et al., 2009). Place aversion and social avoidance occurring with repeated stress is mediated via dynorphin activation in ventral striatum, an effect which can be mimicked by KOR agonists and blocked by KOR antagonists (Land et al., 2009; Schindler et al., 2012). KOR antagonists also block stress-related impairment in elevated plus maze spontaneous exploration (Peters et al., 2011).

Non-stress anxiety paradigms also suggest that KOR antagonists are likely to have anxiolytic effects. Prodynorphin knockouts (prodynorphin is the precursor protein for dynorphins) and KOR antagonists have been found to have anxiolytic-like effects in the novelty-induced hypophagia test, the defensive burying tests, the elevated plus maze test, fear-potentiated startle test, open-field test, and light-dark test (Carr and Lucki, 2010; Knoll et al., 2011; Wittmann et al., 2009).

A larger literature suggests that KOR antagonists are likely to have anti-depressant effects and may prevent the depression-like consequences of stress. Dynorphin mediates the dysphoric aspects of stress via binding to KOR and this effect is prevented by knocking out dynorphin or administering a KOR antagonist (Land et al., 2008). The depression-like behaviors caused by chronic stress, uncontrollable stress, and social-defeat stress are mediated by kappa opiate receptors and can be mimicked by KOR agonists (McLaughlin et al., 2006; Knoll and Carlezon, 2010). KOR antagonist treated mice and KOR knockout mice show a reduction of stress-induced depression-like behavior (McLaughlin et al., 2006; Knoll and Carlezon, 2010). Stress has also been found to trigger KOR activation of dorsal raphe neurons which project to nucleus accumbens and decrease dopamine release thereby diminishing reward and increasing drug seeking (Lemos et al., 2012).

More generally, rodent studies show that administering KOR antagonists or knocking out the prodynorphin gene leads to antidepressant-like effects as assessed by reduced immobility in the forced swim test and reduced learned helplessness (via nucleus accumbens and hippocampal mediated mechanisms), whereas KOR agonists have depressogenic effects in conjunction with decreasing nucleus accumbens dopamine release (Reindl et al., 2008; Shirayama et al., 2004; Carlezon et al., 2006; McLaughlin et al., 2003; Mague et al., 2003; Todtenkopf et al., 2004; Chartoff et al., 2012; Chartoff et al., 2009).

Perhaps the best recognized effect of KOR antagonists in animal models is to prevent the development of anhedonic-like states. The literature suggesting that KOR antagonists have such effects speaks to the potential of these agents to have therapeutic effects on anhedonia in humans, which is a core symptom of mood and anxiety disorders that cuts across diagnostic boundaries. In this regard, there is evidence that KOR stimulation inhibits dopamine release in the striatum (nucleus accumbens and caudate) and induces a negative mood state (Bruijnzeel, 2009). Consistent with this model, a series of studies indicate that KOR agonists decrease phasic dopamine release in the nucleus accumbens and increase intracranial self-stimulation (a model of anhedonia), whereas KOR antagonists have the opposite effect, increasing nucleus accumbens dopamine release and decreasing self-stimulation (Ebner et al., 2010; Muschamp et al., 2011; Carlezon et al., 2006; Maisonneuve et al., 1994). Further, KOR agonists block cocaine's anti-anhedonic effect on intracranial self-stimulation (Tomasiewicz et al., 2008) and block the reinforcing/rewarding effects on drugs of abuse (Wee and Koob, 2010), whereas, giving a KOR antagonist prior to cocaine withdrawal prevented anhedonic-like intracranial self-stimulation responses (Chartoff et al., 2012).

Although data in humans on the effects of selective KOR antagonists are lacking, preliminary data from humans are consistent with the animal data in suggesting that this

target is likely to have therapeutic effects in mood and anxiety spectrum disorders. In an open-label study, 6 patients who had failed antidepressant medications and ECT were found to improve with buprenorphine (a KOR antagonist and partial mu agonist) treatment (Nyhuis et al., 2008). Findings of a double-blind, placebo-controlled, pilot study in 32 patients with treatment-resistant depression treated with the combination of buprenorphine and a mu receptor antagonist (simulated kappa opiate receptor antagonist) indicate that this combination had a significant antidepressant effect (Ehrich, 2012).

Together, the available data provide a compelling indication that KOR antagonists are likely to have therapeutic effects in patients with mood and anxiety spectrum disorders. These data point to the adverse effects of stress and particularly anhedonia as therapeutic targets of interest with these agents.

### 1.2.2. Rationale for Studying Effects of KOR Antagonist on Anhedonia

We chose to focus on anhedonia as an endpoint for this study because: 1) the available data suggest that anhedonia is the dimension of mood and anxiety spectrum disorders that is most likely to be improved by KOR antagonists (see above); 2) anhedonia is associated with measurable neurobiological mechanisms which can be studied with available methodologies that could be used to establish POC in terms of engagement of relevant neural circuitry (Wacker et al., 2009; Pizzagalli et al., 2004, 2009; Treadway et al., 2012; Stoy et al., 2012; Ossewaarde et al., 2011); and 3) anhedonia allows us to accomplish our goal of studying an important aspect of dysfunction that cuts across mood and anxiety spectrum disorders, consistent with the NIMH's RDoC framework.

The data indicating that KOR antagonists are likely to improve anhedonia are strong relative to the data indicating that there will be other therapeutic effects of KOR antagonists. As a result, it seems likely that, if there are any therapeutic effects of these agents in those with mood and anxiety spectrum disorders, a therapeutic effect on anhedonia would be evident. As a result, anhedonia is an appropriate primary endpoint for a POC study with a KOR antagonist the treatment of patients with mood and anxiety spectrum disorders. Failure to demonstrate a therapeutic effect of a KOR antagonist on the neural circuitry related to anhedonia using a dosage that had been demonstrated to have acceptable kappa opiate receptor occupancy would be a reasonable indication to fail KOR antagonism as a treatment for mood and anxiety disorders.

### 1.2.3. Properties of CERC-501

This study will evaluate CERC-501 (LY2456302), a high-affinity, selective kappa opioid receptor antagonist. Its favorable pharmacologic and adverse effects profiles support its use in this study.

#### 1.2.3.1. Clinical Pharmacology

CERC-501 (LY2456302) is a structurally unique kappa-selective opioid receptor antagonist, as highlighted in Tables 1 and 2. CERC-501 selectively occupies kappa opioid receptors in vivo, as shown in Figure 1.

**Table 1 – Receptor Binding Affinity, hKi (nM)**

| Compound | Mu | Kappa | Delta |
|---|---|---|---|
| FP3FBZ | 35.8 | 0.57 | 211 |
| **CERC-501** | **24.0** | **0.81** | **155** |
| norBNI | 34.7 | 0.13 | 9.49 |
| JDTic | 11.5 | 0.06 | 188 |
| Naltrexone | 0.58 | 1.81 | 12.4 |

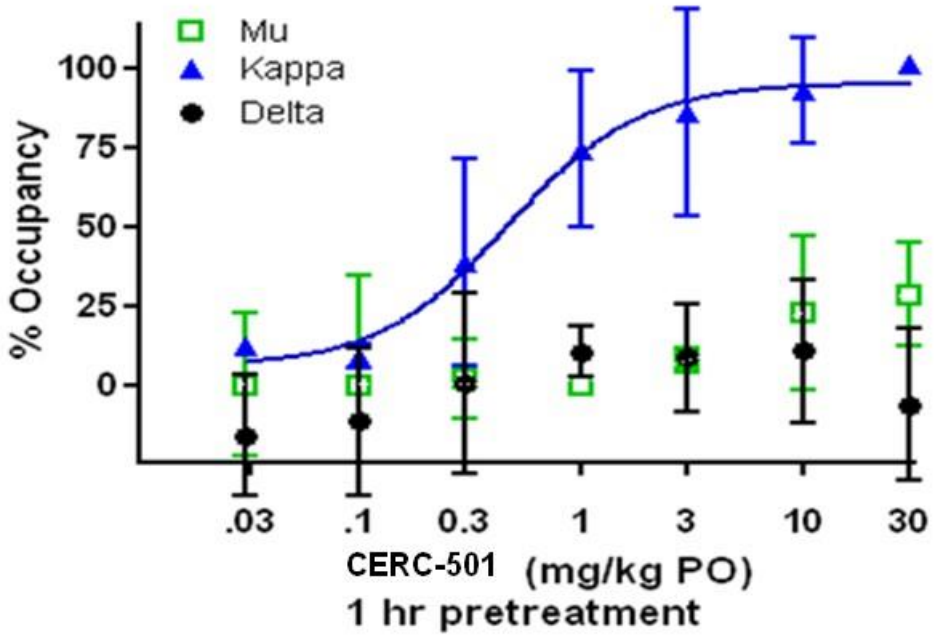
All data represent the mean of n=3 exp on separate occasions.

**Table 2 – Antagonist Potency, hKb (nM)**

| Compound | Mu | Kappa | Delta |
|---|---|---|---|
| FP3FBZ | 21.3 | 1.57 | 293 |
| **CERC-501** | **17.4** | **0.81** | **110** |
| norBNI | 32.9 | 0.80 | 14.1 |
| JDTic | 6.58 | 0.10 | 168 |
| Naltrexone | 0.47 | 2.26 | 11.3 |

All data represent the mean of n=3 exp on separate occasions.

**Figure 1 – Occupancy of kappa opioid receptors in rats in vivo**



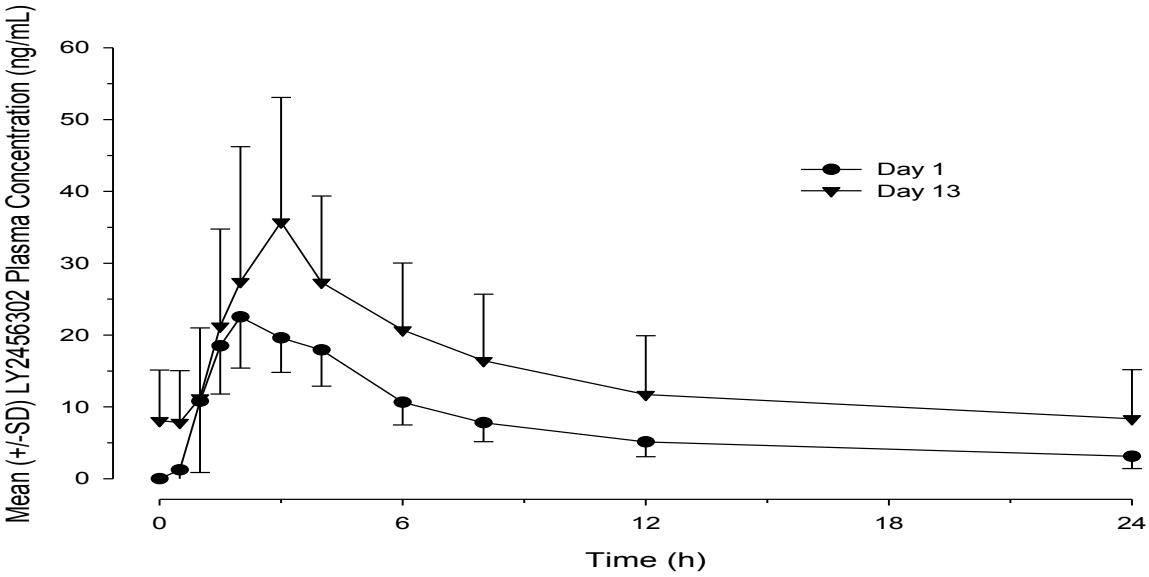

N = 3 male SD rats per dose group; Mu: naltrexone administered at 10 mg/kg IV; Kappa: GR103545 administered at 1.5 mg/kg IV; Delta: naltriben administered at 10 mg/kg IV.

### 1.2.3.2. Pharmacokinetic Studies and Drug-Drug Interactions

Exposures increased approximately proportionally with dose and steady state was reached within 7 to 8 days of once per day (QD) dosing. Moderate accumulation of CERC-501 (LY2456302) - average accumulation ratio (AUC) across dose groups was calculated to be 1.8 (range: 1.8 to 1.9). Degree of accumulation after multiple dosing was consistent with the half-life observed after single dose (see Figure 2). Excretion is mostly through the urine.

There is no definitive drug-drug interaction study yet. However, the risk of drug-drug interactions is currently perceived as relatively low. There was no irreversible inhibition of CYP2C8; however, competitive reversible inhibition was observed with inhibitory constant (Ki) = 3.7 uM. Given a projected maximum drug concentration at steady state (Cmax,ss) = 143 ng/mL (0.34 uM) following multiple dosing at 35 mg, the highest dose to be tested in Study LAFB, a value of Cmax,ss/Ki = 0.1 indicates the possibility of an interaction with CYP2C8 substrates. For this reason, medications that are primarily metabolized by cytochrome P450 2C8 (CYP2C8) (cerivastatin, paclitaxel, repaglinide, sorafenib, and torsemide) will be exclusionary for all studies with CERC-501 until definitive drug-drug interaction studies are carried out.

**Figure 2 – Mean (+/- SD) of CERC-501 Plasma Concentration**



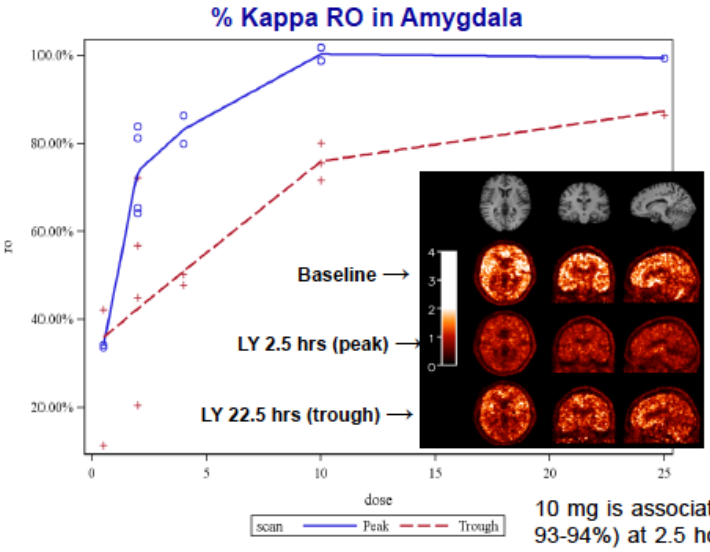

### 1.2.3.3. Selectivity for the Kappa-Opioid Receptor

While CERC-501 (LY2456302) is a selective kappa opioid receptor antagonist in the efficacy dose range, in vivo rat receptor occupancy studies have shown that CERC-501 exhibits mu opioid receptor antagonist activity at higher doses. Therefore, the potential mu antagonist activity of CERC-501 was assessed using an intravenous (IV) agonist challenge with the mu agonist fentanyl, which is known to induce miosis. These agonist-induced effects can then be blocked by mu opioid antagonists. CERC-501 reversal of fentanyl-induced miosis was assessed over a dose range determined from the safety and PK data of CERC-501 in Part 1 of the study. CERC-501 was tested up to 60 mg PO and the effect on fentanyl-induced miosis was linear. CERC-501 blocked fentanyl-induced miosis at 60 mg to modest degree, indicating kappa selectivity at < 10 mg. The dose that produces 50% of Emax (ED50) estimate was 21.9 mg, suggesting the doses of 25 mg and 60 mg have some moderate effect. Naltrexone at the 50 mg dose completely reversed fentanyl-induced miosis (Lilly Study GCAQ; steady state).

### 1.2.3.4. Kappa-Opioid Receptor Occupancy in the Human Brain

KOR Occupancy in the human brain is based on a Positron Emission Tomography (PET) Study (See Figure 3). The novel PET tracer [$^{11}$C]PKAB (LY2879788) was validated as a specific kappa antagonist PET tracer in non-human primate studies and human clinical trials in collaboration with the Yale PET Center. The clinical trial I2Z-MC-LAFC assessed brain kappa OR occupancy after single oral doses of 0.5-25 mg CERC-501, measured by PET with the radioligand LY2879788 after single oral doses of CERC-501 of 0.5, 2, 4,10, 25 mg in 13 healthy subjects. Kappa-opioid receptor occupancy (RO) was measured in high uptake regions (amygdala, anterior cingulate, frontal cortex, and insula) with the specific kappa-opioid antagonist PET tracer LY2829788 at two time-points post-dose, at around Tmax (approximately 2.5 hours post-dose; “peak scan”) and on day 2 at around 22.5 hours post-dose (“trough scan”). As shown in Figure 3, 10 mg is associated with mean peak RO of 94% (range: 93-94%) at 2 hours post-dose, and a mean trough RO of 72% (range 68-75%) at 24 hours post-dose.

**Figure 3 – Kappa receptor occupancy in humans after a singe oral dose of CERC-501**




| | | | scan | |
|---|---|---|---|---|
| | | | Peak | Trough |
| | | | ro | ro |
| ROI | dose | | | |
| amygdala | 0.5 | N | 2 | 2 |
| | | Mean | 0.34 | 0.27 |
| | | Std | 0.00 | 0.22 |
| | 2 | N | 4 | 4 |
| | | Mean | 0.74 | 0.49 |
| | | Std | 0.10 | 0.22 |
| | 4 | N | 2 | 2 |
| | | Mean | 0.83 | 0.49 |
| | | Std | 0.05 | 0.02 |
| | 10 | N | 2 | 3 |
| | | Mean | 1.00 | 0.76 |
| | | Std | 0.02 | 0.04 |
| | 25 | N | 1 | 1 |
| | | Mean | 0.99 | 0.86 |
| | | Std | . | . |

10 mg is associated with mean peak RO of 94% (range: 93-94%) at 2.5 hours post-dose, and a mean trough RO of 72% (range 68-75%) at 22.5 hrs post-dose

***Data obtained from study I2Z-MC-LAFC, a cooperation of Lilly with the Yale PET Center***

Brain kappa opioid RO after a single oral dose of CERC-501 (LY2456302) as measured by PET and [$^{11}$]C-PKAB in healthy subjects.

### 1.2.3.5. Nonclinical Safety Pharmacology

The toxicity profile of CERC-501 (LY2456302) has been characterized in rat, mouse, dog, and rabbit through a package of in vitro, repeat-dose (up to 6 months in dogs and rats and up to 2 weeks in mice), reproductive and genotoxicity studies and through safety pharmacology studies. No adverse effects were observed in dogs at a high dose of 2000 mg/kg/day. In the 6-month rat study, the only adverse findings were dose-related histopathologic changes in the stomach, primarily decreased parietal cells and inflammation at all dose levels. Although a NOAEL was not observed, the gastric findings were minimal to moderate in severity, not life threatening, and partially reversible over a 1-month recovery period. Dose levels of 2000 mg/kg/day in mice, ≥800 mg/kg/day in rats, or ≥200 mg/kg/day in rabbits exceeded the maximum tolerated dose (MTD) resulting in adverse clinical signs, decreased food consumption and body weight, and mortality/euthanasia. Convulsions were also noted after 1 or 2 doses of CERC-501 at 2000 mg/kg (mice), 1500 mg/kg (rats), or 1000 mg/kg (rabbits). CERC-501 was not genotoxic in a standard battery of genotoxicity tests. These nonclinical toxicology results demonstrate an acceptable safety profile for the proposed clinical study of CERC-501. Following 6 months of treatment, the margin of safety (MOS) based on the area under curve (AUC) is 196-fold for the dog and 59-fold for the rat, for all effects, excluding the stomach findings in rats (Table 5.3 of the Investigator's Brochure). A NOAEL for the stomach changes in the rat was not established. The MOS for convulsions is 38-fold based on Cmax, in the most sensitive species (rabbit), and is ≥38-fold based on both Cmax and AUC for convulsions in a mouse ethanol withdrawal model. In nonclinical safety pharmacology studies with CERC-501, there were no effects on the central or peripheral nervous systems or respiratory function in rats given doses up to 800 mg/kg. In dogs, there was an apparent trend for increased QT and QTc intervals following a single

dose of 1000 mg/kg compared to vehicle, although the increases were not statistically significant.

### 1.2.3.6. Clinical Safety Data

In total, 73 healthy subjects have been exposed to single or multiple doses of CERC-501 (LY2456302) in 3 completed clinical pharmacological studies (Studies LAFA, LAFB, and LAFC). No significant safety concerns were encountered in Study LAFA after single-dose administration of CERC-501 up to 60 mg, in Study LAFB after multiple-dose administration of CERC-501 up to 35 mg, and in Study LAFC after single-dose administration of CERC-501 up to 25 mg. In these studies, there were no serious adverse events (SAEs).

In Study LAFA, 1 subject, a 47-year-old white male participating in Part 1, was discontinued from the study due to a treatment-related adverse event (AE) of mild ventricular tachycardia. This subject experienced a mild 5-beat ventricular tachycardia approximately 8 hours after receiving a single 25-mg dose of CERC-501 in Part 2. The subject was asymptomatic during the AE. Follow-up telemetry monitoring for 24 hours did not reveal any clinically significant findings; overall, there was no clear dose dependency observed, as no ventricular tachycardia was observed after administration of a higher (60 mg) dose of CERC-501.

No subjects were discontinued from Study LAFB or Study LAFC. In Study LAFA, 126 AEs were reported by 20 of 32 subjects. Of these 126 AEs, 23 were considered by the investigator to be related to CERC-501 with the most common (occurring >1 incidence) in CERC-501-treated subjects being: headache (6 events), diarrhea (4 events), nausea (3 events), and anxiety (2 events). Most of the 126 AEs were of mild-to-moderate severity; only 2 events (vomiting and flushing), not related to CERC-501 administration, as determined by the investigator, were severe.

In Study LAFB, 52 AEs were reported by 19 of 37 subjects. Of these 52 AEs, 22 were considered by the investigator to be related to CERC-501, with the most common (occurring >1 incidence) in CERC-501-treated subjects being dyspepsia (5 events), pruritus generalized (3 events), headache (2 events), diarrhea (2 events), and abdominal pain (2). Of note, 2 subjects receiving 10 mg CERC-501 and 1 subject receiving 35 mg CERC-501 experienced a total of 24 AEs or more than half of all reported. All AEs were of mild severity, with the exception of 1 moderate AE of hemorrhoids, not related to CERC-501, as determined by the investigator, which occurred after administration of 10 mg CERC-501.

In Study LAFC, 21 AEs of mild-to-moderate severity were reported by 11 of 13 subjects; none of the AEs were considered by the investigator to be related to CERC-501. Most events were related to the study procedure.

There have been no clinically significant values, changes, or trends in clinical laboratory data, with the following exception: 1 subject in Study LAFA experienced mildly elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels (>2 times the upper limit of normal), which were not considered by the investigator to be related to study drug, after receiving a single dose of 4 mg CERC-501 in Part 2 of Study LAFA.

Given the results of the 6-month rat study, where there were adverse dose-responsive findings in the glandular stomach included parietal cell loss, inflammation and mucus cell hyperplasia, FDA expressed safety concerns for the stomach lesions. Minimal to slight effects were observed at the low dose. There was no NOAEL. No other adverse effects in the rat and no adverse effects in dogs at a dose of 2000 mg/kg. These findings are of low risk (nonclinical) and may be species-specific (rat only). Both the FDA Division of Psychiatry Products (DPP) and Division of Anesthesia, Analgesia, and Addiction Products (DAAAP) agreed that studies in a third species would provide considerable weight-of evidence to support the conclusion that the effect was species specific; however, there was still concern that CERC-501 may exacerbate alcohol-induced gastrointestinal pathology. The FDA agreed that a negative second non-rodent species chronic toxicology study would provide a weight-of-evidence argument that GI findings in 6-month rat study are species-specific. This study may obviate the need to conduct routine endoscopies with biopsy in future clinical studies. The FDA concluded that since GI findings were only seen in rats (but not dogs), after 6 months of dosing (but not after 1 month of dosing) and were essentially reversible, the NOAEL/LOAEL provided an adequate safety margin for the proposed dose of 10 mg and duration of an 8-week study with CERC-501. The FDA has agreed that an 8-wk POC study in MDD patients can be conducted without routine endoscopies and biopsies.

### 1.2.4. Rationale for Studying 10 mg Dosage of CERC-501

In the single-ascending dose study, dose escalation was halted at 60 mg based on PK analysis of this dose level. This analysis indicated that the Cmax for CERC-501 (LY2456302) was close to the 180-ng/mL limit required to maintain a 30-fold MOS to the NOAEL for convulsions observed in rabbits. Data from the completed multiple-dose study showed that the mean C (CV%) after multiple (14) QD doses of 35 mg was 81 ng/mL (23%). This is well below the maximum desired concentration of 180 ng/mL. The PET study carried out with the Kappa Opiate Receptor tracer LY2879788 demonstrated that single, oral doses of 0.5 mg up to 25 mg CERC-501 penetrated the blood-brain barrier, led to specific target engagement, and blocked significantly and in a dose-related manner, kappa opioid receptors in the brain (Zheng et al., 2013) (See Figure 3). Approximately 2.5 hours postdose (“peak scan”), brain kappa opioid receptors were almost saturated at doses of 10 mg or more (96.7% receptor occupancy), whereas at trough the receptor occupancy was >60%. Sustained and substantial target engagement was observed for at least 22.5 hours.

An additional consideration motivating the choice of 10 mg of CERC-501 are potential effects on mu opioid receptors (MOR) which may be depressogenic. CERC-501 is a potent, high-affinity KOR antagonist with no appreciable affinity at several non-opioid cell surface receptor targets, including monoaminergic, muscarinic, cholinergic, and adrenergic receptors, as well as the central benzodiazepine binding site, ion channels, or transporters. However, in vitro binding and functional assays suggest that this agent does have some MOR antagonist effects, though the affinity for KOR is over 6 times greater. In vivo receptor occupancy and pharmacology assays indicate that oral administration of CERC-501 exhibits at least a 30-fold functional kappa selectivity over mu and delta opioid receptors (See Figure 1). The potential that CERC-501 might have some mu antagonist effects at the dosage chosen is of concern for studies of the use of this agent in

patients with depression because of several lines of evidence suggesting that mu antagonists might be depressogenic (Berrocoso et al., 2009; Escriba et al., 2004; Kennedy et al., 2006). As a result, pupillometry was carried out following administration of the mu agonist fentanyl to determine the degree to which various dosages of CERC-501 would block the mu agonist effects of fentanyl as indicated by pupil diameter (See 1.2.3.3 above). This test was carried out in a human multiple-ascending dose study. Moderate mu antagonist effects of CERC-501 were seen with the 60 mg dosage but were felt to be "minimal" at dosages below 60 mg (See Investigator Brochure). Based on these data, the 10 mg dose was felt to represent the best tradeoff between kappa and mu effects. It provides sufficient KOR antagonism while at the same time having relatively minimal MOR blockade based on pupillometry.

A further consideration supporting the choice of the 10 mg dosage of CERC-501 is that this is also the highest dosage where completed toxicity studies support carrying out a trial as long as 8 weeks of daily dosing in humans. On these bases, the 10 mg dose was chosen for evaluation in this Phase 2a study.

### 1.3. Public Health Significance

The available treatments for patients with mood and anxiety spectrum disorders have significant limitations (Rush, 2007; Denys and de Geus, 2005). This study will contribute significantly to public health by taking steps to address these limitations by: 1) expeditiously testing a promising new treatment for mood and anxiety spectrum disorders; 2) evaluating a potential target in the brain which could serve as the basis for development of additional new candidate compounds for the treatment of patients with mood and anxiety spectrum disorders; 3) establishing more expeditious methods for evaluating potential new therapies for patients with mood and anxiety spectrum disorders; and 4) specifically establishing methods for the development of new therapies targeting anhedonia, an important RDoC endpoint.

## 2. STUDY OBJECTIVES

The **Primary Objectives** of this study are:

- **Specific Aim 1:**
  Establish POC for KOR antagonism by evaluating the impact of CERC-501 10 mg relative to Placebo (PBO) on reward-related neural circuitry in terms of ventral striatal fMRI activation during anticipation of reward during the Monetary Incentive Delay (MID) Task.

The **Secondary Objectives** of this study are:

- **Secondary Aim 1:**
  Clinical Anhedonia Measure: To determine if 10 mg of CERC-501 is superior to PBO in improving a clinical self-report measure of anhedonia, the Snaith Hamilton Pleasure Scale (SHAPS).
- **Secondary Aim 2:**
  Behavioral Anhedonia Measure: To evaluate the impact of CERC-501 relative to PBO on a behavioral measure of anhedonia, the Probabilistic Reward Task (PRT).

The **Exploratory Objectives** of this study are;

- To assess the effects of CERC-501 relative to placebo on:
  - Ventral striatal fMRI activation during anticipation of loss during the MID Task
  - Resting state delta EEG current density in the rostral anterior cingulate
  - Resting state fMRI connectivity
  - Self-rated affective responses to cues and feedback during the MID Task
  - The Effort-Expenditure for Rewards Task (EEfRT)
  - The Visual Analogue Scale for Anhedonia (VAS)
  - The Temporal Experience of Pleasure Scale (TEPS)
  - The Hamilton Depression Rating Scale (HAM-D)
  - The Hamilton Anxiety Scale (HAM-A)
  - The Cognitive and Physical Functioning Questionnaire (CPFQ)
  - Clinical Global Impression Severity and Improvement (CGI-S and CGI-I) Ratings
- To assess the safety and tolerability of CERC-501 on systematically collected and spontaneously reported adverse events

# 3. SUBJECTS

## 3.1. Description of Study Populations

We will enroll 90 subjects in this trial. The inclusion and exclusion criteria are listed below. We will stratify enrollment to ensure that there is ample cross-diagnostic representation so that the therapeutic effects of CERC-501 on anhedonia may be assessed independently of its effect on any particular mood and anxiety spectrum disorder. This will be accomplished by attempting to randomize subjects who meet the anhedonia entry criteria and have an anxiety disorder but do not currently meet major depressive disorder (MDD) criteria based on the MINI (subjects with a past history of MDD but who do not currently meet diagnostic criteria could be included). Anhedonia is a core symptom of MDD, and one of its diagnostic criteria, but this is not the case for anxiety disorders. As a result, we are including a requirement for inclusion of patients with anxiety disorders because without such a criterion, we run the risk of only including subjects with anhedonia occurring in the setting of MDD, which would result in our being unable to distinguish an antidepressant effect which included a beneficial effect on anhedonia from an anhedonia-specific effect. However, because there has been no prior work establishing the prevalence of the target subgroup (those who meet our anhedonia entry criterion and have an anxiety disorder but do not currently meet MDD criteria) it is unclear how difficult it will be to randomize these individuals. As a result, we will not stratify enrollment to ensure a fixed number of subjects of this type as this could jeopardize the ability of the study to randomize the total target number of subjects (90) and, thereby, impede accomplishing the primary aims. Instead, we will seek to include approximately 33% of the total number randomized (30 subjects) of this type but will prioritize meeting the primary aims of the study (randomizing 90 total subjects) over this goal.
See Appendix 1 for an Eligibility Checklist.

## 3.2. Inclusion Criteria

1. Age between 21 and 65 years of age (inclusive)
2. Must meet DSM-IV TR diagnostic criteria for: Major Depressive Disorder, Bipolar I or II Depressed, Generalized Anxiety Disorder, Social Phobia, Panic Disorder, or Post Traumatic Stress Disorder
3. Snaith-Hamilton Pleasure Scale (SHAPS) score $\geq 20$
4. Reliable and willing to be available for the duration of the study
5. Willing and able to give written informed consent to participate
6. Able to understand and comply with instructions
7. If female of childbearing potential, must agree to use dual methods of contraception and be willing and able to continue contraception for 6 weeks after the last dose of study drug. Females using oral contraception must have started using it at least 2 months prior to the Baseline Visit
8. If male of childbearing potential, must have undergone surgical sterilization (such as a vasectomy) or agree to use a condom used with a spermicide during participation in the study and for 1 month after the last dose of study drug

## 3.3. Exclusion Criteria

1. Expected to require any hospitalization during the course of the study
2. Current/history of a psychotic disorder, current manic or mixed episode, autism spectrum disorders, mental retardation
3. Met DSMIV-TR criteria for substance abuse within the last 3 months or substance dependence within the last 6 months, excluding caffeine and/or nicotine
4. History of unstable or untreated serious medical condition based on physician evaluation, medical history, and screening laboratory testing
5. Active suicidal intent or plan, or history of attempt within the past 3 months based on physician evaluation and Columbia Suicide Severity Rating Scale (C-SSRS)
6. Use of any antidepressant, antipsychotic, anxiolytic, anticonvulsant, mood stabilizing, muscle relaxant, centrally acting antihistaminergic, stimulant or insomnia medications (See Appendix 2) within 5 half-lives of baseline or at any time during after baseline
7. Use of any medication that is primarily metabolized by Cytochrome P450 2C8 within 14 days of baseline or at any time during the study. This includes: Cerivastatin, Paclitaxel, Repaglinide, Sorafenib, Rosiglitazone, Trimethoprim, Amodiaquine, Morphine, Amiodarone, Cabazitaxel, Carbemazepine, Chloroquine, Ibuprofen, Teprostinil, Torsemide.
8. Any contraindications to the magnetic resonance imaging procedures
9. Positive urine drug screen at Screening Visit
10. Use of any investigational medication within 3 months prior to the start of this study or scheduled to receive an investigational drug other than the study drug during the course of this study
11. Known hypersensitivity to CERC-501
12. History of severe allergies or multiple adverse drug reactions
13. History of gastric disease (including peptic ulcer disease, gastritis, upper GI bleeding, or any GI precancerous condition), current clinically evident gastrointestinal complaints.
14. Current use of a proton pump inhibitor or histamine 2 blocker, or a history of

chronic NSAID use
15. History of use of Salvia divinorum or use of Salvia divinorum at any time during the study
16. Any other condition that in the opinion of the investigator would preclude participation in the study
17. Any smoking of cigarettes or use of other nicotine containing products within the last month or at any time during the study
18. Pregnant or lactating

# 4. STUDY DESIGN AND METHODS

## 4.1. Study Overview

This study will be a six-site randomized, double-blind, PBO-controlled, parallel-group mono-therapy study to assess the effects of CERC-501 compared to PBO in adults age 21-65 years with mood and anxiety spectrum disorders. We will recruit a total of 90 subjects, of which 45 will be randomized to CERC-501 and 45 to placebo for 8 weeks of treatment.

Following phone screening subjects will come to the research unit/clinic for a face-to-face screening visit. Subjects who sign informed consent will undergo rigorous screening including: a medical, psychiatric, medication, and treatment history, vital signs, physical examination, urine drug screen, MINI, clinical diagnostic interview for psychiatric disorders, beta-HCG serum pregnancy test (women), ECG, Snaith-Hamilton Pleasure Scale (SHAPS), Visual Analog Scale for Anhedonia, Temporal Experience of Pleasure Scale (TEPS), CSSRS, CGI-S, CGI-I and a battery of clinical laboratory tests (See Table 3 for Schedule of Assessments). All study events, their corresponding dates, and results of these examinations will be reported on the corresponding case report forms (CRFs). All subjects will be off of all antidepressant, antipsychotic, anxiolytic, anticonvulsant, mood stabilizing, muscle relaxant, centrally acting antihistaminergic, stimulant or insomnia medications (See Appendix 2) within 5 half-lives of baseline or at any time during after baseline and will have to remain off of all such medications other than study medication during the trial.

Subjects who require tapering off of medications will discuss a medication tapering plan with a study physician. The study physician will develop a plan for safely decreasing and stopping medications that is tailored to each subject to minimize risks and discomfort and will be conducted in consultation with the subject's treating physician when applicable. This plan will be communicated to the subject including a detailed indication of the times at which medications are to be decreased, the possible side-effects that they may experience with decreases in medication dosage, the possible worsening of symptoms they may experience as medication dosage is lowered, and a plan for how to proceed if they develop side-effects or symptoms worsen. A study physician will be responsible for monitoring subjects during the washout including scheduling assessment visits if needed and addressing any side effects or worsening of symptoms that occur. Subjects who develop clinically significant adverse effects or worsening will receive appropriate assessment and care by a study physician which may include referral for

evaluation/treatment. This plan will be designed so that subjects are free of all excluded medications at least 5 half-lives prior to the baseline visit (see Exclusion Criteria above). Subjects will also undergo a mock MRI scanning session at this visit.

Subjects will return to the research unit/clinic for a baseline assessment visit up to 30 days after the screening visit. At this visit subjects will undergo interval history, physical examination, ECG, vital signs, battery of laboratory tests, MRI, EEG, SHAPS, TEPS, VAS, CSSRS, PRT, EEfRT, HAM-D, HAM-A, CPFQ, CGI-I and CGI-S and will have the option of having blood collected to be sent to Rutgers for potential genetic analysis or other future analysis. Blood will also be collected for tests for assessment for gastric adverse events including gastrin and pepsinogen I/II levelsAt the end of this visit, subjects who continue to qualify will be randomized to CERC-501 10 mg or placebo in a 1:1 ratio using IVRS randomization within the EDC.

IVRS Randomization will be carried out using the Simple Internal Randomization Engine (SIRE), which is a tool developed by the DCRI to randomize study patients. SIRE is fully integrated with the EDC database. A randomization list in hardcopy will also be kept under lock and key by the statistician as a back-up should the computer system be down. In case of system failure, the statistician will provide the individual dispensing the medication at a site with the medication allocation over the phone. It will also allow study physicians to obtain the randomization information from the statistician and procedures to break the blinding in cases of emergencies. All individuals having interactions with the subjects will remain blind to treatment group assignment other than in cases of emergencies.

Subjects will then return for treatment visits at weeks 2, 4, 6 and 8 following randomization during which the following study assessments will take place: Interval history, battery of lab tests, Vital signs, physical examination, ECG, SHAPS, TEPS, VAS, CCRS, CGI-I, and CGI-S, Adverse Effects Assessment with Patient Reported Inventory of Side-Effects (PRISE), and blood will be collected for determination of CERC-501 levels. At the week 4 and week 8 visits blood will also be collected for tests for assessment for gastric adverse events including gastrin and pepsinogen I/II levels.

In addition at the 8 week post-randomization visit subjects will also undergo MRI, EEG, PRT, EEfRT, HAM-D, HAM-A, and CPFQ and will have the option of having blood collected to be sent to Rutgers for potential genetic analysis or other future analysis. Following this visit study drug will be discontinued and subjects will be instructed to return in 4 weeks for a final visit.

At the final visit, subjects will undergo: vital signs, interval history, SHAPS, TEPS, VAS, CSSRS, CGI-I, CGI-S and a clinical interview for adverse effects. At the conclusion of this visit subject participation in the study will end.

**Table 3. Schedule of Assessments, Outcome Measures, Labs, and Procedures**

| | Screen | Baseline | Follow Up | Double-Blind Treatment Period | | | | Follow Up |
|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screen** | **A0** | **Phone** | **A1 †** | **A2 †** | **A3†** | **A4†** | **1 Month†** |
| **Weeks from Baseline** | **Up to -4** | **0** | **1** | **2** | **4** | **6** | **8** | **12** |
| Informed Consent | X | | | | | | | |
| Physical Exam / Medical History / Demographics | X | X | | X | X | X | X | |
| Treatment History | X | X | | X | X | X | X | X |
| Mini-International Neuropsychiatric Interview (MINI) | X | | | | | | | |
| Clinical Interview/Interval History | X | X | | X | X | X | X | |
| Electrocardiogram (ECG) | X | X | | X | X | X | X | |
| Urine drug screen | X | | | | | | | |
| HCG (serum) | X | | | | | | | |
| Labs* | X | X | | X | X | X | X | |
| | | | | | | | | |
| Medication Adherence | | | | X | X | X | X | |
| fMRI with Monetary Incentive Delay Task, Resting State Connectivity | | X | | | | | X** | |
| Snaith-Hamilton Pleasure Scale (SHAPS) | X | X | | X | X | X | X | X |
| Probabilistic Reward Task (PRT) | | X | | | | | X | |
| Effort Expenditure for Rewards Task (EEfRT) | | X | | | | | X | |
| Quantitative EEG (QEEG) | | X | | | | | X | |
| Temporal Experience of Pleasure Scale | X | X | | X | X | X | X | X |
| Visual Analogue Scale for Anhedonia | X | X | | X | X | X | X | X |
| HAM-D | | X | | | | | X | |
| HAM-A | | X | | | | | X | |
| Cognitive and Physical Functioning Questionnaire (CPFQ) | | X | | | | | X | |
| Vital signs | X | X | | X | X | X | X | X |
| Columbia Suicide Severity Rating Scale (CSSRS) | X | X | X | X | X | X | X | X |
| Blood Collection for Rutgers | | X | | | | | X | |
| Blood Collection for Gastric Adverse Events** | | X | | | X | | X | |
| Blood Sample for Drug Blood Level Testing | | | | X | X | X | X | |
| Adverse Effects Assessment with Patient Reported Inventory of Side-Effects (PRISE) | | X | X | X | X | X | X | X |
| Clinical Global Impression–Severity (CGI-S) | X | X | | X | X | X | X | X |
| Clinical Global Impression – Improvement (CGI-I) | | X | | X | X | X | X | X |
| Randomization | | X | | | | | | |
| Mock MRI Scanning Session | X | | | | | | | |

*Complete blood count with differential, Electrolytes, Metabolic Panel, Thyroid Function Tests, Urinalysis. **Tests for assessment of gastric adverse events include: gastrin, and pepsinogen I/II levels.

** Not performed if subject terminates from drug prior to week 8.

† ±4 days

## 4.2. Recruitment

Subjects will be recruited at each study site. The information collected at that time is used simply to rule out any obvious exclusion criteria, such as age and substance abuse/medical history, as well as to collect some simple demographic information. If a participant is deemed ineligible or decides not to participate in the study, their information is destroyed, i.e., shredded. If an individual appears to meet enrollment criteria and is interested in participating, a face-to-face interview is conducted by one of the project investigators. A release of information is obtained for review of any available historical and clinical data. The nature of the project, procedures, relative risks and benefits, and alternatives to participation in the project are discussed with the individual. Following this discussion, the individual is given a copy of the consent form to review, and any questions are answered. The process of informed consent will be obtained in accordance with 21CFR50 and local IRB standards by study personnel who have participated in institutionally approved training in human subject protection. Upon obtaining voluntary, written, informed consent, medical and psychiatric screening procedures will be used to confirm study eligibility. Documentation regarding the informed consent process and the subject's study participation will be made in the subject's medical record and/or study source documents.

In order to ensure a high rate of enrollment in the proposed study a number of procedures will be implemented under the direction of the Contract PI Dr. Krystal and implemented via the weekly Coordinating Committee (CC) meetings that include all of the Site PIs, the DCRI Project Leader, and the Leader of the Assessment/Signal Detection Core for the study, Richard Keefe, Ph.D. As part of this effort, each site will submit a site recruitment plan which will be reviewed by the CC prior to the commencement of the trial. The Committee will discuss each plan and make suggestions for ways to facilitate recruitment at each site. Throughout the trial it will be the responsibility of Dr. Krystal or his designee, to track recruitment closely at each site and to present these data at the regular CC meetings for review. This review will include discussing the recruitment at each site in the context of the recruitment strategies they are employing in order to make ongoing modifications of the recruitment strategies of slower recruiting sites. This will also provide a forum for PIs of sites that are more successful in recruiting to share the strategies that have been most effective with the PIs of slower recruiting sites. In general, we will attempt to optimize recruitment and retention by promoting practices that are associated with effective recruitment across the sites and minimize practices which are unsuccessful through careful planning, establishing input and PI and site staff buy-in, adaptive recruitment strategies to address site idiosyncrasies, and adopting comprehensive and active community outreach. Assessment of recruitment will be based on the rate of enrollment needed to meet our enrollment target. This target requires us to enroll 90 subjects in 9 months with 6 sites. As a result, each site will have to enroll 1.7 subjects per month for us to successfully meet our recruitment goal. Because it is understood that sites will vary in startup speed it is assumed that some sites will have to enroll more rapidly than 1.7 subjects per month for us to meet our recruitment target. As a result, we will assess sites against a benchmark of enrolling 2 subjects per month. All sites who do not meet this benchmark for more than 1 month and all sites that fall below 1.0 subject per month in any month will be the focus of a recruitment evaluation which

will result in the formation of a recruitment plan for that site which will be followed up with ongoing close monitoring. In the case where a site falls below a recruitment rate of one subject per month for 3 months a thorough evaluation of the site and the factors that might be responsible for the under-recruiting of subjects will be carried out. Such sites will be discussed with the NIMH Contracting Officer's Representative (COR) and considered for being dropped from the study. In this case the DCRI, in coordination with the NIMH COR, will need to determine what steps should be taken including assessing the wisdom of closing a low enrolling site and initiate a new one. Likewise, we will discuss with NIMH COR the need to add additional sites, if that proves necessary.

It is anticipated that we will enroll twice as many women as men mirroring the gender distribution of mood and anxiety spectrum disorders. Because we have geographically diverse sites, we anticipate that the study population will have the ethnic distribution of the general U.S. population. A strong effort will be made to achieve these racial/ethnicity distribution enrollment targets. Each site has experience recruiting minorities through a concentrated effort in communities that have high representation of ethnic and racial minorities and through advertisements in newspapers and radio stations whose readers/listeners are predominantly of specific racial or ethnic minorities. The CC will monitor the gender and racial/ethnic composition of the subjects enrolled throughout the study to assess whether the planned targets are being met. If not, the CC will work together to formulate a plan to address identified problems.

## 4.3. Screening

**Screening Visit**: **Day -30 to Day -1 (4 - 6 hours)**
Once a subject has signed an inform consent for this study subjects will undergo a set of screening tests to determine if they meet the inclusion/exclusion criteria for the study. These screening tests will include:

- Medical, psychiatric, and medication history
- Vital signs (temperature, blood pressure, and pulse)
- Physical examination
- Pregnancy Test (beta-HCG serum pregnancy test – females only)
- Electrocardiogram (ECG)
- Urine drug screen
- Mini-International Neuropsychiatric Interview (MINI)
- Snaith-Hamilton Pleasure Scale (SHAPS)
- Temporal Experience of Pleasure Scale (TEPS)
- Visual Analogue Scale for Anhedonia (VAS)
- Columbia Suicide Severity Rating Scale (CSSRS)
- Clinical Global Impression – Severity (CGI-S)
- Battery of clinical laboratory tests including: Complete blood count with differential, Electrolytes, Metabolic Panel, Thyroid Function Tests, Urinalysis
- Mock MRI scanning session

Those who qualify to continue with the study based on the screening assessment will be asked to return 1-30 days later for additional evaluations. Subjects who require medication tapering will discuss a medication tapering plan with a study physician during

this visit (for details see Section 4.1. Study Overview). This plan will be designed so that subjects are free of all excluded medications at least 5 half-lives prior to the baseline visit (see Section 3.3. Exclusion Criteria). Qualifying subjects will be instructed about not using any prohibited medications during the study including substances of abuse. They will be specifically instructed about not using Salvia divinorum, which has kappa opioid agonist effects and is not an illegal substance.

A potential subject who previously screen failed due to a positive breath test ONLY may be brought back for re-screening. Subject should sign a new informed consent and all screening procedures must be repeated.

## 4.4. Study Procedures

**Baseline: Day 0 Visit (6 – 8 hours):**
Following rigorous screening, subjects will return to the clinic/research unit up to 30 days later and undergo the following assessments and procedures:

- Structural MRI, fMRI during Monetary Incentive Delay Task, Resting State Connectivity fMRI
- Interval history
- Battery of clinical laboratory tests including: Complete blood count with differential, Electrolytes, Metabolic Panel, Thyroid Function Tests, Urinalysis.
- EEG
- Vital signs (temperature, blood pressure, and pulse)
- Physical examination
- Electrocardiogram (ECG)
- Snaith-Hamilton Pleasure Scale (SHAPS)
- Temporal Experience of Pleasure Scale (TEPS)
- Visual Analogue Scale for Anhedonia (VAS)
- Columbia Suicide Severity Rating Scale (CSSRS)
- Probabilistic Rewards Task (PRT)
- Effort Expenditure for Rewards Task (EEfRT)
- Hamilton Depression Rating Scale (HAM-D)
- Hamilton Anxiety Rating Scale (HAM-A)
- The Cognitive and Physical Functioning Questionnaire (CPFQ)
- Clinical Global Impression – Severity (CGI-S)
- Clinical Global Impression – Improvement (CGI-I)
- Adverse events assessment with the Patient Reported Inventory of Side-Effects (PRISE)
- Blood sample collection for tests for assessment for gastric adverse events including gastrin, and pepsinogen I/II levels.
- Blood sample collection to be sent to Rutgers for potential genetic analysis or other future analysis (optional)

At the end of this visit subjects will be randomized to CERC-501 10 mg or placebo, will be provided with enough study medication for a 2-week period and will be instructed to take the medication every morning. Subjects will also be instructed to bring all of their study drug packaging and unused study drug with them to each visit.

**Week 1 – Telephone Follow Up:**
Subjects will be called after one week to assess for any safety concerns. Any safety issues identified will be addressed as appropriate.

**Week 2 Visits (4 – 6 hours per visit): +/- 4 days**
At this visit subjects will undergo the following assessments and procedures:

- Interval history
- Battery of clinical laboratory tests including: Complete blood count with differential, Electrolytes, Metabolic Panel, Thyroid Function Tests, Urinalysis
- Vital signs (temperature, blood pressure, and pulse)
- Physical examination
- Electrocardiogram (ECG)
- Snaith-Hamilton Pleasure Scale (SHAPS)
- Temporal Experience of Pleasure Scale (TEPS)
- Visual Analogue Scale for Anhedonia (VAS)
- Columbia Suicide Severity Rating Scale (CSSRS)
- Clinical Global Impression – Severity (CGI-S)
- Clinical Global Impression – Improvement (CGI-I)
- Adverse events assessment with the Patient Reported Inventory of Side-Effects (PRISE)
- Inspection of medication blister packs to assess medication adherence
- Blood sample for drug blood level testing

At the end of this visit subjects will receive enough study medication for another 2 week period, and will be instructed to take the medication each morning, to bring all study drug packing and unused study drug to the next visit, and to return to the clinic/research unit in 2 weeks.

**Week 4 Visit (4 – 6 Hours): +/- 4 days**
At this visit subjects will undergo the following assessments and procedures:

- Interval history
- Battery of clinical laboratory tests including: Complete blood count with differential, Electrolytes, Metabolic Panel, Thyroid Function Tests, Urinalysis.
- Vital signs (temperature, blood pressure, and pulse)
- Physical examination
- Electrocardiogram (ECG)
- Snaith-Hamilton Pleasure Scale (SHAPS)
- Temporal Experience of Pleasure Scale (TEPS)
- Visual Analogue Scale for Anhedonia (VAS)
- Columbia Suicide Severity Rating Scale (CSSRS)
- Inspection of medication blister packs to assess medication adherence
- Blood sample for drug blood level testing
- Clinical Global Impression – Severity (CGI-S)
- Clinical Global Impression – Improvement (CGI-I)

- Adverse events assessment with the Patient Reported Inventory of Side-Effects (PRISE)
- Blood sample for tests for assessment for gastric adverse events including gastrin, and pepsinogen I/II levels.

At the end of this visit subjects will receive enough study medication for another 2 week period, and will be instructed to take the medication each morning, to bring all study drug packing and unused study drug to the next visit, and to return to the clinic/research unit in 2 weeks.

**Week 6 Visit (4 – 6 Hours): +/- 4 days**
At this visit subjects will undergo the following assessments and procedures:

- Interval history
- Battery of clinical laboratory tests including: Complete blood count with differential, Electrolytes, Metabolic Panel, Thyroid Function Tests, Urinalysis.
- Vital signs (temperature, blood pressure, and pulse)
- Physical examination
- Electrocardiogram (ECG)
- Snaith-Hamilton Pleasure Scale (SHAPS)
- Temporal Experience of Pleasure Scale (TEPS)
- Visual Analogue Scale for Anhedonia (VAS)
- Columbia Suicide Severity Rating Scale (CSSRS)
- Inspection of medication blister packs to assess medication adherence
- Blood sample for drug blood level testing
- Clinical Global Impression – Severity (CGI-S)
- Clinical Global Impression – Improvement (CGI-I)
- Adverse events assessment with the Patient Reported Inventory of Side-Effects (PRISE)

At the end of this visit subjects will receive enough study medication for another 2 week period, and will be instructed to take the medication each morning, to bring all study drug packing and unused study drug to the next visit, and to return to the clinic/research unit in 2 weeks.

**Week 8 Visit (6 – 8 Hours): +/-4 days**
At this visit subjects will undergo the following assessments and procedures:

- Structural MRI, fMRI during Monetary Incentive Delay Task, Resting State Connectivity fMRI
- Interval history
- Battery of clinical laboratory tests including: Complete blood count with differential, Electrolytes, Metabolic Panel, Thyroid Function Tests, Urinalysis
- Electroencephalogram (EEG)
- Vital signs (temperature, blood pressure, and pulse)
- Physical examination
- Electrocardiogram (ECG)
- Snaith-Hamilton Pleasure Scale (SHAPS)

- Temporal Experience of Pleasure Scale (TEPS)
- Visual Analogue Scale for Anhedonia (VAS)
- Columbia Suicide Severity Rating Scale (CSSRS)
- Probabilistic Rewards Task (PRT)
- Effort Expenditure for Rewards Task (EEfRT)
- Hamilton Depression Rating Scale (HAM-D)
- Hamilton Anxiety Rating Scale (HAM-A)
- The Cognitive and Physical Functioning Questionnaire (CPFQ)
- Blood sample collection to be sent to Rutgers for potential genetic analysis or other future analysis (optional)
- Clinical Global Impression – Severity (CGI-S)
- Clinical Global Impression – Improvement (CGI-I)
- Blood sample for tests for assessment for gastric adverse events including gastrin and pepsinogen I/II levels.
- Adverse events assessment with the Patient Reported Inventory of Side-Effects (PRISE)
- Inspection of medication blister packs to assess medication adherence
- Blood sample for drug blood level testing

At the end of this visit study drug will be discontinued and subjects will be instructed to return to the clinic/research unit in 4 weeks.

**Week 12 Visit: Post Medication Follow-up (2 - 4 hours) +/- 4 days**
At this visit subjects will undergo the following assessments:

- Vital signs
- Interval history
- Snaith-Hamilton Pleasure Scale (SHAPS)
- Temporal Experience of Pleasure Scale (TEPS)
- Visual Analogue Scale for Anhedonia (VAS)
- Columbia Suicide Severity Rating Scale (CSSRS)
- Adverse events assessment with the Patient Reported Inventory of Side-Effects (PRISE)
- Clinical Global Impression – Severity (CGI-S)
- Clinical Global Impression – Improvement (CGI-I)

At the conclusion of this visit subject participation in the study will end.

## 4.5. End of Participation / Early Termination

At the end of the week 12 visit, participation in the study will end. The results of all of the laboratory assessments and EEGs, ECGs, and physical examinations that could affect the subject's health care will be shared with them and their health care provider if they would like this to occur. Subjects will meet with the study physician and options for treatment will be reviewed. Participants will not be informed as to their treatment assignment at this meeting.

In case a subject is lost-to-follow-up, every possible effort must be made by the study site

personnel to contact the subject and determine the reason for withdrawal. The measures taken to follow up must be documented.

Subjects have the right to withdraw from the study at any time for any reason. If a subject withdraws from the study before Week 8, the subject should have a complete evaluation performed at the time of withdrawal that includes all Week 8 procedures **EXCEPT fMRI** (see Table 3 Schedule of Assessments). Subjects will be followed through Week 12 follow up unless they specifically withdraw their consent for further contact or for medical records review. When a subject withdraws before completing the study, the reason for withdrawal is to be documented on the eCRF and in the source document. Subjects who withdraw will not be replaced.

# 5. STORAGE OF DATA AND SAMPLES

After the database is closed, data will be stored in the secure server located at North Pavilion Datacenter, 2400 Pratt Street, Durham NC. The server is named GNEISS. The data will stored in \\gneiss\ct. All data will be stored as SAS version 9 datasets. The software is maintained and managed by the Duke Clinical Research Institute (DCRI) Information Technology (IT) group. The Director of Statistical Operations is responsible for granting access to the database as per our Standard Operating Procedures (SOPs). Once a year, the Director of Statistical Operations or Associate Director of Statistics reviews all trial access permissions and remove any trial access that is no longer appropriate. The data will be stored in gneiss for a maximum of 6 years. After six years, it will be archived. The Director of Statistical Operations, the Associate Director, Statistical Group for Manuscript Analysis (SIGMA) & Faculty and Fellows Affairs, and the Lead Biostatistician for the study or designee will approve the archival of the files from the DCRI network. The final datasets will be stripped of identifiers prior to release for sharing.

Blood samples for mailing to Rutgers for storage for potential genetic or other future analyses will be collected at baseline and after 8 weeks of treatment with study drug. These samples will be handled, processed, and mailed in accordance with applicable state, federal, and international regulations.

All samples will be logged into protected computer databases with all coded identifiers received from the FAST-MAS sites. A unique in-house RU ID # will be assigned as samples are logged into the RUCDRLIMS. This RU ID # for each specimen will be used to track the sample throughout processing, transformation and DNA and Plasma extraction. This new ID # that is unrelated to sample origin provides an additional level of security for maintaining sample anonymity. The FAST-MAS ID # and RU ID # will be cross-referenced in the computer database. The Alternate ID # will not be entered into the clinical or genetic databases. It will be used solely by the RUCDR as a secondary ID to help resolve labeling discrepancies that may occur during sample collection and submission.

# 6. RISKS AND DISCOMFORTS

The main potential risks (and burdens) to the subjects can be categorized as follows:

## 6.1. Risks of Discontinuing Medications for Study Participation

In order to participate in this study all subjects must be free of all antidepressant, antipsychotic, anxiolytic, anticonvulsant, mood stabilizing, muscle relaxant, centrally acting antihistaminergic, stimulant or insomnia medications (See Appendix 2) within 5 half-lives of baseline or at any time during after baseline and will have to remain off of all such medications other than study medication during the trial. There are risks associated with discontinuing medications which depend on the type of medication stopped, and the dosage and duration of use. Adverse effects requiring medical attention could also occur including seizures.

## 6.2. Risks of Being Without Therapy During the Study

During participation in this study subjects will go through periods of time without receiving treatment for their mood and anxiety spectrum disorder. This will occur because subjects will need to be off of all antidepressant and anxiolytic medications for 5 half-lives prior to starting the trial and during the trial, they may receive placebo during the study, the study medication may lack therapeutic effects for their condition, and all subjects will be off of study medications during weeks 8-12 of the study and will not be allowed to take other medications during this period. Risks of going without therapy include significant worsening of the symptoms of their mood and anxiety spectrum disorder.

## 6.3. Risks to Confidentiality

Patients will grant the experimenters access to protected health information. They will also reveal sensitive information about themselves, which will include their psychiatric status and any history of substance abuse. Subjects may feel uncomfortable revealing this information, and there is a risk of losing confidentiality. Subjects will also be informed in the consent document that confidentiality will be limited in cases where the subject reveals intentions to harm themselves or others, and the investigator feels that the proper authorities may need to be notified in order to prevent the occurrence of harm to the subject, or others.

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. The study protocol, documentation, data, and all other information generated will be held in strict confidence. All files will be kept secure and only authorized research personnel will have access. Only staff members who have a need to know personal identifying information will have access to this information.

All subject data will be kept strictly confidential, and no subject-identifying information will be released to anyone outside the project. Confidentiality will be through several mechanisms. First, each subject will be assigned a unique subject number which will then be used on all study forms, including the electronic data capture system (EDC). Secondly, any study forms, blood samples, or paper records that contain subject information will be

kept at the clinical sites in secured areas, and identified by the unique subject number. Once blood is collected, there will be no subject identifiers placed on blood samples, only the unique subject number and the date of sample collection. Third, access to all subject data and information, including laboratory specimens, will be restricted to authorized personnel. In the case of computerized data, this restricted access will be assured through user logon IDs and password protection. This protects forms from unauthorized view and modifications as well as inadvertent loss or damage.

Any data, forms, reports, and other records that leave the site will not contain the subject's name or any other direct identifier. The subject will be identified by a unique identifier to maintain subject confidentiality. Information will not be released without written permission of the participant, except as necessary for monitoring by the Institutional Review Board (IRB), the NIMH DSMB, the FDA, the government Office of Human Research Protections (OHRP) and/or any other government officials, safety monitors/committees that may need the information to make sure that the study is done in a safe and proper manner, learn more about side effects, and/or analyze the results of the study.

All subject data will be kept strictly confidential, and no subject-identifying information will be released to anyone outside the project. Confidentiality will be through several mechanisms. First, each subject will be assigned an anonymous study unique subject number which will then be used on all study forms, including the electronic data capture system (EDC). Secondly, any study forms, blood samples, or paper records that contain subject information will be kept at the clinical sites in secured, locked areas, and identified by the coded by unique subject number. Once blood is collected, there will be no subject identifiers placed on blood samples, only the unique subject number and the date of sample collection. Third, access to all subject data and information, including laboratory specimens, will be restricted to authorized personnel. In the case of computerized data, this restricted access will be assured through user logon IDs and password protection. This protects forms from unauthorized view and modifications as well as inadvertent loss or damage.

## 6.4. Risks Associated with Treatment

The most important risk identified in studies carried out to date is a potential risk of worsening of peptic ulcer disease in patients with gastric infection with Helicobacter pylori. This is based on findings of decreased parietal cells and inflammation in the stomach of rats.

In addition, subjects with a history of peptic ulcer disease or gastritis will also be excluded from participating in the study.

Other risks associated with the study drug include: headache, diarrhea, nausea, vomiting, flushing, anxiety, upset stomach, itchiness, and ventricular tachycardia (rapid heartbeat that requires immediate medical attention which was observed in 1 subject in the prior

studies). There is also the possibility that CERC-501 might lead to symptoms of depression based on the fact that this agent might have some mu opioid antagonist effects which several lines of evidence suggest might be depressogenic (Berrocoso et al., 2009; Escriba et al., 2004; Kennedy et al., 2006).

In addition to these risks there may be other risks of the study medication which are currently unknown as the number of individuals treated with this medication so far is limited.

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 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 a prescribed medication within 30 days of the first study day, or nonprescription medication except vitamins and acetaminophen (up to 4 g/day) within 15 days of starting study drug, or need to continue any medication during the study; 5) taken an investigational medication within 3 months prior to the start of this study; 6) smoked cigarettes within the last month or at any time during the study; or 7) a history of severe allergies or multiple adverse drug reactions.

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 Columbia Suicide Severity Rating Scale (CSSRS) and the CGI-S. If subjects experience an adverse reaction, appropriate medical care will be provided. This may include referral to an appropriate practitioner outside of the study, transfer to an Emergency Room, or hospital admission. Subjects will be monitored regularly until the adverse reaction is resolved. Unexpected adverse reactions occurring during the period of participation in the study or that are relevant to subjects after their participation in the study will be communicated to them. All adverse reactions will be reviewed by the NIMH Data and Safety Monitoring Board. They will make ongoing evaluations of the risks of study participation and can recommend that the study be terminated in cases where the risks are deemed to be excessive.

## 6.5. Risks of Venipuncture

Subjects will undergo venipuncture 6 times during the study. Complications of drawing blood from a vein occur approximately 2% of the time and include discomfort at the site of puncture; possible bruising and swelling around the puncture site; rarely an infection; and, uncommonly, faintness from the procedure.

In order to minimize the risks of drawing blood from a vein, sterile technique will always be used and these procedures will be carried out by individuals with sufficient training.

## 6.6. Risks Associated with fMRI Scanning

The risks associated with fMRI scanning include the development of anxiety inside the scanner (occurs in 5-10%) and the possibility of injury due to movement of a magnetic

object in the scanner room. In order to reduce the risk of developing fear during MRI procedures, subjects will have a practice MRI scanning session before having any MRI scans to help them get used to being inside the MRI scanner. Rarely people develop anxiety inside the scanner and decide not to continue in the study. This session will provide subjects with an opportunity to make sure they are comfortable with proceeding with MRI scanning.

Risks of injury during MRI will be reduced by evaluating subjects for risk factors including having implanted metal devices. If such risk factors are identified subjects would not be allowed to participate in the study. In addition, the area around the MRI scanner is kept free of objects that could pose a risk to subjects.

## 6.7. Risks associated with EEG and ECG studies

Risks associated with EEG and ECG studies: The risks associated with undergoing EEG and ECG studies include skin irritation at the sites electrodes are applied/removed and the general inconvenience of having to set aside time to undergo such monitoring. The risk of skin irritation will be minimized by having highly experienced individuals attach the electrodes and using methods that decrease the likelihood of irritation.

## 6.8. Risks to Those of Reproductive Potential

### 6.8.1. Females

Being a part of this study while pregnant may expose the unborn child to significant risks, some of which may be currently unforeseeable. In order to protect subjects from this risk, pregnant women will be excluded from the study. For all women of childbearing potential, a blood pregnancy test will be done and it must be negative before subjects can continue in this study. If sexually active, subjects must agree to use appropriate contraceptive measures for the duration of the study and for 1 month afterwards. Medically acceptable contraceptives include: (1) surgical sterilization (such as a tubal ligation or hysterectomy), (2) approved hormonal contraceptives (such as birth control pills, patches, implants or injections), (3) barrier methods (such as a condom or diaphragm) used with a spermicide, or (4) an intrauterine device (IUD). Contraceptive measures such as Plan B (TM), sold for emergency use after unprotected sex, are not acceptable methods for routine use. If subjects do become pregnant during this study or if they have unprotected sex, they will be instructed to inform the study physician immediately.

### 6.8.2. Males

Participation in this research has the potential to damage sperm, which could cause harm to a child that a subject might father while on this study. Such harm may be currently unforeseeable. As a result, in order to protect subjects, sexually active male subjects will have to agree to use a medically acceptable form of birth control during and 1 month after this study in order to participate. Medically acceptable contraceptives include: (1) surgical sterilization (such as a vasectomy), or (2) a condom used with a spermicide. Contraceptive measures such as Plan B (TM), sold for emergency use after unprotected sex, are not acceptable methods for routine use. Subjects will be instructed to inform their

partners of the potential for harm to an unborn child and that they should immediately inform the study doctor if pregnancy occurs.

### 6.8.3. Pregnancy

This protocol does not allow for currently pregnant women to be included in the study, due to the unknown risk to the fetus with this medication. At study entry, all women must have a negative pregnancy test to be allowed to enroll into the study, and must practice an acceptable method of birth control while in the study. ßHCG serum pregnancy tests will be performed for all women, unless surgically sterile, at the screening visit and if clinically indicated at any time during the study. All women of childbearing potential will be instructed to notify the Investigator if they become pregnant at any time during the study or within 30 days of last study evaluation. The pregnancy will be followed until delivery. If any subject is found to be pregnant during the study, study drug will be immediately discontinued. The subject will remain in the study for continued follow up of the pregnancy. If the pregnancy occurs in a female subject or the partner of a male subject during the study or within 30 days of the end of the study the investigator will inform the subject of their right to receive treatment information. If the female subject or partner agrees to the primary care physician being informed, the investigator will notify the primary care physician that the subject or her partner was participating in a clinical study at the time she became pregnant and provide details of treatment the subject received. All reported pregnancies will be followed up to final outcome. The outcome, including any premature termination, will be reported on the appropriate CRF page.

# 7. SUBJECT SAFETY MONITORING

The Principal Investigator or a Co-Investigator at each of the sites will monitor all subjects for adverse effects throughout their participation in the study.

## 7.1. Parameters to be Monitored

The Site Principal Investigator (PI) or a Co-Investigator (Co-I) at each site will monitor all subjects for adverse effects throughout their participation in the study. This will include carrying out a clinical interview for adverse events at each visit which will consist of asking the subject whether they experienced any new symptoms or changes since the last visit. In addition, a series of safety assessments will be carried out and the findings reviewed by the site PI or a Co-I. These safety assessments will include: the Columbia Suicide Severity Rating Scale (CSSRS) (Posner, 2011), vital signs (height, weight, blood pressure, and pulse), physical examination, Beta-HCG serum pregnancy test, urine drug screen, complete blood count with differential, electrolytes, comprehensive metabolic panel including liver function tests, thyroid function tests, urinalysis, CGI-I, and ECG.

These data will be used to assess for adverse event type, severity, expectedness, and causal relationship to study drug as defined in Section 16 below. The plan for documentation and reporting of Adverse Events is also included in Section 16 below.

In terms of monitoring for suicidality, subjects will be assessed at every visit with the Columbia Suicide Severity Rating Scale (CSSRS). Subjects who answer "yes" to item 5 of the CSSR-S ("Active suicidal ideation with specific plan and intent) and are considered by clinical interview to be at risk are immediately removed from the study and appropriate intervention is implemented. In case of ambiguity regarding suicidal plans, the patient is removed from the study and provided appropriate treatment. Any subject with an increase in suicidality will undergo thorough assessment by the study psychiatrist who will 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. If necessary, the study psychiatrist will seek consultation from the study PI or medical monitor. Subjects for whom continued participation is deemed not to be in their best interest will be discontinued from the study.

The site PI will follow all Adverse Events until resolution or they no longer believe that it is clinically significant. AEs ongoing at the time of the last dose of study drug will be followed up for as long as necessary to adequately evaluate the participant's safety, until they are resolved, or until it is determined by the site PI or designated Co-Investigator not to be clinically significant.

### 7.2. Criteria for Stopping Procedures in an Individual

If a participant experiences a severe adverse event (see Section 16 below), study procedures, including taking study medication, will be stopped for that individual. This will also occur if:

- A subject is assessed with a CGI-I score greater than 5
- In the opinion of the site PI or Co-I that it is in the participant's best interest to discontinue study procedures.

## 8. OUTCOME MEASURES

### 8.1. Primary Outcome Measures

- **Reward-Related Circuit Engagement POC Outcome** for the study will be task-related fMRI ventral striatal (e.g., nucleus accumbens) activation occurring with reward and anticipation during the Monetary Incentive Delay (MID) Task

### 8.2. Secondary Outcome Measures

- **Clinical Anhedonia Outcome** for this study will be the total score Snaith-Hamilton Pleasure Scale (SHAPS)

- **Behavioral Anhedonia Outcome** for this study will be Response Bias and Reward Learning Scores on the Probabilistic Reward Task (PRT)

## 8.3. Exploratory Outcome Measures

- Ventral striatal fMRI activation during anticipation of loss during the MID Task
- Resting state delta EEG current density in the rostral anterior cingulate
- Resting state fMRI connectivity
- Self-rated affective responses to cues and feedback during the MID Task
- The Effort-Expenditure for Rewards Task (EEfRT)
- The Visual Analogue Scale for Anhedonia (VAS)
- The Temporal Experience of Pleasure Scale (TEPS)
- The Hamilton Depression Rating Scale (HAM-D)
- The Hamilton Anxiety Scale (HAM-A)
- The Cognitive and Physical Functioning Questionnaire (CPFQ)
- Clinical Global Impression – Severity (CGI-S)
- Clinical Global Impression – Improvement (CGI-I)
- Safety and tolerability of CERC-501 on systematically collected and spontaneously reported adverse events

## 8.4. Description of Outcome Measures

### 8.4.1. Monetary Incentive Delay Task-related fMRI

fMRI during the Monetary Incentive Delay (MID) Task will yield the primary outcome measure for this study with which we will evaluate whether we can establish POC for KOR antagonists by assessing whether CERC-501 engages the neural circuitry that mediates reward-related function (Wacker et al., 2009; Pizzagalli et al., 2004, 2009; Knutson et al., 2008). We will specifically assess whether CERC-501 leads to greater ventral striatal activation than placebo during anticipation of rewards with MID Task-related fMRI.

Evidence supporting the use of the MID Task-related fMRI for this purpose is that depressed patients were found to have decreased ventral striatal activation to anticipated reward with this task (Stoy et al., 2012) and that this measure has been demonstrated to be sensitive to changes occurring with antidepressant/anxiolytic therapies (SSRI and SNRI) in small open-label studies including those with major depression and healthy controls and a double-blind placebo-controlled study in healthy controls (Stoy et al., 2012; Ossewaarde et al., 2011).

The MID fMRI will be obtained on Day 0 (baseline) and after 8 weeks of daily treatment with study drug (Visit A4). The test will be carried out as previously described (Knutson et al., 2008). The MID task was designed to elicit neural responses to monetary incentive anticipation and outcomes. To test the specificity of the findings, both reward and penalties will be included. It will be administered in two task runs each of which will consist of 90 6-second trials. For each trial, subjects will be presented with one of nine possible cue shapes for 250 msec while their gaze is fixed on a crosshair on a computer screen. The period of waiting for the cue shape will vary from 2000-2500 msec. These cue shapes will signal whether the upcoming trial has the potential for monetary gain

(n=72, denoted by circles), potential for monetary losses (n=72; denoted by squares), or that no response is required (n=36; denoted by triangles). Gain cues will signal the possibility of winning $0.00 (n=18; no lines), $0.20 (n=18; one horizontal line), $1.00 (n=18; two horizontal lines), or $5.00 (n=18; three horizontal lines). Similarly, loss cues will signal the possibility of losing $0.00 (n=18; no lines), $0.20 (n=18; one horizontal line), $1.00 (n=18; two horizontal lines), or $5.00 (n=18; three horizontal lines). "No response" trials (n=36; a triangle) will indicate that the subject should not respond during that trial, and instead should wait until the cue signaling the next trial appeared. Trial types will be pseudo-randomly ordered within each run, and runs will be counterbalanced across subjects.

When subjects are presented with the cue shape they will be instructed to respond by pressing a button when they are presented with a white target for a period of 160-360 msec. They will also be instructed that on incentive trials, subjects could either gain or avoid losing money by pressing the button during target presentation. 1650 msec after the white target disappears, subjects will be notified as to how much money they had gained or lost on that trial and their total winnings (or losses).

Subjects will undergo at least 10 minutes of training and practice prior to testing. They will also see the money that they could potentially win prior to fMRI testing. Task difficulty will be based on reaction times collected during practice sessions and will aim to allow subjects to successfully press the button during target presentation on incentive trials on 66% of the trials.

Scan Acquisition: All scans will be conducted on research dedicated 3.0 Tesla MRI scanners running the latest software version using an advanced 32-channel RF coil. MR compatible video projection system with vision correction lenses, high quality headphones and a button box will be used for fMRI task presentation and response recording. Physiological monitoring is performed and digitally recorded during all scans.

Resting state connectivity will also be assessed with fMRI for exploratory analysis.

**MRI acquisition sequences (use 32-channel headcoil)**:

(1) Localizer (Time: ~ 15 s);
(2) EPI-Monetary Incentive Delay Task (Time: 4 min 32 s): Gradient-echo echo-planar (EPI) fMRI scans with physiological monitoring, axial, TR/TE: 2000/30 ms, flip angle: 70 deg, FOV: 25.6 cm, matrix: 64x64, 32 axial slices, acceleration factor = 2, voxel size: 4x4x4 mm, 137 fMRI time points + 4 dummy scans at the beginning (total 141 points/TRs), total scan time 4 min 42 seconds (141 x 2 s) for each run. This duration is same as that of the E-prime stimulus file. Repeat the same fMRI run (corresponding to individual E-prime stimulus files), totaling five fMRI runs.
(3) EPI-resting state connectivity fMRI: Same as 2, except no task will be used, and only one run is needed. Subjects are instructed to keep eyes closed, and try to think nothing in particular.
(4) DTI: Spin-echo EPI acquisition at128x128 matrix, acceleration factor =2, voxel size: 2x2x2 mm, 30 directions, b=1000, 3 baseline b0 scans, TE minimum (should be near

~80 ms), TR = 8 s (or shortest possible to accommodate all slices within), 64 slices, total scan time 4 min 24 seconds.

(5) High resolution T1-weighted volume (Time: <5min) for registration and morphometric characterization (3D MP-RAGE or IP-SPGR), 128 axial slices to cover whole brain, voxel size: 1x1x1 mm;

(6) Axial PD/T2 TSE, to rule out incidental findings (Time: <5 min): 64 axial slices from apex, voxel size: 1x1x2mm.

Note: brain volume must be kept the same for all series (series 2-6)

### 8.4.2. The Snaith-Hamilton Pleasure Scale (SHAPS)

The Snaith-Hamilton Pleasure Scale (SHAPS) (Snaith et al., 1995) is a well-validated 14-item questionnaire used to assess anhedonia. It asks participants to agree or disagree with statements of hedonic response in pleasurable situations (e.g., "I would enjoy my favorite television or radio program"). Four responses are possible: Strongly disagree, Disagree, Agree, or Strongly agree. 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." Therefore, scores on the SHAPS can range from 14 to 56, with higher scores corresponding to higher levels of anhedonia.

The SHAPS covers four domains of hedonic experience: interest/pastimes, social interaction, sensory experience, and food/drink (Snaith et al., 1995). Participants completing the SHAPS are instructed to respond based on their ability to experience pleasure "in the last few days." This scale has shown adequate overall psychometric properties in clinical and student samples (Gilbert el al., 2002; Snaith et al., 1995). The SHAPS convergent validity has been supported by its correlations with MADRS Hedonic Tone item, the Mood and Anxiety Symptom Questionnaire Anhedonic Depression subscale, and Positive and Negative Affect Schedule-Positive Affect subscale (Gilbert et al., 2002; Snaith et al., 1995). Its discriminant validity has been supported by its lack of association with MADRS Depressed Mood and Anxiety items (Snaith et al., 1995). The SHAPS has satisfactory test-retest validity in healthy participants over an interval of three weeks (intraclass correlation coefficient between test and retest: $r = .70$, $p < .001$; Franken et al., 2007). Besides being well-validated, the SHAPS is of utility for our study because there is an established cutoff for clinical significance. Although this is also true for the Anhedonic Depression subscale of the Mood and Anxiety Symptoms Questionnaire (MASQ), we wanted a scale that assessed anhedonia and was not depression specific or specific to any other disorder given the goal of using it across diagnostic boundaries. ROC using the MADRS anhedonia item score (cutoff was clinically significant level) as a discriminator suggests that a SHAPS score of $\geq 20$ corresponds to clinically significant anhedonia (Snaith et al., 1995). This was validated in 30 volunteers from the general population. It should be noted that the original ROC analysis was done with dichotomous ratings rather than the current 4 option rating system. The original finding was that 3 or more negative answers indicated clinical significance which corresponds to a score of at least 20 with the current rating system.

Another important consideration that supports the use of the SHAPS in this study is that it is the only anhedonia measure that we are aware of that has been found to significantly improve with the administration of a treatment in at least one clinical trial (Di Giannantonio and Martinotti, 2012; Martinotti et al., 2011). In a study of alcohol dependent individuals, treatment with acetyl-l-carnitine led to statistically significantly greater improvement in the SHAPS than placebo by 10 days after initiating treatment (Martinotti et al., 2011). In an 8-week trial comparing the effects of venlafaxine XR and agomelatine treatment in depressed patients, significant improvement from baseline on the SHAPS was observed in both groups with significantly greater improvement being found for the agomelatine treated patients (Di Giannantonio and Martinotti, 2012). Finally, SHAPS scores among healthy participants have been related to reward-related activation within frontostriatal pathways (see e.g., Wacker et al., 2009), providing an additional reason for its inclusion in the current study.

The SHAPS will be collected at all study visits. It will be used to screen subjects for having anhedonia as part of inclusion/exclusion assessment. It will also be compared in subjects receiving CERC-501 and PBO in secondary analysis to assess the effects on a clinical self-rated measure of anhedonia.

### 8.4.3. Probabilistic Reward Task

The Probabilistic Reward Task (PRT) (adapted from Tripp & Alsop (1999)) was designed to objectively assess participants' propensity to modulate behavior as a function of reinforcement history. This task has been validated in multiple independent samples (e.g., Barr et al., 2008; Bogdan and Pizzagalli, 2006, 2009; Pizzagalli et al., 2005, 2007, 2008a,b; Vrieze et al., 2013a,b). Participants will complete two blocks of 100 trials, in which they will be asked to determine whether a briefly presented mouth on a cartoon face was 'long' or 'short', and report their decision by pressing one of two corresponding keys on a computer keyboard ('z' or '/'). Importantly, the brief presentation time (100 ms) and the minimal difference in length between the two target stimuli (11.5 vs. 13 mm) make it difficult for participants to perceptually distinguish which stimulus is presented. Moreover, an asymmetrical reinforcement ratio is implemented across the two blocks so that one of the two stimuli (the 'rich' stimulus) is consistently rewarded ("Correct!! You Won 20 Cents") three times more frequently than the 'lean' stimulus (30 vs. 10 times per block). Reinforcement allocation and key assignments will be counterbalanced across participants. Participants are instructed to respond as quickly and accurately as possible to maximize monetary rewards, and that not all correct responses will be followed by rewards. In healthy participants, such asymmetric reinforcement schedule has been found to induce a systematic preference (response bias) for choosing the stimulus paired with more frequent reward (Macmillan and Creelman, 1996). Healthy controls are able to successfully modulate their behavior based on their experience, resulting in a significant response bias for the more frequently rewarded stimulus. In contrast, individuals with elevated depressive symptoms (Pizzagalli et al., 2005) or MDD (Pizzagalli et al., 2008b) – specifically MDD subjects with anhedonia (Vrieze et al., 2003) – show a blunted response bias, and overall reduced reward learning. Of note, reward learning negatively correlated with anhedonic symptoms and predicted them one month later (Bogdan and Pizzagalli, 2006; Pizzagalli et al., 2005) and predicted the persistence of a MDD diagnosis after 8-week antidepressant treatment (Vrize et al., 2013). Moreover, trial-by-

trial probability analyses revealed that MDD subjects were impaired at integrating reinforcements over time and expressing a response bias in the absence of immediate reward. This impairment correlated with anhedonic symptoms, even after considering anxiety and distress symptoms (Pizzagalli et al, 2008b).

Data reduction and analyses: After removing trials with outlier reaction times (see Pizzagalli et al., 2005 for detail), response bias (log b) will be computed according to the following formula:

$$\log b = \frac{1}{2}\log\left(\frac{Rich_{correct} * Lean_{incorrect}}{Rich_{incorrect} * Lean_{correct}}\right)$$

To avoid issues related to having a zero in one cell of the formula, 0.5 will be added to every cell of the detection matrix (Hautus and Collins, 2003). As evident from the formula, response bias reflects the participants' preference for the stimulus paired with more frequent rewards. Reward learning will be operationalized as the change in response bias from Block 1 to Block 2 (RB = Response Bias [Block 2] – Response Bias [Block 1]).

The results of a number of studies provide the rationale for using the PRT in this study. This includes a study identifying that PRT score predicts clinical outcome to naturalistic antidepressant treatment. In this study, 69 inpatients and 63 healthy controls performed the PRT at baseline (i.e., at intake for patients), and patients were administered the task again after 8 weeks of antidepressant treatment (Vrieze et al., 2013a). Relative to controls, MDD patients showed reduced reward learning ($F[2,280]=3.53$, $p=0.03$). Critically, this blunting was found only in patients with high anhedonia ($p=0.007$). Of note, Hamilton Depression Rating Scale (HDRS) scores were not related to reward learning. Also SHAPS scores uniquely predicted reward learning even when controlling for depression severity (HRSD scores) and anxiety comorbidity ($\beta=0.33$, $t=3.08$, $p=0.003$). Further, reward learning did not correlate with overall depressive symptoms (adjusted Beck Depression Inventory [BDI] score computed without anhedonic items) ($p>.71$) (Pizzagalli et al., 2005). Critically, the link between anhedonia and blunted reward learning was confirmed when entering the adjusted BDI scores as a covariate in a partial correlation ($r=-.38$, $p=0.008$).

Additional findings of interest are that, in healthy controls, reward learning is reduced (1) under acute stress (Bogdan and Pizzagalli, 2006; Bogdan et al., 2011) and during naturalistic stressors (Nikolova et al., 2012); and (2) by a pharmacological challenge (single low dose of the D2/D3 agonist pramipexole) affecting DA (Pizzagalli et al., 2008a). Notably, the deleterious effects of stress on reward learning were largest in subjects reporting anhedonic symptoms in daily life (Bogdan and Pizzagalli, 2006) and carrying genetic markers previously associated with depression risk (Bogdan et al., 2010, 2011; Nikolova et al., 2012). Blunted reward learning in subjects receiving pramipexole was assumed to result from activation of presynaptic DA autoreceptors, leading to decreased phasic DA bursts in response to unpredictable rewards. Conversely, reward learning was increased by a nicotine patch (Barr et al., 2008), a manipulation assumed to activate mesocorticolimbic DA neurons (Kenny and Markou, 2006). The heritability of reward learning is substantial, 48% (Bogdan and Pizzagalli, 2009), and reward learning is

associated with reward-related ACC and striatal activation and DA release (Santesso et al., 2008; Vrieze et al., 2013b).

The PRT will be carried out at baseline and after 8 weeks of double-blind treatment. The change from baseline in CERC-501 and PBO groups will be compared in secondary analysis to assess the effects on a behavioral outcome measure that assessed reward-related function.

### 8.4.4. Other Efficacy Assessments

Other efficacy assessments carried out in this study to be employed in exploratory analyses include resting state delta EEG current density in the rostral anterior cingulate, the Visual Analogue Scale for Anhedonia (VAS), The Effort Expenditure for Rewards Task (EEfRT), the Temporal Experience of Pleasure Scale (TEPS), The Hamilton Rating Scale for Depression (HAM-D), The Hamilton Rating Scale for Anxiety (HAM-A), and the Cognitive and Physical Functioning Questionnaire (CPFQ). The VAS-Anhedonia, TEPS, and CPFQ are self-report instruments. The EEfRT is a behavioral task. The HAM-D and HAM-A are clinician administered assessements which will be administered by appropriately trained and monitored raters. The training, assessment, and maintenance of inter-rater reliability will be managed by the Duke Signal Detection Team. Clinical raters, neuropsychological technicians, and all staff involved in collection and analysis of clinical measures will be blinded to treatment assignment and to clinical status. They will have no interactions with subjects other than the administration of the specific tests they are assigned to administer. Raters and patients will complete best-guess forms so that the adequacy of the blind can be assessed. Best-guess forms will be used to formally assess the adequacy of the blind for patients and raters.

#### 8.4.4.1. Resting state delta EEG current density in the rostral anterior cingulate

We will obtain resting state, eyes-closed quantitative EEG (QEEG) in order to provide an additional, secondary circuit-based measure of hedonic function. We will obtain resting state current density using Low Resolution Electromagnetic Magnetic Tomography (LORETA) based on evidence that using this method, greater resting EEG delta (1.5- 6 Hz) current density (i.e., lower brain activity) in the rostral anterior cingulate is correlated with higher anhedonia scores with the MASQ anhedonic depression subscale (Wacker et al., 2009; Pizzagalli et al., 2004).

The recordings will consist of at least 32 channels of EEG data with electrodes located according to the Modified International 10-20 System. Right infra-orbital and left outer canthus electrodes will be used to monitor eye movements. Recordings will be obtained with a referential montage employing a linked-ear reference. EEG files will be named according to a standardized convention and processed in order to ensure that there are no subject identifiers included with the recorded data. The data digitization rate will be at least 256 Hz. Electrode impedances will be maintained at below 5 kOhm during the recordings. Standard square-wave and bio-calibrations will be performed at the beginning of each study. The EEG data will be recorded with subjects in a semi recumbent position, with eyes closed in a maximally alert state in a sound-attenuated room with subdued lighting. Data collection will occur for 20 minutes (10 minutes with eyes closed and 10

minutes with eyes opened) following calibration with filter settings of 0.5 and 70 Hz. Analysis will be carried out using Low Resolution Electromagnetic Magnetic Tomography (LORETA) (see Statistical Analysis Section) to produce estimates of resting state EEG delta current density in the rostral anterior cingulate which has previously been reported to correlate with a self-rated anhedonia measure (Wacker et al., 2009; Pizzagalli et al., 2004).

The Duke QEEG Core will receive the data collected at each of the sites, and it will undergo analysis blinded to subject and study time point and entered manually. The studies will first undergo epoch-by-epoch visual artifacting with confirmation by a second rater. In cases of discrepancies, a third rater will be employed for resolution. QEEG analysis will only be carried out if a minimum of 30 seconds of waking, artifact-free data are available. The data will undergo Laplacian transformation to produce current density estimates (Wacker et al., 2009; Pizzagalli et al., 2004). The current density data will undergo spectral analysis via Fast Fourier Transformation (FFT) with boxcar windowing to generate power estimates in the following frequency bands: delta (1.5–6.0 Hz), theta (6.5–8.0 Hz), alpha1 (8.5–10.0 Hz), alpha2 (10.5–12.0 Hz), beta1 (12.5–18.0 Hz), beta2 (18.5–21.0 Hz), beta3 (21.5–30.0 Hz), and gamma (36.5–44.0 Hz). This will be carried out with the LORETA software, at each voxel (n = 2394; voxel resolution = 7 mm3), current density will be computed as the squared magnitude of the intracerebral current density within each of the eight frequency bands (unit: amperes per square meter, A/m2). Current density estimates will be intensity-normalized to unity and log-transformed before statistical analyses. This will be carried out in 2-second segments and averaged over the available artifact-free, waking data. These data will be used to estimate resting state EEG current density. The primary QEEG outcome will be EEG delta current density in the rostral anterior cingulate which has found to correlate with self-rated anhedonia (Wacker et al., 2009; Pizzagalli et al., 2004). Resting state EEG current density power estimates in other frequency bands will be analyzed to evaluate the specificity of possible findings. Exploratory analysis for patterns of EEG activity that are predictors and correlates of outcome will be carried out using EEG power in all bands derived from all of the electrodes, employing principal components analysis (Krystal et al., 1995).

#### 8.4.4.2. Exploratory Anhedonia Measures

To complement the SHAPS we include 3 secondary anhedonia measures: the Effort-Expenditure for Rewards Task (EEfRT), the Visual Analogue Scale for Anhedonia (VAS-Anhedonia) and the Temporal Experience of Pleasure Scale (TEPS). These will be compared in subjects receiving CERC-501 and PBO in exploratory analysis.

The **Effort-Expenditure for Rewards Task** (EEfRT; Treadway et al., 2009) is intended to assess the motivation to pursue rewards, one important dimension of reward-related function. It has been validated in a study of 60 individuals who were screened to have anhedonia with the SHAPS (Treadway et al., 2009). The EEfRT was found to be correlated with the Beck Depression Inventory (BDI) anhedonia scale and the Chapman anhedonia scale. The EEfRT task is a multi-trial game in which participants are given an opportunity on each trial to choose between two different task difficulty levels in order to obtain monetary rewards. For all trials, participants make repeated manual button presses

within a short period of time. Each button press raises the level of a virtual "bar" viewed onscreen by the participant. Participants are eligible to win the money allotted for each trial if they raise the bar to the "top" within the prescribed time period. Each trial presents the subject with a choice between two levels of task difficulty, a 'hard task' and an 'easy task.' Successful completion of hard-task trials requires the subject to make 100 button presses, using the non-dominant little finger within 21 seconds, while successful completion of easy-task trials requires the subject to make 30 button presses, using the dominant index finger within 7 seconds. For easy-task trials, subjects are eligible to win the same amount, $1.00, on each trial if they successfully complete the task. For hard-task choices, subjects are eligible to win higher amounts that vary per trial within a range of $1.24 – $4.30 ("reward magnitude"). Subjects are not guaranteed to win the reward if they complete the task; some trials are "win" trials, in which the subject receive the stated reward amount, while others are "no win" trials, in which the subject receives no money for that trial. To help subjects determine which trials are more likely to be win trials, subjects are provided with accurate probability cues at the beginning of each trial. Trials have three levels of probability: "high" 88% probability of being a win trial, "medium" 50% and "low" 12%. Probability levels always apply to both the hard task and easy task, and there are equal proportions of each probability level across the experiment. Each level of probability appears once in conjunction with each level of reward value for the hard task. All subjects receive trials presented in randomized order.

All trials begin with a 1-second fixation cross, following a 5-second choice period in which subjects are presented with information regarding the probability of receiving reward and the reward magnitude of the hard task. Subjects are told that if they did not make a choice within 5 seconds, they will be randomly assigned to either the easy or the hard task for that trial. After making a choice, subjects are then shown a 1-second "Ready" screen and then they complete the task. Following task completion, subjects are shown a 2 second feedback screen informing them that the task was successfully or unsuccessfully completed. If subjects successfully complete the task, then a second feedback screen appears for 2 seconds in which subjects are told whether they had won money for that trial (reward feedback). In total, easy-task trials take approximately 15 seconds, whereas hard-task trials take approximately 30 seconds.

Subjects are told that they will receive a base-rate of compensation for their participation. In addition, they are told that two of their win trials will be randomly selected at the end of the experiment as "incentive trials," for which they will receive the actual amount won on those trials. Subjects are informed that they have twenty minutes to play as many trials as they can. Since hard-task trials take approximately twice as much time to complete as easy-task trials, the number of trials that the subject is able to play depends in part on the choices that he or she makes. This means that making more hard-task trials toward the beginning of the experiment could reduce the total number of trials, which could in turn mean that the subject does not get a chance to play high-value, high-probability trials that might appear towards the end of the playing time. This trade-off is explained clearly to the subject. Importantly, subjects are not provided with any information regarding the distribution of trial types. The goal of this trade-off is to ensure that neither a strategy of always choosing the easy or the hard option could lead to an 'optimal' performance on the task. Moreover, the complexity of variables (with varying monetary reward levels, probability, and loss of time for future trials), does not lend itself to a formal calculation

of an optimal response selection, and subjects are required to make decisions within a brief amount of time. This is done to help ensure that subject decisions reflected individual differences in the willingness to expend effort for a given level of expected reward value.

The **VAS-Anhedonia** is a standard VAS assessment of anhedonia severity which is included because it provides a global anhedonia indicator which takes very little time to obtain and which was found to be sensitive to change with treatment in a prior placebo-controlled trial in alcohol dependent subjects (Martinotti et al., 2011). The test consists of making a rating on a 100 mm scale in response to the directive: "Make a mark on the line below that indicates how much pleasure you experience from food, sexual behavior, and meeting friends". At the left end of the scale is the anchor "No Pleasure" and at the right end of the scale is the anchor "Extreme Pleasure."

We also include the **Temporal Experience of Pleasure Scale** (TEPS) because it provides different information about reward-related function than the SHAPS and has been found to be correlated with activation in the key circuits of interest (nucleus accumbens and putamen) in Monetary Incentive Delay Task-related fMRI (Stanford et al., 2011). 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’). Both components are currently believed to be relevant to measuring anhedonia.

#### 8.4.4.3. Other Exploratory Measures

The **Hamilton Rating Scale for Depression** (HAM-D) 17-item version (Hamilton, 1967) will be included in exploratory analysis to provide confirmatory support for changes in depression severity with treatment. This interviewer-administered semi-structured interview is one of the most widely used instruments in depression treatment studies.

The **Hamilton Rating Scale for Anxiety** (HAM-A) is a rating scale designed to measure the severity of anxiety symptoms (Hamilton, 1959). It is widely used in both clinical and research settings. The scale consists of 14 items, each defined by a series of symptoms, and measures both psychic anxiety (mental agitation and psychological distress) and somatic anxiety (physical complaints related to anxiety). It has been demonstrated to acceptable reliability, validity and sensitivity to change (Maier, 1988). Each item is scored on a scale of 0 (not present) to 4 (severe), with a total score range of 0–56, where <17 indicates mild severity, 18–24 mild to moderate severity and 25–30 moderate to severe. This instrument will be included in secondary analysis to provide confirmatory support for changes in anxiety severity with treatment.

The **Cognitive and Physical Functioning Questionnaire** (CPFQ) is a 7-item self-report instrument was intended to be a brief scale for measuring cognitive and executive dysfunction in patients with mood and anxiety disorders (Fava et al., 2009). This scale

has been demonstrated to have strong internal consistency, good temporal stability and sensitivity to change with treatment (Fava et al., 2009).

The **Clinical Global Impression - Severity** (CGI-S) is a widely administered clinician rated global measure of subject overall illness severity. 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 all subject visits.

The **Clinical Global Impression - Improvement** (CGI-I) 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**.

# 9. STATISTICAL ANALYSIS

## 9.1. Analysis of Data/Study Outcomes

### 9.1.1. Analysis of data/ study outcomes

#### 9.1.1.1. Data Management

All data management activities, including establishing a trial electronic case report form and procedures for randomization will be done within the framework of the Duke Clinical Research Institute (DCRI) and will conform to FDA GCP requirements.

Electronic data validation checks with data entry discrepancy flags will utilized. The flags will be triggered by missing values, logically inconsistent values, and values beyond limits. Data checks will be automated and occur daily and lead to the generation of queries in the EDC system which are communicated to the sites. The sites will then review the queries and make appropriate clarifications and corrections. The EDC system will also track queries and produce reports of outstanding queries.

As the sites are entering data, on-screen edit checks run real-time to catch missing values, out of range values, and logical inconsistencies. These will be specified by the DCRI Clinical Data Specialist (CDS) working in conjunction with the statistician. The sites will be trained on reliably entering data. A final database audit on a representative sample of the data will also be carried out.

#### 9.1.1.2. Interim Analysis.

There will be no interim analysis carried out.

#### 9.1.1.3. Primary Outcome Measures

- **Reward-Related Circuit Engagement POC Outcome** for the study will be task-related fMRI ventral striatal (e.g., nucleus accumbens) activation occurring with reward and anticipation during the Monetary Incentive Delay (MID) Task

#### 9.1.1.4. Secondary Outcome Measures

- **Clinical Anhedonia Outcome** for this study will be the total score Snaith-Hamilton Pleasure Scale (SHAPS)

- **Behavioral Anhedonia Outcome** for this study will be Response Bias and Reward Learning Scores on the Probabilistic Reward Task (PRT)

#### 9.1.1.5. Exploratory Outcome Measures

- Ventral striatal fMRI activation during anticipation of loss during the MID Task
- Resting state delta EEG current density in the rostral anterior cingulate
- Resting state fMRI connectivity
- Self-rated affective responses to cues and feedback during the MID Task
- The Effort-Expenditure for Rewards Task (EEfRT)
- The Visual Analogue Scale for Anhedonia (VAS)
- The Temporal Experience of Pleasure Scale (TEPS)
- The Hamilton Depression Rating Scale (HAM-D)
- The Hamilton Anxiety Scale (HAM-A)
- The Cognitive and Physical Functioning Questionnaire (CPFQ)
- Clinical Global Impression - Severity (CGI-S)
- Clinical Global Impression – Improvement (CGI-I)
- Safety and tolerability of CERC-501 on systematically collected and spontaneously reported adverse events

### 9.1.2. Planned analyses and statistical methodology to be used

We will employ “Intention-to-Treat” (ITT) principles. Statistical tests will be performed based on a two-sided test at the 5% level of significance. Supporting analyses will also be performed. A “per protocol” analysis will restrict the analysis to participants who receive minimal exposure to the intervention, have a minimal number of longitudinal evaluations, and/or are free of major protocol violations. A “completer” analysis will only include participants who completed acute treatment without exiting or prematurely terminating from their assigned treatment. Because participants may be removed differentially across the treatment arms in these analyses, both approaches are vulnerable to differential selection bias and will be interpreted cautiously.

Most of the study outcomes are observed repeatedly at well-defined time-points post-randomization, so that statistical methods for repeated measures data will be applied. This includes the linear model with structured covariance matrices and the mixed effects models as implemented in PROC MIXED in SAS. Site will also be included as an independent variable in order to account for variability among the study sites.

Covariates in Regression Models: The baseline value of the outcome, centered about its sample mean, will be included in each analysis. Otherwise, only age and sex will be entered as covariates.

Missing Values: We will proactively monitor the amount of missing data, and ensure that we respond quickly to emerging problems. Statistically, missing data will be addressed through the use of mixed models analysis which will be carried out with PROC MIXED in SAS. We will carry out an assessment of the degree to which the structured covariance matrix in mixed effects models fits the data.

Other Confounders Arising Post-Randomization: Logistic regression analysis will be carried out to investigate: (a) adherence to the assigned study intervention (this will include serum levels of study drug and blister pack check data), (b) co-administration of psychotropic medications, and (c) discontinuation from the study intervention. First, we will determine whether these effects occur differentially across the treatment arms suggesting that a differential selection bias may have influenced the results. Then, we will investigate whether baseline characteristics are predictive of any behavior. This will include demographic characteristics, initial severity as measured by the baseline values of the outcome variables, and clinical characteristics such as diagnoses and medications taken at baseline.

Magnitude of the Clinical Effect: To evaluate the clinical significance of the impact of treatment on outcome, effect sizes (Hedge's g) will be calculated as (ME – MC) / SD pooled, where ME represents the adjusted mean of experimental treatment, MC represents the adjusted mean of the comparison treatment, and SD pooled represents pooling of the standard deviations from within both groups. We will also assess how the estimated magnitude of treatment effect and the estimated standard deviation deviate from those postulated in sample size planning.

fMRI Analysis: The monetary incentive delay task entails three key task phases: anticipating making a response, anticipating an outcome, and receiving that outcome. Functional brain responses to these three task phases will be identified using a General Linear Model based statistical analyses (GLM). Here, a statistical test is carried out between the actual data (fMRI time series) and a predicted model (event timings and parametric modulations by behavior). If the fit between the predicted and actual data is good, then a relationship is concluded to exist between the actual data (voxel intensity values) and task-related factors (anticipation, response, receipt). Having tested the fit at the single subject level for each experimental session, the data will be combined into a second-level, random-effects, repeated-measures analysis of variance. Statistical inferences about task effects will be made after appropriate correction for multiple comparisons. Regions of interest or ROIs will be defined with reference to a standard brain atlas (that of Talaraich and Tournoux). The fMRI primary analysis (see below) will focus on the ventral striatum. However, exploratory analyses will be carried out with other ROIs. Cluster-wise parameter estimates for each condition (anticipation, response, receipt) will be extracted for the ventral striatum (and from the other predefined ROIs for exploratory analysis) and correlated with receptor occupancies. In this manner our regression analyses will be robust to non-independence error.

#### 9.1.2.1. Primary Analysis

Point estimate and the corresponding confidence interval for the primary study outcome (fMRI ventral striatal activation during reward anticipation of reward during the MID) of

the adjusted predicted score will be obtained. A mixed effects model analysis will be carried out for the primary outcome of the study, task-related fMRI activation in the ventral striatum during anticipation of reward occurring during the Monetary Incentive Delay Task. This analysis will test the hypothesis that CERC-501 will lead to a greater increase in ventral striatal activation during anticipation of reward than placebo.

#### 9.1.2.2. Secondary Analysis

A mixed effects model analysis will also be carried out for the secondary outcomes of the study, SHAPS score and PRT-derived Reward Learning and Response Bias. These analyses will test the hypothesis that CERC-501 will: 1) decrease SHAPS score to a greater degree than PBO; and 2) increase PRT-derived reward learning and response bias to a greater degree than PBO.

#### 9.1.2.3. Exploratory Analysis

Exploratory mixed effects model analyses will also be carried out with the exploratory outcomes of the study. This includes: ventral striatal fMRI activation during anticipation of loss during the MID Task; resting state delta EEG current density in the rostral anterior cingulate; resting state fMRI connectivity; self-rated affective responses to cues and feedback during the MID Task; EEfRT total reward; VAS score; TEPS anticipatory and consummatory pleasure scores; HAM-D score; HAM-A score; CGI-S, CGI-I, and CPFQ score.

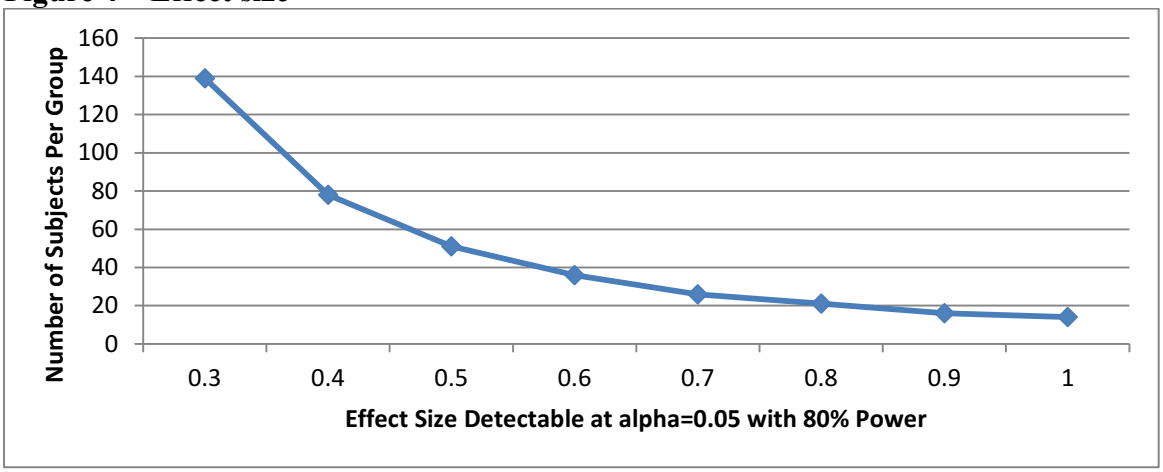
## 9.2. Power Analysis

A total of 90 subjects will be randomized: 45 subjects randomized to CERC-501 and 45 to placebo and included in the primary efficacy analysis. We conservatively anticipate that we will have incomplete data on up to 20% of subjects due to subject drop out or loss of data due to factors such as poor scan quality. As a result, we estimate that we will have complete data on as few as 72 subjects and as many as 90 subjects. In the section above, we outline how we will address the issue of missing data including carrying out analyses on the intent-to-treat (ITT) population and employing mixed effect models. If we assume the worst case scenario of 72 subjects, for a one tailed t-test at an alpha value of 0.05, power is 80% to identify an effect size of 0.58. For a one tailed t-test at an alpha value of 0.10 power is 80% to identify an effect size of 0.50. In Figure 4, it is indicated the effect that could be detected with 80% power at the alpha=0.05 level as a function of number of subjects.

Our capacity to estimate the expected effect size on our primary outcome measure is limited by the studies that have been carried out with this measure to date. The most relevant study was carried out by Stoy and co-workers (2011) who evaluated 15 patients with major depression and 15 controls and administered open-label escitalopram to both groups for 6 weeks and assessed outcome with fMRI activation of the ventral striatum during the MID. However, it must be kept in mind that estimates of power based on this study are likely overly optimistic because this was an open-label treatment study and only included 15 depressed subjects. We can estimate the expected effect size for our study from the ventral striatal activation to reward anticipation subject group by time interaction observed in this study. The effect size for a greater increase in those with depression compared with controls is 0.88. We can also estimate effect size from the pre-

to-post treatment difference in ventral striatal activation during reward anticipation and reward during the MID with fMRI within the depressed group which are 1.1 and 1.0 respectively. These effect sizes suggest that we are likely to have more than sufficient power to detect a significant effect with our primary outcome measure in the proposed study at the alpha=0.05 level with 80% power.

**Figure 4 – Effect size**




# 10. HUMAN SUBJECTS PROTECTION

## 10.1. Subject Selection

### 10.1.1. Statement of equitability

Subjects in this study will all be recruited within the vicinity of each of the sites by the site teams. We expect that the racial and sexual distributions of patients enrolled in this study will reflect those of the general population in each region. All of the sites have experience recruiting minorities through a concentrated effort in communities that have high representation of ethnic and racial minorities and through advertisements in newspapers and radio stations whose readers/listeners are predominantly of specific racial or ethnic minorities.

### 10.1.2. Justification for exclusion of children

Children (those under age 21) will not be included in this study because insufficient data are available with CERC-501 in adults to judge potential risk in children.

### 10.1.3. Justification for exclusion of other vulnerable subjects

Because the aims of this study can be achieved with individuals who are not members of vulnerable groups (those who lack capacity for consent, prisoners, pregnant women etc.), we will not have to include any vulnerable subjects.

### 10.1.4. Safeguards for vulnerable populations

In order to protect vulnerable populations, they will be excluded from participation in this study. This includes pregnant women. In order to identify women who are pregnant, all women of childbearing potential will undergo a blood pregnancy test and it must be negative before subjects can continue in this study. If sexually active, female subjects must agree to use appropriate contraceptive measures for the duration of the study and for 1 month afterwards. Medically acceptable contraceptives include: (1) surgical sterilization (such as a tubal ligation or hysterectomy), (2) approved hormonal contraceptives (such as birth control pills, patches, implants or injections), (3) barrier methods (such as a condom or diaphragm) used with a spermicide, or (4) an intrauterine device (IUD). Contraceptive measures such as Plan B (TM), sold for emergency use after unprotected sex, are not acceptable methods for routine use. If subjects do become pregnant during this study or if they have unprotected sex, they will be instructed to inform the study physician immediately.

Participation of sexually active men has the potential to damage sperm, which could cause harm to a child that a subject might father while on this study. As a result, in order to protect subjects, sexually active male subjects will have to agree to use a medically acceptable form of birth control during and 1 month after this study in order to participate. Medically acceptable contraceptives include: (1) surgical sterilization (such as a vasectomy), or (2) a condom used with a spermicide. Contraceptive measures such as Plan B (TM), sold for emergency use after unprotected sex, are not acceptable methods for routine use. Subjects will be instructed to inform their partners of the potential for harm to an unborn child and that they should immediately inform the study doctor if pregnancy occurs.

### 10.1.5. Qualifications of Investigators

The overall principal investigator for this study is Dr. Andrew D. Krystal, MD, MS. He is highly experienced in serving as the principal investigator for single-site and multi-site clinical trials with patients with mood and anxiety spectrum disorders. This includes those employing fMRI scanning as will be carried out in the current study. Each one of the site PIs was selected because they too are highly experienced in serving as PIs for studies of patients with mood and anxiety spectrum disorders and they themselves or those at their site also have experience and expertise in carrying out the assessments that will be carried out in this study.

## 11. ANTICIPATED BENEFIT

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 which 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. However, the drug will not be available to subjects after the study concludes. The information that we get from this study may help us to better treat future patients with mood and anxiety disorders.

The available treatments for patients with mood and anxiety spectrum disorders have significant limitations. This study has the potential to take steps to address these

limitations by: 1) testing a promising new treatment for mood and anxiety spectrum disorders; 2) evaluating a potential target in the brain which could serve as the basis for development of additional new candidate compounds for the treatment of patients with mood and anxiety spectrum disorders; 3) establishing more expeditious methods for evaluating potential new therapies for patients with mood and anxiety spectrum disorders; and 4) specifically establishing methods for the development of new therapies targeting anhedonia, an important endpoint.

# 12. CLASSIFICATION OF RISK *(for the study as a whole)*

## 12.1. Risk Classification

This study is associated with more than minimal risk

## 12.2. Overall Risk and Benefit Consideration

The risks of the study are reasonable in relation to the anticipated benefit described in Section 11.

# 13. CONSENT DOCUMENTS AND PROCESS

## 13.1. Consent Procedures

All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing.

# 14. DATA AND SAFETY MONITORING

## 14.1. Data and Safety Monitor

Data and safety will be monitored by the NIMH Data and Safety Monitoring Board (DSMB). The NIMH DSMB functions include, among many, the assessment of interim data to determine whether the treatment interventions show a clear risk when compared with placebo. The main purpose of the NIMH DSMB is to assess safety of the interventions by determining whether an increased number of adverse events occur among study participants receiving the intervention compared to the participants receiving placebo treatment. Data will be reviewed every 4 months after the NIMH DSMB approval of the protocol and consent form.

## 14.2. Data and Safety Monitoring Plan

As described above, an independent DSMB will be established by NIMH that will meet regularly to review trial data in order to protect subject safety and data integrity. The

DSMB will meet prior to the start of the study to review the protocol and consent form. After the study commences, the DSMB will meet at least every 4 months.

Prior to each DSMB meeting that takes place after the study commences, the Principal Investigator will generate and circulate a full report to the NIMH DSMB members and the study biostatistician, summarizing recruitment, patient status and safety data for the study to be reviewed at that DSMB meeting including SAE reports. These reports will be prepared illustrating patient status and safety data. Patient status reports will summarize the number of subjects screened, eligible, randomized, treated, and completing the study -- by month and overall to-date. Detailed tables will be provided on reasons for ineligibility, and reasons for early termination of enrolled subjects. Safety reports will include summary data on all subjects experiencing the emergence or worsening of any adverse event (including abnormal laboratory values) during the course of a study, and during each study phase (screening, treatment, follow-up) -- each relative to the number of subjects in that phase. More detailed reports will provide information on the specific types of adverse events experienced, and the number that constituted Serious Adverse Events (SAEs) or led to termination from the study. Details of all SAEs occurring during a study will be provided to the DSMB for review of the nature and outcome of each SAE, as well as that all appropriate follow-up and required reporting was done in a timely manner. Tabulation and descriptions for protocol deviations will also be included in the report.

In addition to reviewing these data, the DSMB will also review procedures for ensuring that data are collected and analyzed per protocol privacy and that subject confidentiality is being maintained.

The DSMB will also carry out an overall assessment of the risk-benefit ratio of the study based on the available data. If there is a concern that the risks might outweigh the benefits, then enrollment will be suspended pending a more detailed review. However, if it is clear that the risks outweigh the benefits, then they will recommend permanently stopping the study.

# 15. QUALITY ASSURANCE

## 15.1. Program Management Committee

The Program Management Committee (PMC) will have primary responsibility for Quality Assurance Monitoring for this study. This committee will be chaired by the DCRI Project Leader and Co-Chaired by the FAST-MAS PI, Andrew Krystal, MD, MS. This committee will also include several teams: 1) Site Management – this includes a Clinical Research Associate (CRA), who, along with the Project Lead will have primary site management responsibility; 2) Data Management – this includes the Lead Biostatistician, and the Program Manager of electronic data system (EDC) and data management group; 3) Assessment/Signal Detection – Richard Keefe, Ph.D. will lead oversight of assessments and quality of data collection; 4) Magnetic Resonance Imaging (MRI) – Allen Song, Ph.D. will lead oversight for ensuring the quality of MRI methods, MRI data collection, and MRI data analysis.

## 15.2. Quality Assurance Plan

The PMC will meet regularly via teleconference (weekly from the beginning of study start up until the end of the study) to provide operational leadership to ensure that the study is implemented and managed efficiently and effectively and to oversee Quality Assurance. In this effort, they will work collaboratively with the NIMH Program Team. The Quality Assurance Plan includes the PMC carrying out the following key activities.

### 15.2.1. Monitoring and Managing Study Timelines

One of the most important responsibilities of the PMC will be to monitor study timelines by assessing whether key timeline milestones are being met by each of the sites. The PMC will be responsible for collecting ongoing data on whether milestones are being met. Dr. Krystal will then lead forming a strategy to address problems in meeting recruiting or other milestones and will work directly with the site PI in the implementation of that strategy.

### 15.2.2. Monitoring for Protocol Deviations

Another important function of the PMC will be to monitor for protocol deviations. These will be identified via review of electronic case report by the Site Management and Data Management teams. In addition each site will report all protocol deviations to the Site Management team. The PMC will monitor the protocol deviations and institute plans for addressing ongoing problems/deficiencies where needed.

### 15.2.3. Site Training

Site Training will take place in a number of steps. This will include training from the Site Management team on many study procedures, training from Dr. Keefe on completing subject assessments, training from Dr. Song on MRI procedures, and EDC training (see following section). The procedures to be carried out at the sites will be reviewed. This will also be the case for requirements for training of staff and documentation that those carrying out tasks for this study have the appropriate training documented.

Dr. Keefe will implement processes that he and his group have developed for rater training and data quality review to promote the collection of high quality data. These processes will be employed in this FAST-MAS study and are outlined below:

a. Develop a protocol-specific guide for the MINI, HAM-D, HAM-A and C-SSRS.
b. Provide all testing materials to the sites.
c. Pre-screen 2 raters per site, including assessing the sites for their rater training/credentials, extent of rater experience, and experience with clinical populations, and experience with the specific instruments used in this study.
d. Implement training procedures, including preparations for training, sending protocol-specific procedures, and providing access to clinical rating training videos for raters to review prior to the investigator's meetings.
e. Individual certification of at least one rater per site.
f. Review of testing procedures by Dr. Keefe via a presentation to the site investigators.
g. Either in-person or phone training and certification for all raters.

### 15.2.4. Site Electronic Data Capture (EDC) System Training

The Data Management team will conduct a web-based training on the EDC systems for site personnel. The site teams will enter data in the training environment during the training session, ask questions, and receive feedback on their work. In addition to the training session, the Data Management team will be available on a continuing basis to assist site personnel over the phone with questions related to remote data entry. Throughout the trial, for new study coordinators training will be delivered via a web-based program and telephone.

### 15.2.5. Research Ethics Training

The CC will ensure that all faculty, clinical staff having direct patient contact, and all personnel involved in site and data management involved in this study will meet human subjects training requirements.

### 15.2.6. Ongoing Site Management/Monitoring

Ongoing Management/Monitoring of each site will be carried out by the DCRI Site Management team which consists of the DCRI Project Leader, and a Clinical Research Associate (CRA). Site Management will include both on-site and in-house monitoring, routine communication with the site personnel, and collection of site-related data and documents. A particular focus will be to work with the site on effective recruiting strategies. The CRA will also provide ongoing monitoring of the recruitment at the site and report this to the FAST-MAS PI along with suggestions for improving enrollment. The CRA will carry out the same type of monitoring and trouble-shooting with protocol implementation and quality of adherence to study procedures. Dr. Keefe and the Signal Detection team will also play an important role in ongoing monitoring in order to maintain high quality in study assessments. Their activities will include:

a. Monitoring frequency of assessments carried out by raters.
b. Raters not administering assessments for 4 months will be required to complete refresher training.
c. Carrying out a phone certification process for replacement raters when there is site rater turnover.
d. Participate in biweekly conference calls to prepare for training meetings, review data collection processes, and address all study-related assessment issues as they arise over the course of the study.

# 16. ADVERSE EVENT AND UNANTICIPATED PROBLEM REPORTING

All adverse events serious and non-serious, expected and unexpected, related and unrelated to study drug will be documented and reported to the sponsor.

## 16.1. Plan for Assessment for Adverse Events

The Principal Investigator or a Co-Investigator at each site will monitor all subjects for adverse effects from the time of signature on the informed consent throughout their participation in the study. This will include carrying out a clinical interview for adverse events at each visit and administering the Patient Reported Inventory of Side Effects (PRISE) . In addition, a series of safety assessments will be carried out and the findings reviewed by the site PI or Co-I. These safety assessments will include: the Columbia Suicide Severity Rating Scale (CSSRS) (Posner, 2011), vital signs (height, weight, blood pressure, and pulse), physical examination, Beta-HCG serum pregnancy test, urine drug screen, complete blood count with differential, electrolytes, comprehensive metabolic panel including liver function tests, thyroid function tests, urinalysis, the Clinical Global Impression – Improvement (CGI-I), the Clinical Global Impression – Severity (CGI-S)and ECG and EEG ( obtained at baseline, at the end of 8 weeks of daily treatment with study drug). In addition, because gastritis was noted in some of the rats administered CERC-501 and some gastric-related side-effects were observed in the Phase 1 human studies, specific assessment for gastric adverse events will be carried out in this study. This will consist of collecting blood at baseline, 4 weeks, and 8 weeks of double-blind treatment from which gastrin and pepsinogen I/II levels will be determined.

These data will be used to assess for adverse event type, severity, expectedness, and causal relationship to study drug as defined below.

### 16.1.1. Adverse Events

An adverse event (AE) is defined as any untoward medical occurrence associated with the use of the study drug in a subject whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign, symptom or disease temporally associated with the use of the drug. Any change in clinical status, ECGs, laboratory tests, physical examinations, etc., that is considered clinically significant by the site investigators.

### 16.1.2. Serious Adverse Events (SAE)

An SAE is any adverse event where any of the following is the case:

- Is associated with death.
- Is life-threatening: Places the subject at immediate risk of death at the time of the event as it occurred. This includes suicide attempts. It does not include an AE that, had it occurred in a more severe form, might have caused death.
- Causes persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Is associated with inpatient hospitalization or prolongation of hospitalization.
- Is associated with a congenital anomaly or birth defect.
- Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition above. This determination is based on the opinion of either the investigator or sponsor (e.g., if either believes it is serious, it must be considered serious).

### 16.1.3. Assessment of Adverse Event Severity

The determination of adverse event severity will be made by the site PI or site Co-I. The severity of AEs will be graded using the following definitions:

- **Mild:** Participant is aware of symptoms or has minor findings, but tolerates them well and no or minimal intervention is required
- **Moderate**: Participant experiences enough symptoms or findings to require intervention
- **Severe:** Participant experiences symptoms or findings that require significant intervention or is life threatening (participant is at immediate risk of death from symptoms or findings that require aggressive medical management)

### 16.1.4. Assessment of Causal Relationship to Study Drug

The site PI or Co-Investigator will rate the causal relationship of any adverse event to the study drug using the following:

- **Not related**: There is not a reasonable causal relationship to the investigational product and the adverse event
- **Unlikely related:** An AE that has some possibility of being related to the study drug but this possibility is remote.
- **Possibly related**: An AE that has little or no relationship to the study drug and there exists a more likely alternative cause
- **Probably related**: An AE that is likely to be related to the administration of the study drug and an alternative cause less likely when compared to the study drug
- **Definitely Related**: An AE that has a strong temporal relationship to the study drug. AE will recur with continued or repeated use of the study drug, and another cause is unlikely or less likely

### 16.1.5. Expectedness of Adverse Event

Any adverse event, the specificity, nature, or severity of which is not consistent with the investigator brochure will be considered an unexpected AE. AEs that are expected based on the investigator brochure consist of peptic ulcer disease, gastritis, headache, diarrhea, nausea, vomiting, flushing, anxiety, upset stomach, and itchiness.

## 16.2. Documentation of Adverse Events

All events determined to be an AE, based on physical examination, laboratory findings, or other means, will be recorded. Each AE will be entered into the electronic data capture (EDC) system by the site PI or qualified designee. The investigator will provide date of onset and resolution, assessment of severity, intensity, and frequency, action(s) taken, changes in study drug dosing, relationship to study drug, and outcome. When additional relevant information related to an AE becomes available, the site PI or Co-I will record follow-up information according to the same process used for reporting the initial event as described above. They will follow all Adverse Events until resolution or they no longer believe that it is clinically significant. AEs ongoing at the time of the last dose of study drug will be followed up for as long as necessary to adequately evaluate the participant's safety, until they are resolved, or until it is determined by the investigator not to be clinically significant. All women of childbearing potential will be instructed to notify the Investigator if they become pregnant at any time during the study or within 30 days of last study evaluation. The pregnancy will be followed until delivery. Any

associated AEs or SAEs that occur to the mother or fetus/child will be recorded in the EDC system.

## 16.3. Reporting of Adverse Events

Any SAE entered into the EDC system will generate automatic notification to the DCRI Safety Surveillance Team. The DCRI Safety Surveillance Team will review all SAEs at the time they are reported and immediately report them to the Principal Investigator Dr. Krystal. Dr. Krystal or his designee will be responsible for notifying the NIMH Contract Officer's Representative (COR), NIMH DSMB and Cerecor within 1 – 2 business days of receipt of all site reported SAEs. Dr. Krystal or his designee will be responsible for forwarding written IND Safety reports (for those events identified as serious, related and unexpected) to the NIMH COR, NIMH DSMB, Cerecor, the site PIs, and the Duke IRB within 7 days (fatal/life threatening) and/or 15 days (for all other event determined to meet expedited reporting criteria).

The individual site principal investigators will be responsible for reporting SAEs occurring in subjects at their respective sites to their local Institutional Review Boards (IRB) per local IRB reporting requirements.

Dr. Krystal or his designee will be responsible for notifying the FDA in a written IND safety report of all SAEs determined to meet expedited reporting criteria within 7 days (for fatal/life threatening events) and/or 15 days (for all other event determined to meet expedited reporting criteria) per 21 CRF 312. 32. Sites will be responsible for submitting IND Safety Reports to their respective IRBs per their local requirements. Documentation will be retained by each investigative site.

Pregnancy occurring during a clinical investigation, although not considered a serious adverse event, must be reported to the DCRI within the same timelines as a serious adverse event. The pregnancy will be recorded on the appropriate pregnancy form and will be followed until final outcome. Any associated AEs or SAEs that occur to the mother or fetus/child will be recorded on the appropriate AE or SAE eCRF form and processed accordingly. The DCRI Safety Group will notify the NIMH COR, NIMH DSMB, and Cerecor of any pregnancy occurring during the study within the same timelines as a serious adverse event. The site principal investigators will be responsible for notifying the respective IRB of any pregnancies occurring at their sites.

In addition, Dr. Krystal or designee will promptly report to the NIMH COR, NIMH DSMB and the site principal investigators any unanticipated problems that are not adverse events within 1–2 business days of becoming aware of an unanticipated and life threatening problem or within 15 days of becoming aware of all other unanticipated problems. Unanticipated problems are any event that is: unexpected in nature, frequency or severity; related or possibly related to participation in the research; or suggest that the risk of harm to subjects is increased.

Expected or non-serious adverse events will be reported to the NIMH COR, NIMH DSMB prior to each Board meeting.

# 17. ALTERNATIVES TO PARTICIPATION

Subjects will be informed that they do not have to participate in this study to receive treatment for their condition. Instead of taking part in this study, subjects could receive an FDA approved treatment available by prescription to treat their mood or anxiety disorder.

- **Alternative treatments and procedures.** In some, cases patients will have failed or partially responded to conventional treatment. Their main alternative to participation will be treatment with another traditional treatment which includes antidepressant or anxiolytic medication, and/or psychotherapy, a newer form of treatment such as repetitive transcranial magnetic stimulation (rTMS), or a more intensive form a treatment like ECT. The risks and benefits of the alternatives are those of the possible treatments.

# 18. CONFLICT OF INTEREST

## 18.1. Distribution of NIH Guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

## 18.2. Conflict of Interest

All investigators will abide by the conflict-of-interest policies of their own institution.

## 18.3. Role of a Commercial Company or Sponsor

Cerecor will be providing study medication for this study. No personal identifiers of participants will be shared with Cerecor.

# 19. TECHNOLOGY TRANSFER

A Clinical Trial Support Agreement exists between Cerecor, who is providing study medication (CERC-501) for this study, and who owns the intellectual property for CERC-501, and Duke University. This Agreement includes a confidentiality provision – participant personal identifiers will not be shared with Cerecor.

# 20. RESEARCH AND TRAVEL COMPENSATION

All subjects will be compensated for time and research-related inconveniences. The amount of compensation will be prorated for parts of the study completed if subjects do not complete the study. Local site and institutional policy will be used to determine the amount of compensation.

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# APPENDICES

Appendix 1. Eligibility Checklist
Appendix 2. List of Prohibited Medications

## Appendix 1. Eligibility Checklist

- ☐ Age 21 through 65 years of age
- ☐ Meets DSM-IV TR diagnostic criteria for: Major Depressive Disorder, Bipolar I or II Depressed, Generalized Anxiety Disorder, Social Phobia, Panic Disorder, or Post Traumatic Stress Disorder
- ☐ Snaith-Hamilton Pleasure Scale (SHAPS) score $\geq 20$
- ☐ Reliable and willing to be available for the duration of the study
- ☐ Willing and able to give written informed consent to participate
- ☐ Able to understand and comply with instructions
- ☐ If female of childbearing potential, must agree to use dual methods of contraception and be willing and able to continue contraception for 6 weeks after the last dose of study drug. Females using oral contraception must have started using it at least 2 months prior to the Baseline Visit
- ☐ If male of childbearing potential, must have undergone surgical sterilization (such as a vasectomy) or agree to use a condom used with a spermicide during participation in the study and for 1 month afterward
- ☐ Not expected to require hospitalization during the course of the study
- ☐ No current/history of a psychotic disorder, current manic or mixed episode, autism spectrum disorders, mental retardation
- ☐ Did not meet DSMIV-TR criteria for substance abuse within the last 3 months or substance dependence within the last 6 months, excluding caffeine and/or nicotine
- ☐ No history of unstable or untreated serious medical condition based on physician evaluation, medical history, and screening laboratory testing.
- ☐ No active suicidal intent or plan, or history of attempt within the past 3 months based on physician evaluation and Columbia Suicide Severity Rating Scale (C-SSRS)
- ☐ No use of any antidepressant, antipsychotic, anxiolytic, anticonvulsant, mood stabilizing, muscle relaxant, centrally acting antihistaminergic, stimulant or insomnia medications within 5 half-lives of baseline or at any time during after baseline
- ☐ No use of any medication that is primarily metabolized by Cytochrome P450 2C8 within 14 days of baseline or at any time during the study. This includes: Cerivastatin, Paclitaxel, Repaglinide, Sorafenib, Rosiglitazone, Trimethoprim, Amodiaquine, Morphine, Amiodarone, Cabazitaxel, Carbemazepine, Chloroquine, Ibuprofen, Teprostinil, and Torsemide
- ☐ Without magnetic resonance imaging contraindications
- ☐ No positive urine drug screen at any time during the study
- ☐ No use of any investigational medication within 3 months prior to the start of this study or scheduled to receive an investigational drug other than CERC-501 during the course of this study
- ☐ No known hypersensitivity to CERC-501
- ☐ No history of severe allergies or multiple adverse drug reactions
- ☐ History of gastric disease including peptic ulcer disease or gastritis, upper GI bleeding, or any GI precancerous condition, clinically evident gastrointestinal complaints.
- ☐ Current use of a proton pump inhibitor or histamine 2 blocker or a history of chronic NSAID use.

- ☐ History of use of Salvia divinorum or use of Salvia divinorum at any time during the study.
- ☐ Without any other condition that in the opinion of the investigator would preclude participation in the study
- ☐ No smoking of cigarettes or use of any nicotine containing products within the last month or at any time during the study
- ☐ Not pregnant or lactating

## Appendix 2. List of Prohibited Medications

| Generic | Trade Name | | | | |
|---|---|---|---|---|---|
| **Benzodiazepines and Non-Benzodiazepine hypnotics** | | | | | |
| **Alprazolam** | Xanax | | | | |
| **Brotizolam** | | | | | |
| **Chlordiazepoxide HCl** | Librium | | | | |
| **Clonazepam** | Klonopin | Paxam | Rivotril | | |
| **Clorazepate dipotassium** | Apo-Clorazepate | Gen-Xene | Novo-Clopate | Tranxene(-SD) | |
| **Estazolam** | ProSom | | | | |
| **Flunitrazepam** | Rohypnol | | | | |
| **Flurazepam** | Dalmane | | | | |
| **Diazepam** | Valium | Diastat | Diazemuls | Antenex | Ducene |
| | Novo-Dipam | Vivol | Dizac | | |
| **Loprazolam** | | | | | |
| **Lorazepam** | Apo-Lorazepam | Ativan | Novo-Lorazem | Nu-Loraz | |
| **Lormetazepam** | | | | | |
| **Midazolam** | Aldurazyme | Versed | | | |
| **Nitrazepam** | Mogadon | | | | |
| **Oxazepam** | Novoxapam | Serax | | | |
| **Quazepam** | Doral | | | | |
| **Temazepam** | Restoril | | | | |
| **Triazolam** | Halcion | | | | |
| **Zaleplon** | Sonata | | | | |
| **Zolpidem** | Ambien | | | | |
| **Antidepressants** | | | | | |
| **Bupropion** | Wellbutrin (SR) | Zyban | | | |
| **Citalopram** | Celexa | Seropram | Cipramil | | |
| **Clomipramine** | Anafranil (SR) | Placil | | | |
| **Duloxetine** | Cymbalta | | | | |
| **Doxepine** | Sinequan | | | | |
| **Escitalopram** | Cipralex | Lexapro | | | |
| **Desipramine** | Norpramin | Pertofran(e) | | | |
| **Fluoxetine** | Prozac | Lovan | Erocap | Sarafem | Zactin |
| **Fluvoxamine** | Fevarin | Luvox | | | |
| **Imipramine** | Apo-Imipramine | Impril | Janimine | Norfranil | Novopramine |
| | Tipramine | Tofranil | Tofranil-PM | | |
| **Mianserine** | Tolvon | | | | |
| **Mirtazapine** | Remeron | | | | |
| **Nefazodone** | Dutonin | Serzone | | | |
| **Paroxetine** | Paxil (CR) | Aropax | Seroxat | Deroxat | |
| **Sertraline** | Zoloft | Lustral | | | |
| **Trazodone** | Desyrel | Dividose | Molipaxin | Trazon | Trialodine |
| **Protriptyline** | Triptil | Concordin | Vivactil | | |
| **Reboxetine** | Edronax | | | | |
| **Venlafaxine** | Effexor (TR) | | | | |

| Generic | Trade Name | | | | |
|---|---|---|---|---|---|
| **Centrally active sedating antihistamines** | | | | | |
| **Azelastine HCl** | Astelin | Optivar | | | |
| Dimenhydrinate | Dramamine | | | | |
| **Diphenhydramine** | Benadryl | Sominex | Nytol | Tylenol | |
| **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** | | | | | |
| **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 | | | | | |
| **Lithium** | Camcolit | Carbolith | Duralith | Eskalith | |
| | Lituane | Lithicarb | Lithizine | Lithobid | |
| | Lithonate | Lithotabs | Priadel | Cibalith-S | |
| **Melatonin** | | | | | |
| **Modafinil** | Provigil | | | | |
| **Phenelzine sulfate** | Nardil | | | | |
| **St. John's Wort** | | | | | |
| **Sodium oxybate** | Xyrem | | | | |
| **Tranyleypromine sulfate** | Parnate | | | | |
| **Stimulants** | | | | | |
| **Dextroamphetamine** | Dexedrine | | | | |
| **Laevoamphetamine (amphetamine sulfate)** | Alderall | Benzedrine | | | |
| **Methamphetamine** | Methedrone | Desoxyn | Gradumet | | |
| **Methylphenidate** | Ritalin | Metadate | Methylin | Concerta | |
| **Modafinil** | Provigil | | | | |
| **R-Modafinil** | Nuvigil | | | | |

| Generic | Trade Name | | | | |
|---|---|---|---|---|---|
| **Medications Primarily Metabolized by Cytochrome P450 2C8** | | | | | |
| **Cerivastatin** | | | | | |
| **Paclitaxel** | | | | | |
| **Repaglinide** | | | | | |
| **Sorafenib** | | | | | |
| **Rosigilatazone** | | | | | |
| **Trimethoprim** | | | | | |
| **Amodiaquine** | | | | | |
| **Morphine** | | | | | |
| **Cabazitaxel** | | | | | |
| **Amiodarone** | | | | | |
| **Carbemazepine** | | | | | |
| **Chloroquine** | | | | | |
| **Ibuprofen** | | | | | |
| **Teprostinil** | | | | | |
| **Torsemide** | | | | | |
| **Alpha-1 andAlpha-2-agonists / antagonists** | | | | | |
| **Clonidine** | Aruclonin | Atensina | Barclyd | Catapres | Catanidin |
| **Prazosin** | Minipress | | | | |
| **Methyldopa** | Aldoclor | Aldomet | | | |
| **Decongestants** | | | | | |
| **Ephedrine** | Amesec | Aminomal con Antiasmatico | Adalixin | Argyrophedrine | Apracur |
| **Phenylephrine** | Accuhist | Aclophen | Aerohist Plus | AH-chew | Ak-Dilate |
| **Pseudoephedrine** | 4 cold & cough-30 MG-Capsule | Acamol | Accuhist DM | Acetaminophe and Pseudoephedrine | Actagen |
| **Phenylpropanolamine (PPA)** | Acutrim | Alamine | Alcinal | Aler-Releaf | Alka-Seltzer plus cold |
| **Xylometazoline** | Aforinol | Amidrin | Imidin | Nasan | Nasben |
| **Respiratory Stimulants** | | | | | |
| **Levodopa** | Sinemet | | | | |
| **Pramipexole** | Mirapex | | | | |
| **Ropinorole** | Requip | | | | |
| **Rotigotine** | Neupro | | | | |
| **Anti-Seizure Medications** | | | | | |
| **Gabapentin** | Neurontin | | | | |
| **Valproic Acid** | Depakote | | | | |
| **Carbemazepine** | Tegretol | | | | |
| **Lamotrigine** | Lamictal | | | | |
| **Pregabalin** | Lyrica | | | | |
| **Opioids** | | | | | |
| **Buprenorphine** | Suboxone | | | | |
| **Dihydrocodeinone** | Vicodin | | | | |
| **Methadone** | Methadone | | | | |
| **Morphine** | Morphine | | | | |
| **Oxycodone** | Oxycontin | | | | |
| **Tramadol** | Ultram | | | | |
| **(Dextro)propoxyphene** | Darvon | | | | |
| **Meperidine** | Demerol | | | | |

| Generic | Trade Name | | | | |
|---|---|---|---|---|---|
| **Sympathicomimetics** ***(bronchodilators: e.g., albuterol, salmeterol, terbutaline; xanthines: e.g., theophylline)*** | | | | | |
| **Tertbutaline** | | | | | |
| **Salmeterol** | Serevent | | | | |
| **Clenbuterol** | Spiropent | | | | |
| **Ephedrine** | Bronkaid | | | | |
| **Levosalbutamol** | Xopenex | | | | |
| **Salbutamol/albuterol** | Proventil | Ventolin | | | |
| **Theophylline** | Elixophyllin | Norphyl | Theo-Dur | Uniphyl | |
| **Aminophylline** | Phyllocontin | Truphylline | Minomal R | | |