

Protocol

EMBARC

Establishing Moderators and Biosignatures of Antidepressant Response for Clinical Care

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1. Introduction and Purpose

1.1. Study Overview

The timely selection of the best treatment for patients with depression is critical to the goal of improving remission rates. Due to the biological heterogeneity and variable symptom presentation of depression, it is unlikely that a single clinical or biological marker can guide treatment selection. Rather, a biosignature developed from a systematic exploration of a group of several clinical and biological markers is more likely to be successful. Two types of biosignatures are needed to achieve improved outcomes: 1) biosignatures to maximize the selection of optimal treatment for individual patients at beginning of treatment (moderators) and 2) biosignatures to identify indicators of eventual outcomes early in treatment (mediators). This approach has great potential to personalize treatment and to begin to characterize the biology of treatment response.

We propose a randomized, placebo-controlled trial of a serotonin selective reuptake inhibitor sertraline (SERT) and placebo (PBO) for participants with major depressive disorder or dysthymic disorder (for purposes of inclusion in this study, collectively referred to as MDD) in which we will assess a comprehensive array of carefully selected clinical (e.g., anxious depression, early life trauma, gender) and biological (i.e., neuroimaging, electrophysiology and behavioral neuropsychiatric) moderators and mediators of outcome. Using innovative statistical approaches the identified moderators and mediators will then be used to develop a differential depression treatment response index (DTRI).

The proposed study is a randomized two-stage trial design with 400 MDD patients randomly assigned to one of two treatments under masked conditions (SERT vs. PBO; n=200 each). At the end of Stage 1, nonresponders to 8 weeks of SERT will be switched to bupropion (BUP), and nonresponders to PBO will be switched to SERT. This two-stage approach is similar to a Sequential Multiple Assignment Randomized Trial (SMART) design, which allows for the exploration of multiple treatments in individual patients.

This study will be conducted under the joint leadership from Columbia University and University of Texas Southwestern Medical Center together with Massachusetts General Hospital, University of Michigan, University of Pittsburgh and McLean Hospital. This study brings together researchers with extensive experience in conducting large clinical trials together with experts at the forefront of the neurobiology of depression, including: clinical trials (Trivedi, Weissman, McGrath, and Fava), neuroimaging (Phillips, Parsey, and Buckner), neurophysiology (Bruder and Pizzagalli), clinical predictors (Weissman, Trivedi, McGrath, Fava, Kurian, Morris, and Oquendo). This team will also be guided by a highly qualified group of biostatisticians (Petkova, Kraemer, Ogden, and Carmody), with specific expertise in emerging statistical methods to develop disease biomarkers using complex biomarker data.

This study will examine multiple carefully selected clinical and biological markers, using both existing state-of-the-art technologies as well as pioneering, innovative approaches. Evaluation of the usefulness of these markers in a carefully conducted clinical trial comparing an antidepressant to placebo will assist in developing a depression treatment response index (DTRI) to help clinicians match treatments to patients with MDD, resulting in timely selection of treatments best suited for individual patients and thus approaching personalized treatment.

1.2. Specific Aims

1.2.1. Moderator Aims

Aim 1: To identify baseline clinical, neuroimaging, neurophysiological, and behavioral moderators of differential treatment outcome (mean symptom change and tolerability) for sertraline (SERT, a serotonergic antidepressant) versus placebo (PBO) for the treatment of MDD. Symptom change will be measured using mean change from baseline in the 17-item Hamilton Rating Scale for Depression (HRSD₁₇).¹ Tolerability will be measured using the Frequency, Intensity, and Burden of Side Effects Rating (FIBSER) and the Treatment Emergent Symptom Scale (TESS).

Aim 1A: Clinical Moderators – To assess the extent to which baseline clinical variables – including anxious depression, melancholic depression, anger attacks, Axis II disorder, hypersomnia/fatigue, and atypical depression – will moderate treatment outcomes to SERT and PBO.

Aim 1B: Neuroimaging Moderators – To assess the extent to which the following baseline neuroimaging measures – fMRI response to two tasks (implicit emotion processing and regulation and reward processing) resting state connectivity, pulsed arterial spin labeling (PASL), diffusion tensor imaging (DTI), and structural MRI measures of cortical thickness – will differentially moderate treatment outcomes to SERT and PBO.

Aim 1C: Behavioral Phenotyping Moderators – To assess the extent to which the following baseline behavioral measures – pre-treatment psychomotor slowing, cognitive control, working memory performance, and reward responsiveness – will differentially moderate treatment outcomes to SERT and PBO.

Aim 1D: Electrophysiology Moderators – To assess the extent to which the following pretreatment electrophysiology measures – resting EEG alpha and theta power, source localization measures of theta activity in the rostral ACC, and loudness dependence of auditory evoked potentials (LDAEP) – will differentially moderate treatment outcomes to SERT and PBO.

1.2.2. Mediator Aims

Aim 2: To identify early phase (week 1) changes in neuroimaging, neurophysiological, and behavioral tasks as mediators of differential treatment outcomes (symptom change, tolerability) to SERT and PBO.

Aim 2B: Neuroimaging Mediators – To assess the extent to which changes in the following neuroimaging measures – fMRI response to two tasks (implicit emotion processing and regulation and reward processing), resting state connectivity, and PASL measures of regional cerebral blood flow from baseline to one week post-treatment – will differentially mediate treatment outcomes to SERT and PBO.

Aim 2C: Behavioral Phenotyping Mediators – To assess the extent to which changes in the following behavioral measures – psychomotor speed, cognitive control, working memory performance, and reward responsiveness from baseline to one week post-treatment – will differentially mediate treatment outcomes to SERT and PBO.

Aim 2D: Electrophysiology Mediators – To assess the extent to which changes in the following electrophysiology measures – resting EEG alpha and theta power, theta activity in the rostral ACC, and loudness dependence of auditory evoked potentials (LDAEP) from baseline to one week post-treatment – will differentially moderate treatment outcomes to SERT and PBO.

1.2.3. Main Treatment Effects Aim

Aim 3: To compare the 8-week outcomes of SERT vs. PBO using mixed model regression analysis to maximize power to discriminate treatment efficacy differences.

1.2.4. Methodological Innovation Aims

Aim 4: To develop methods to examine the complex interactions of clinical, neuroimaging, neurophysiology, and behavioral moderators and mediators of differential treatment outcomes (mean symptom change, tolerability) for SERT and PBO. We provide an initial example of four possible complex interaction aims as illustrations. Secondary analyses will be conducted to explore further interactions.

Example: To assess the interaction between fatigue, psychomotor retardation, baseline imaging measures of activity and connectivity in reward processing neural systems, and task-specific reaction times to differentially moderate SERT vs. PBO treatment outcomes.

Aim 5: To develop a depression differential treatment response index (DTRI) that integrates moderators across clinical and biological variables. In developing markers for inclusion in the DTRI, we propose that any individual marker must independently or interactively predict treatment response. Based on available

literature, a model that can provide 20% improvement using DTRI selected treatments over the currently used nonspecific treatment selection approach could signify a clinically meaningful treatment prediction.⁵⁵

Aim 6: To develop a composite scale of treatment acceptability (which incorporates depressive symptom outcomes, treatment tolerability, and the patient's functional status) and compare 8-week outcomes for SERT and PBO.

Aim 7: To explore the clinical, neuroimaging, electrophysiology, and behavioral phenotype biosignatures of differential treatment outcome identified for first step responders, among second step treatment responders (i.e., first step nonresponders).

1.3. Study Risks/Potential Benefits

The largest clinical trial of MDD ever conducted, STAR*D, indicates that 2/3 of patients treated with a first step antidepressant do not achieve remission of symptoms.² Furthermore, successive treatment steps lead to diminishing remission rates.³ Additionally, a large number of patients discontinue treatment prematurely due to side effects.² The trial and error method currently used in clinical practice often leads to repeated failures before an effective treatment is identified.

Given this relative ineffectiveness of treatments for depression and resulting practice of trial and error multiple treatment step algorithms, there is an urgent need to identify factors that can be used to personalize treatment (i.e., markers that maximize effectiveness and minimize the risk for toxicity). Similar efforts have been successfully used in treating other medical illnesses.⁴⁻⁹ The National Institute of Mental Health's (NIMH) RFA-MH-10-040 recognizes the need to develop personalized treatments for individuals with MDD and "discover panels of promising potential biomarkers (i.e., biosignatures) that are predictive of treatment outcomes." This study aims to discover novel biosignatures that are predictive of treatment outcome for individual patients with MDD, defined from clinical and biological markers taken from several domains.

The inclusion of a placebo control group (PBO) is essential to differentiate between nonspecific changes that are due to environmental or psychological factors versus those that are the result of an active treatment. Furthermore, including a PBO assists in identifying changes, which occur in the earlier stages of treatment that mediate treatment response. It will also allow for the identification of baseline predictors of a treatment response, although identification of moderators or mediators that are specific to a treatment requires that an alternative active treatment be part of the design. In addition, it may allow for the identification of markers that indicate that recovery from the current depressive episode is likely without an active antidepressant medication.

While the risks involved include the possibility of being randomized to receive placebo, the results from this study may provide significant benefits to future patients with depression (i.e., personalizing the selection of treatments). Recent evidence also indicates that some depressed patients may not benefit from antidepressant medications, and this study may assist in identifying those individuals early in the course of treatment. Lastly, the study treatments may provide new treatment strategies for depression, and information gained from this research could lead to better care for patients with MDD.

2. Background

2.1. The Purpose of this Research

2.1.1. Burden of MDD:

Depressive disorders are one of the leading causes of disability-adjusted life years, worldwide.¹⁰ According to the World Health Organization (WHO) depressive disorders are the fourth leading cause of disability-adjusted life years, worldwide and by 2020 are estimated to be second only to ischemic heart disease.¹¹ This debilitating illness will affect up to 16.2% of Americans at some point in their lifetime.¹²

2.1.2. Low Remission Rates:

The goal of treatment for those suffering from MDD is remission (i.e., the absence of depressive symptoms),¹³ and yet, two-thirds of patients treated with a first step antidepressant do not achieve remission of symptoms⁴ and successive treatment steps lead to diminishing remission rates.³ Moreover, large numbers of patients either discontinue treatment prematurely due to side effects, or become discouraged and drop out of treatment altogether.⁴ Advances in antidepressant drug treatment discovery have been very limited, and simply waiting for the next big breakthrough is certainly not a sure bet.¹⁴ In fact, disease heterogeneity¹⁵ is likely to complicate pharmacological approaches going forward, even in face of novel drug development. Establishing methods for identifying which patient is likely to respond (and have fewer side effects) to current treatment options is an essential priority for our field.¹⁶

2.1.3. Personalized Treatment:

Studies of cardiovascular disease, asthma, breast cancer, lung cancer, multiple sclerosis, macular degeneration and other medical illnesses have been successful in identifying important moderators of treatment response, leading to the development of personalized treatment approaches.⁴⁻⁹ Given the ineffectiveness of first-step treatments for MDD, there is a clear and urgent need to identify factors that can be used to individualize treatment (i.e., markers that maximize effectiveness and minimize risk for toxicity).^{16, 17} Personalizing treatments for MDD is likely accomplished by utilizing an array of clinical and biological markers that differentiate treatment selection for individual patients.

2.2. Current Standard of Care

The current standard of care for treating patients with MDD is an antidepressant medication (Olfson et al). However, as stated above, remission rates for patients treated with a first step antidepressant are low⁴, leaving the vast majority with residual symptoms. In addition, a recent analysis indicates that antidepressant medications are unlikely to differentiate from placebo response in patients with mild to moderate depression (Fourier et al).

2.3. How Biomarkers May Improve the Standard of Care

2.3.1. Goals of the Current Study:

This study embarks on a novel initiative, which compares an active antidepressant treatment with placebo, as well as a second step switch for nonresponders to either treatment, and for the first time assesses clinical, neuroimaging, neurophysiological, behavioral, and genetic/plasma moderators (and the interrelationship of each group) of treatment outcome. It is our intention to develop a predictive model that integrates the best current evidence to provide personalized treatment, which can be further modified as new evidence emerges.

2.3.2. Significance and Importance:

This study represents, to the best of our knowledge, the first attempt to construct a comprehensive biosignature for the personalized care of MDD. While necessary to choose two or more distinct treatments to construct the personalized care model, the study has greater implications for future studies because: 1) it details, for the first time, how to collect, process, and analyze a wide array of clinical and biological markers in the context of a clinical trial, which can be extended to other competing treatments and disorders; 2) it has the potential to produce, for the first time, a clinically useful metric to guide treatment; 3) uses a systematic process to choose the best clinical, neurobiological and neurophysiological moderator candidates, a process that can be extended to other psychiatric disorders; and 4) the extensive clinical and biomarker database can be used as a national resource for other investigators in the future as the field develops to identify other potential moderators.

2.4. Status of Drugs Included in this Study

Both active antidepressants to be utilized in this study (sertraline and bupropion) have an FDA approved indication for MDD. Furthermore, the dosing guidelines and delivery of treatment in this study follow established guidelines.

2.5. Biomarker Research in Depression

2.5.1. Rationale for Selection of Markers for EMBARC:

Most previous research has evaluated clinical and biological markers for treatment response for individual treatments. While this research provides indicators of those likely to respond to an active treatment, the context of these studies does not allow us to determine which, if any, of these markers is specific to active treatment, and which are nonspecific markers of placebo response. The clinical and biological markers that we have selected for inclusion in this study are based on a thorough review of the relevant literature. The major advantage of our current approach stems from the ability to account for placebo response as well as evaluate a myriad of clinical and biological markers and their interactions in a single study. In addition, this study will allow us to establish standard procedures for the collection, processing, data management, and analysis of a combination of biological and clinical markers. As such, this study will set a precedent for the field for future definitive biosignature endeavors.

2.5.2. Identifying Candidate Clinical and Biological Markers:

Three approaches were used by our team to identify potential clinical and biological candidates, and prioritize the use of established markers that have been shown to be associated with treatment outcome, though whether as predictors or as moderators has not been adequately investigated. Clinical markers were identified based on best available evidence from the literature, including results from the NIMH-sponsored, multicenter Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study,^{2, 18, 19} with a specific focus on differential treatment outcomes. We carried out a systematic review of the literature on clinical and biological markers of antidepressant response in MDD, including potential neurobiological moderators and mediators based on the best current models of depression.²⁰⁻²⁴ Finally, we have included biological measures for which we have unpublished preliminary data. Through this process, we determined that the most promising clinical and biological marker candidates to assess are:

1. Comprehensive clinical phenotype;
2. Magnetic resonance imaging using MRI measures of cortical structure;
3. Diffusion tensor imaging (DTI) to assess cortical white matter tract integrity;
4. Functional magnetic resonance imaging (fMRI) using multiple tasks to assess brain activation patterns to both emotional conflict and reward-dependent learning tasks;
5. Quantitative electroencephalography (qEEG) to assess cortical and subcortical brain activation patterns, using advanced EEG processing techniques;
6. Cortical evoked EEG potentials;
7. Behavioral neuropsychological tasks to include reaction time, motor processing speed, etc.;
8. DNA, mRNA, and plasma protein samples, before, during and after treatment, to be deposited in the NIMH-designated repositories for future research.

2.5.3. Assessing Biomarkers Collected:

Biomarkers will be assessed in a medication-free state prior to treatment, after a minimum of a three week washout of psychotropic medication (five weeks for fluoxetine). All biomarker assessments (except clinical phenotype and DTI) will be repeated one week after beginning study medication to assess early biomarkers of treatment response (potential treatment mediators). Participants experiencing a response to study medication at week 8 will be continued for 8 additional weeks to assess the stability and completeness of this response. Participants not experiencing a response will be crossed over, under double-blind conditions to an alternate medication treatment: placebo non-responders will be given sertraline and sertraline non-responders will be given bupropion. We plan to analyze this broad array of moderator and mediator biomarkers individually and then assess how these markers are related to each other with innovative statistical methods within a systematic theoretical framework that will be developed through the work of this group. The findings will then be integrated to form the differential depression treatment response index (DTRI). Since it is critical to minimize placebo response in clinical trials, participants in this study will be required to have a history of early onset chronic or recurrent MDD.

2.6. Preliminary studies

2.6.1. Prior Clinical Predictor Trials:

Typically, depression studies identify predictors that explain only a small percentage of the systematic variance, often less than 10%.²⁵⁻²⁷ STAR*D provided clinical information about treatment response throughout a number of treatment levels. In general, for level 1 treatment with the SSRI citalopram, Trivedi and colleagues found that comorbid psychiatric and/or medical conditions, greater initial depressive symptom severity, and lower socioeconomic status (SES) predicted a lower likelihood for achieving remission.² Nonresponders were then randomized to various different pharmacological and psychological treatments in level 2. Rush and colleagues assessed clinical moderators of treatment response among these level 2 treatments. Unfortunately, when examined, no significant clinical moderators (i.e., variables that differentially predicted treatment response to an individual medication) were identified.²⁸

STAR*D also assessed some specific phenotypes that were associated with treatment response. For example, despite the fact that the DSM-IV-TR does not currently include a specifier for depression with anxious features, a number of studies have described this common correlate in the literature.^{29, 30} Based on research from STAR*D, 46% of patients met baseline criteria for anxious depression.²⁹ Patients with MDD and comorbid anxiety are more likely to suffer increased disease burden and longer duration of illness.^{29, 30} Furthermore, most studies, including STAR*D, have shown that patients suffering from anxious depression are less likely to respond and remit following antidepressant treatment than are those with nonanxious depression.³¹

2.6.2. Imaging Predictors/Moderators:

Structural and functional magnetic resonance imaging (fMRI) may be used to help predict treatment response. The poorer responses to treatment observed in patients with comorbid anxiety may be understood through application of recent fMRI paradigms to assess key amygdala-ACC circuitry implicated in implicit emotion processing and regulation. Our collaborative group^{32, 33} has reported that patients with generalized anxiety disorder (GAD) differ significantly from healthy controls in patterns of ACC activity on tasks of implicit emotion processing and regulation, and that patterns of ACC activity to such tasks in patients with MDD predict future outcome to antidepressant treatment. As described below, patients with MDD comorbid with GAD are similar to GAD patients without comorbid depression on these tasks and differ from major depressives without GAD. Employment of an implicit emotion processing and regulation tasks with fMRI is therefore one method for assessing ACC function, that relates to anxiety in major depression, and that could be useful pre-treatment signature for predicting and moderating treatment response in MDD. Our collaborative group has also reported reduced ventral striatal activity to reward in patients with MDD relative to healthy controls³⁴ that may in turn be associated with anhedonia in MDD. Employment of a reward task with fMRI is therefore a method for assessing ventral striatal – and reward circuitry - function, that in turn has potential to be a useful pre-treatment signature for predicting and moderating treatment response in MDD. We will also employ resting state connectivity, pulsed arterial spin labeling (PASL), and diffusion tensor imaging (DTI) to obtain measures of low frequency BOLD fluctuations (LFBF) at rest, regional cerebral blood flow, and white matter integrity, respectively, in our key emotion processing, regulation, and reward neural circuitries of interest. In addition, structural MRI will measure cortical thickness, which may also be useful in pre-treatment signatures predicting and/or moderating treatment response in MDD.

2.6.3. Electrophysiology Predictors/Moderators:

We aim to assess pretreatment EEG measures of brain activity to differentially predict antidepressant response. Pretreatment alpha and theta power have been shown to be associated with antidepressant response.³⁵⁻³⁹ Furthermore, compelling evidence finds a significant association between increased pretreatment resting theta activity in the rostral ACC and antidepressant response (including SSRIs).⁴⁰⁻⁴² In addition, findings suggest that loudness dependence of auditory evoked potential (LDAEP) correlates with serotonergic treatment response. Specifically, prior studies have shown that patients with higher pretreatment loudness dependence – assumed to reflect blunted serotonergic activity – responded well to SSRI, while responders to the selective noradrenergic reuptake inhibitor, reboxetine, had reduced pretreatment loudness dependence.^{38, 43-45} Similarly, response to the SSRI citalopram was associated with strong LDAEP, while response to reboxetine was associated with weak LDAEP.⁴³

2.6.4. Behavioral Predictors/Moderators:

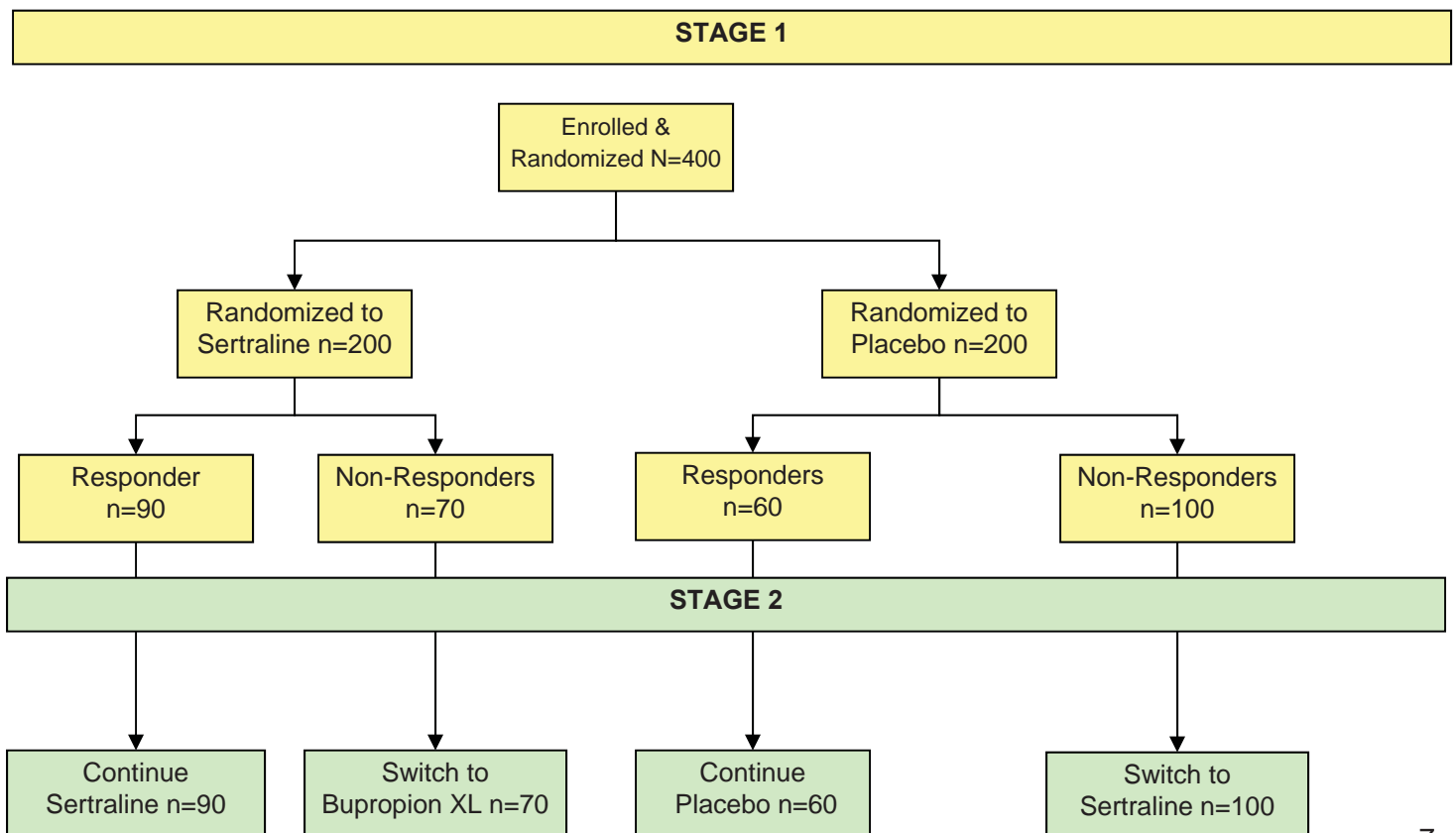
We will measure reaction time (interference and post-error adjustments) during performance of the implicit emotion processing and regulation and reward processing neuroimaging tasks as key behavioral measures associated with neuroimaging measures of ACC- and ventral striatal-centered neural circuitry that in turn have potential to be useful pre-treatment signatures predicting and/or moderating treatment response. In addition, we will measure pre-treatment psychomotor slowing, cognitive control (particularly post-error behavioral adjustments), working memory performance, and reward responsiveness, which have preliminary evidence that they may be able to differentially predict treatment response.

3. Concise Summary of Project

3.1. Study Design

The current study is designed to identify biomarkers for the prediction of differential treatment outcomes between the SSRI antidepressant sertraline (SERT) and placebo (PBO) in a randomized trial for patients with MDD. In addition, a second stage will collect data to explore moderators and mediators of treatment outcomes between pharmacologically distinct active treatment arms: sertraline (SERT), a serotonergic antidepressant or bupropion (BUP), a nonserotonergic antidepressant. To reduce biologic heterogeneity, we will only enroll patients with early onset of DSM IV MDD (before age 30) because these criteria in probands have been shown to be associated with increased familial loading. Patients will also have chronic or recurrent MDD with 2 or more recurrences (including current episode). Additionally, patients will be required to have a current symptom severity score of 14 or more on the Quick Inventory of Depressive Symptomatology – Self Report (QIDS-SR), both at study screening and at the randomization (baseline) visit. In the first stage, patients will receive an 8-week course of treatment in one of the two study arms. As part of the SMART design, patients that have not achieved response at the end of 8 weeks to their stage one treatment, defined by not being at least “much improved” on the Clinical Global Improvement scale (CGI), will be switched to Stage 2 treatment.

Figure 1. Study Flow Diagram



3.2. Study Drug(s)

Dosing will follow the schedule shown below. Dose is raised only if tolerated and the patient has not responded. All medication will be administered in the morning as a single dose of identically matched capsules. The extended release form of BUP (i.e., bupropion XL) will be used to enhance adherence with single daily dosing, which is made safer by using the XL formulation.

| Study Week, Stage 1 | SERT | PBO |
|----------------------------------|-------------------------------|-------------------------------|
| 1 (Days 1-3) | 50 mg | 1 cap |
| 1 (Days 4-7) | 100 mg | 2 caps |
| 2 | 100 mg | 2 caps |
| 3 | 150 mg | 3 caps |
| 4-8 | 200 mg | 4 caps |
| Study Week, Stage 2 [†] | SERT (for PBO non-responders) | BUP (for SERT non-responders) |
| 9 | 50 mg | 150 mg |
| 10 | 100 mg | 300 mg |
| 11 | 150 mg | 450 mg |
| 12-16 | 200 mg | 450 mg [‡] |

[†] To preserve blinding in Stage 2, we will alter the dosing schedule such that PBO non-responders will match SERT non-responders.

[‡] NOTE: The fourth capsule for patients receiving BUP will contain placebo, however for patients receiving SERT it will contain sertraline 50mg.

Both to keep patients in the study and to approximate “real world” practice, study medication will be adjusted depending on clinical judgment in consultation with patient wishes. The maximum dose will be a target dose in patients who tolerate the medication but have not responded.

All participants who do not meet response criteria during Stage 1 treatment will be crossed, double-masked to the other treatment for 8 weeks with all ratings collected at the equivalent time points during treatment as in the initial trial. Patients unable to tolerate Stage 1 treatment will be exited from the study. This will help optimize power to explore predictive biomarkers both for tolerability and efficacy of SERT and BUP, and to allow exploratory examination of a group not responding to either medication.

3.3. Mechanism of Action for Study Drugs

SERT: Based on a recent analysis, treatment with antidepressant medications has steadily increased over the past decade.⁴⁸ Of these, the most commonly prescribed agents are the SSRIs, particularly since all but one of the SSRIs are available in generic formulations. Based on results from STAR*D and other large effectiveness trials, the SSRIs and other newer antidepressant agents are modestly successful at achieving remission, and are mechanistically designed to treat the monoaminergic model of MDD.

The immediate action of the SSRIs is to inhibit the neuronal reuptake of serotonin by blocking the serotonin transporter.^{49, 50} The SSRI paroxetine appears to have mild noradrenergic reuptake inhibition at high

doses,^{49, 50} while the SSRI fluoxetine, particularly its R stereo-isomer, has mild 5HT_{2A} and 5HT_{2C} antagonist activity, which may explain the increase in norepinephrine and dopamine in the prefrontal cortex of animals treated with fluoxetine,⁵¹ as well as mild noradrenergic reuptake inhibition.^{49, 51} The SSRIs have minimal or no affinity for cholinergic receptors, with the exception of paroxetine which is a weak cholinergic receptor antagonist.^{49, 51} The effects of SSRIs on various histaminergic and α -adrenergic receptors are negligible.^{49, 51} While citalopram and escitalopram are probably the most selective SSRIs from a pharmacological standpoint, the FDA reports increasing concerns that optimal citalopram dosing can lead to cardiovascular risks and escitalopram is not available in a generic formulation. Hence we selected SERT, which is an SSRI available in a generic formulation and was used in Level 2 of STAR*D, as one of our treatment arms.

PBO: A placebo control will be used to establish the frequency and magnitude of changes in clinical and biological marker endpoints that may occur in the absence of pharmacologically active treatment. Thus, the inclusion of a PBO group will provide valuable information to characterize treatment specific biological and clinical markers. Lastly, PBO nonresponders provide an enriched sample to further explore biomarkers of SERT response in a group of participants whose PBO response is likely less, and thus is biologically more homogenous.

BUP: The mechanism of action of bupropion (BUP) is unclear but has been postulated to be primarily related to the inhibition of the reuptake of both dopamine and norepinephrine, but it seems fair to characterize it as nonserotonergic and thus distinct from SERT.⁵² BUP and its metabolites have shown to be able to inhibit striatal uptake of the selective DAT-binding radioligand (11)C-beta CIT-FE in vivo achieving DAT occupancy ranging from approximately 14%⁵³ to 26%^{54, 55} at therapeutic doses. BUP has also been reported to have mild affinity for the norepinephrine transporter, although some researchers have argued that the effect of BUP on norepinephrine is primarily through an increase in presynaptic norepinephrine release.⁵⁶ Regardless of the exact mechanism, the overall effect of BUP appears to be a dose-dependent increase in brain extra cellular dopamine and norepinephrine concentrations.⁵⁶⁻⁵⁸ In addition, BUP also appears to noncompetitively inhibit the α 3 β 2, α 3 β 4-, and α 4 β 2- nicotinic acetyl cholinergic receptors in vitro.⁵⁹ At least four stereo isomers of two major metabolites of BUP (S,S-, R,R hydroxybupropion, R,R-threohydrobupropion) were also reported to noncompetitively inhibit α 3 β 4- nicotinic acetyl cholinergic receptors in vitro, although not as potently as BUP.⁵⁹ Because its mechanism of action does not involve serotonin but does appear to involve catecholamines, we have selected BUP as the comparative active treatment arm in Stage 2.

3.4. Study Outcome

The research goal is to develop data to understand whether a wide range of potential biological markers of treatment response to antidepressant medication have predictive value in selecting medication treatment or show early indications of response to such treatment.

3.5. Subjects

3.5.1. Sample:

Feasibility Sample

Of note, citalopram was the original SSRI chosen for this study. However, given recent FDA labeling changes due to increased cardiovascular risks, the EMBARC Steering Committee decided to switch to SERT for the evaluable sample. However, recruitment of a small subgroup of subjects (approximately 3 per site) for study feasibility assessments had been initiated prior to the FDA labeling changes for citalopram. As such, the EMBARC Steering Committee decided to continue to utilize citalopram for the feasibility sample, and adhere to the FDA dosing guidelines. In addition, for the feasibility sample, prospective participants will have a baseline ECG and review of personal and family cardiac history. Participants with a baseline QT_c interval of > 460 msec or requiring concomitant medications known to increase the QT_c interval will be excluded. In vulnerable participants, such as patients over the age of 60 with baseline cardiac disease, an ECG will be done ~ 5 days after a dose increase to 40 mg and medication will be discontinued if the QT_c interval is > 500 msec. Appropriately, two separate consent forms will be utilized, one for the feasibility sample and a second for the evaluable sample.

Biosignatures Sample

For the first stage, 400 depressed outpatients from 4 clinical sites will be randomized into one of the two treatment arms (SERT: 200, PBO: 200). Adults, age 18-65 will be included (see Section 7 for a detailed list of inclusion and exclusion criteria). All patients will meet DSM-IV TR⁶⁰ criteria for nonpsychotic MDD that is established using the SCID-I, to ensure a careful documentation of the phenotype for each patient (this includes recording and entering all symptoms of MDD for each patient), as well as documenting age of onset, number of episodes, and other manifestations of chronicity which have been associated with medication treatment response.⁶¹

3.5.1.1. Stage 1 Treatment Phase:

Outcomes will be assessed in terms of response rates (HRSD₁₇) and tolerability (FIBSER) at the end of Stage 1 (up to 8 weeks of treatment). Treatment will be guided by clinician-rated symptom measures using the Structured Interview Guide for the Hamilton Rating Scale for Depression Scale (HRSD) -32 item (SIGH_D₃₂) and global side effects measures (the Frequency, Intensity, and Burden of Side Effects Rating⁶² or FIBSER) obtained at each treatment visit. Medication treatment visits will occur at baseline and at weeks 1, 2, 3, 4, 6, and 8 to ensure delivery of appropriate, vigorous, and tolerable pharmacotherapy. Those with unacceptable/intolerable side effects despite dose reduction will be removed from the study and given post study treatment. Patients defined as nonresponders (CGI less than “much improved”) will enter Stage 2 Treatment Phase.

3.5.1.2. Stage 2 Treatment Phase:

Nonresponding patients will be switched to Stage 2 treatment, under double blind conditions. PBO nonresponders will receive SERT, and SERT nonresponders will receive BUP. Again, treatment will be guided by symptom measures (the HRSD) and global side effects measures (FIBSER) obtained at each treatment visit. Visit frequency, dose escalation, and treatment monitoring will follow the same procedures used in Stage 1, response and remission will be defined as a 50% improvement and HRSD₁₇ ≤ 7, respectively.

3.5.1.3. Why include Stage 2:

Including the Stage 2 treatment phase results in a design similar to a SMART design.⁶³ This SMART-like design is to switch nonresponders to a treatment they did not receive in Stage 1. Consistent biosignatures in both phases may be suggestive of the strongest biologic correlates of treatment response.

3.5.1.4. Naturalistic Follow-up Phase:

At the end of the treatment phase, patients will be asked to continue as part of a naturalistic follow-up phase, for up to two years. The purpose of the follow-up phase is to gather data on patients that may help inform researchers about their disease course and potential relapses and recurrences of their depressive

disorder. Furthermore, researchers will be able to assess the impact of clinical and biological markers on longer-term treatment outcomes. During the follow-up phase, patients will return to seeing their primary care physician or psychiatrist (or if they do not have one, they will be given referral information) and are not restricted in terms of the medication they can take. During the follow-up phase, patients will be asked to provide information about any treatment they are currently receiving, and their depressive and associated symptoms at the following time intervals: 6 months, 9 months, 12 months, 15 months, 18 months, 21 months, and 24 months. These visits may take place in person, over the phone, or online. For more details regarding the specific assessments given to patients at follow-up, see Section 4.

3.5.1.5. Patient Flow (see Figure 1):

Of the 200 patients randomized to SERT, about 90 will respond to treatment, and roughly 70 will be nonresponders (we estimate that the remaining 40 will be dropouts). For those randomized to PBO, we estimate about 60 responders, and 100 nonresponders. Patient flow estimates are based on our STAR*D experience, and from prior placebo-controlled antidepressant treatment trials.

3.5.2. Local Subjects:

At each Clinical Site, we expect to randomize up to 150 subjects into the study to reach the study's national target of 400 subjects.

3.5.3. Conditions for Study Exit:

A patient will be exited from the study prior to completion for the following reasons:

- Physician Discretion: Participant deemed a potential danger to self or others.
- Patients who develop severe acute or chronic medical or psychiatric conditions that, in the judgment of the investigator, would put the patient at risk for continuing will be discontinued early.
- Participant Choice: Participants may choose not to continue in the study. CRCs will work with the physician to understand reasons for this choice and offer reasonable support and treatment alternatives to the participant. However, some participants will choose to discontinue participation.
- Participant Lost to Follow-up: If a participant has missed several visits and the CRC is unable to contact the participant after multiple attempts at different times of day, the participant is considered "Lost to Follow-up."
- Patients unwilling or unable to comply with the baseline biomarker assessments will be exited from the study.
- Administrative Error: Patients who do not meet all study inclusion or exclusion criteria may enter the study in error. Once this is learned, the participant will need to be exited from the study.
- Patients who are hospitalized for worsening of depression and/or suicidal ideation since hospitalization is likely to lead to treatment changes.
- Symptoms have emerged that leads the clinician to make a change in the primary diagnosis.

When a patient withdraws from the study, the reason(s) for withdrawal will be recorded by the investigator on the case report form (CRF). Whenever possible, all patients who withdraw from the study prematurely will undergo all final efficacy and safety assessments. Patients who fail to return for final assessments will be contacted by the study site by telephone (at least 2 documented follow-up telephone calls), and if necessary, by up to 2 registered letters, in an attempt to have them comply with the protocol.

It is vital to obtain follow-up data on any patient withdrawn because of an AE or SAE. In any case, every effort must be made to undertake protocol-specified, follow-up procedures.

Patients who withdraw, are withdrawn by the study physician, exit per the protocol, or otherwise do not complete the entire trial will not be replaced.

4. Study Procedures

For individuals meeting study inclusion criteria from screening and potentially interested in participating, the study psychiatrist will take a psychiatric history, review eligibility, explain the study procedures, including risks, benefits, and alternatives, answer questions and obtain written informed consent. A research psychiatrist or psychologist will administer the SCID-IV-I, HRSD₃₂ (from which the HRSD₁₇, the principal study outcome measure can be extracted), and CGI. The research assistant will draw blood to test for hematology, liver, thyroid and kidney function, and other general health measures, measure the participant's height, weight, and waist circumference, and obtain a urine specimen for urinalysis and drug screen (as well as a pregnancy test for fecund women). A physician will perform a physical examination. The patient will complete all required self-rating instruments (see Table of Assessments below for details) and schedule for EEG and MRI scanning will be determined based on the participant's availability. The study psychiatrist will review clinical laboratory results to evaluate eligibility for continued participation. Patients not eligible to participate based on these results will be offered a referral for clinical treatment.

For patients who are currently taking antidepressant medications and not doing well, there are two possibilities. At sites with IRB approval to do so, they may be tapered off their medication under close clinical supervision to wash out for study entry. At sites without such IRB-approved washout procedures, they will be referred back to their primary care physicians (PCPs), to have their medications tapered as clinically indicated depending on the drug they are on, with more prolonged taper in the case of medications likely to induce withdrawal symptoms. Participants will need to be antidepressant medication-free for at least 21 days prior to collection of imaging and other biomarker data (five weeks for fluoxetine).

After the drug-free period, eligible participants will undergo baseline visit, which includes: imaging, electrophysiologic and neuropsychological testing, and blood sampling procedures to obtain study biomarker data. Multiple sessions may be scheduled, one for EEG and biobehavioral assessment (~ 2.5 hours, see Section 4.4 for details) and one for the MRI procedures (~ 2- hours, see Section 4.5 for details) to gather this data. Also at baseline, the study psychiatrist will review continued study eligibility and a research psychologist or psychiatrist will complete the required rating instruments and participants will complete self-rating instruments. Patients still meeting study criteria will be randomized to: sertraline (SERT) or placebo (PBO) under double-masked conditions. The EEG, biobehavioral assessment, MRI, and blood collection procedures will be repeated after 1 week of randomized double-masked treatment.

Study visits will be at weeks 1, 2, 3, 4, 6 & 8 for Stage 1 participants and weeks 9 (only for participants crossing over to a new treatment for Stage 2), 10, 12, 14 & 16 for Stage 2 participants. The target treatment duration will be 8 weeks for Stage 1. Participants who have not achieved a response (CGI of "much improved" or "very much improved") will be crossed over to the alternate treatment (SERT for PBO non-responders, BUP for SERT non-responders), which will continue to be masked for an additional 8 weeks (Stage 2). Participants who have responded will continue the randomized treatment at the same dose under masked conditions for 8 more weeks. At each study visit, the study physician will complete the HRSD, and review side effect (TESS), tolerability (FIBSER), adherence (PAQ), and safety (CHRT, CAST, C-SSRS) measures. Patients will complete self-rating forms at each visit. Study assessments at any visit in Stage 1 and Stage 2 that are not associated with blood draws may be conducted by phone, if needed.

All study data will be captured in the StudyTRAX electronic data management system. For complete details about StudyTRAX and clinical visits, please refer to the EMBARC Clinical Procedures Manual.

4.1. Naturalistic Follow-Up Study Procedures

Study visits (in-person or remote) will occur at months 6, 9, 12, 15, 18, 21, and 24. At each visit patients will complete a treatment history form that records the current medications they are taking and they will also complete the following self-report forms: Quick Inventory of Depressive Symptomatology – Self Report (QIDS-SR), Concise Health Risk Tracking (CHRT), Concise Associated Symptom Tracking (CAST), Columbia Suicide Severity Rating (C-SSRS), Altman Self-Rating Mania (ASRM), and the Social Adjustment Scale (SAS). The time necessary to complete these assessments is not expected to exceed 30 minutes. Subjects included in the feasibility sample will not be followed for these visits.

4.2. Study Medication

Study medication will be provided by the NCC to each of the clinical sites. Study medication will consist of capsules containing purchased Sertraline 50mg tablets, donated Wellbutrin XL 150mg tablets (donated by Valeant Pharmaceuticals), and matching placebo for the approximately 400 subjects in the Biosignatures Sample. For the smaller Feasibility Sample study medication will consist of capsules containing purchased Citalopram 20mg tablets, donated Wellbutrin XL 150mg tablets, and matching placebo. All medications will be prepared in sets of matching capsules to mask treatment identity. Based on randomization, StudyTRAX will allocate medication assignment and each site will follow their local site approved methods to select and administer study medications. Medication adherence will be monitored by counts of dispensed and returned pills and recorded in StudyTRAX.

4.3. Visit Windows

StudyTRAX will be programmed to establish visit windows of 3 days before to 3 days after a scheduled visit day. Visits outside of the window will default to the appropriate visit. In the event a visit cannot be scheduled within the visit window, a telephone or Skype visit can be substituted, in order not to miss ratings. NOTE: Participants may log in to StudyTRAX from their home computer to complete ratings. For telephone- or Skype-rated visits, the HRSD and a verbal report of adherence will be rated, at the minimum. For telephone- or Skype-rated visits only, agitation and retardation on the HRSD will be rated based on subject report. Telephone or Skype visits will not be permitted for visits 1, 4, 8, 9, 12, or 16 as these require biomarker collection and/or blood sampling. In addition, if two telephone or Skype visits have been conducted for a given participant, then the site responsible for that participant must receive approval from the NCC to allow any further non-office visits.

| Schedule of Assessments – Treatment Phase | | | | | | | | | | | | | | |
|---|------------------------|---------------------------|----------------------------------|------------|---|---|---|---|---|-----|----|----|----|----|
| | Study Visit | | | | | | | | | | | | | |
| | Prior to Randomization | | | Study Week | | | | | | | | | | |
| <i>Rating or procedure</i> | <i>Evaluation</i> | <i>Biological Markers</i> | <i>Randomization (Baseline)*</i> | 1 | 2 | 3 | 4 | 6 | 8 | 9** | 10 | 12 | 14 | 16 |
| CONSENT | X | | | | | | | | | | | | | |
| ELIGIBILITY FOR ENROLLMENT | X | | | | | | | | | | | | | |
| DEMOGRAPHICS | X | | | | | | | | | | | | | |
| SCID-I | X | | | | | | | | | | | | | |
| ATRQ | X | | | | | | | | | | | | | |
| METAL SCREEN | X | | | | | | | | | | | | | |
| EHI | X | | | | | | | | | | | | | |
| LABS [†] | X | | | | | | | | | | | | | |
| PHYSICAL EXAM | X | | | | | | | | | | | | | |
| HT / WT / BMI / Waist | X | | | | | | | | | | | | | |
| BP | X | | | | | | | | X | X | X | X | X | X |
| EEG/BEHAVIORAL | | X | | X | | | | | | | | | | |
| SSAI | | X | | X | | | | | | | | | | |
| VAMS | | X | | X | | | | | | | | | | |

| | | | | | | | | | | | | | | |
|----------------------------------|---|---|--|---|---|---|---|---|---|---|---|---|---|---|
| Fagerstrom | | X | | X | | | | | | | | | | |
| Resting EEG | | X | | X | | | | | | | | | | |
| Evoked Potential | | X | | X | | | | | | | | | | |
| Word Fluency | | X | | X | | | | | | | | | | |
| Flanker Task | | X | | X | | | | | | | | | | |
| A not B Task | | X | | X | | | | | | | | | | |
| Choice Reaction | | X | | X | | | | | | | | | | |
| Probablistic Reward | | X | | X | | | | | | | | | | |
| MRI | | X | | X | | | | | | | | | | |
| SSAI | | X | | X | | | | | | | | | | |
| MASQ | | X | | X | | | | | | | | | | |
| SHAPS | | X | | X | | | | | | | | | | |
| Visual Acuity | | X | | X | | | | | | | | | | |
| BOLD | | X | | X | | | | | | | | | | |
| ASL | | X | | X | | | | | | | | | | |
| Emotional Recog | | X | | X | | | | | | | | | | |
| Reward task | | X | | X | | | | | | | | | | |
| Structural Imaging | | X | | X | | | | | | | | | | |
| Blood Collection*** | | X | | X | | | X | | X | X | | X | | X |
| QIDS-SR | X | X | | X | | | | | | | | | | |
| ELIGIBILITY FOR RANDOMIZATION | | | | X | | | | | | | | | | |
| FHS | | | | X | | | | | | | | | | |
| SCQ | | | | X | | | | | | | | | | |
| AAQ | | | | X | | | | | | | | | | |
| MDQ | | | | X | | | | | | | | | | |
| CTQ | | | | X | | | | | | | | | | |
| NEO-FFI | | | | X | | | | | | | | | | |
| SAPAS | | | | X | | | | | | | | | | |
| WASI [‡] | X | | | | | | | | | | | | | |
| HRSD | | | | X | X | X | X | X | X | X | X | X | X | X |
| CGI | | | | | | | | | X | | | | | |
| SAS | | | | X | | | | X | X | | | X | | X |
| PFIB | | | | X | X | X | X | X | X | X | X | X | X | X |
| TESS | | | | | X | X | X | X | X | X | X | X | X | X |
| FIBSER | | | | | X | X | X | X | X | X | X | X | X | X |
| CHRT (\pm C-SSRS)**** | | | | X | X | X | X | X | X | X | X | X | X | X |

| | | | | | | | | | | | | | | |
|----------------|-----|-----|----|-----|----|----|----|----|----|----|----|----|----|----|
| SFI | | | X | | | | X | | X | | | X | | X |
| ASRM | | | X | | | | X | | X | | | X | | X |
| CAST | | | X | X | X | X | X | X | X | X | X | X | X | X |
| PAQ | | | | X | X | X | X | X | X | X | X | X | X | X |
| Time (minutes) | 150 | 250 | 70 | 280 | 45 | 30 | 40 | 30 | 50 | 40 | 35 | 45 | 35 | 50 |

- * Randomization
- ** An additional visit is scheduled at Week 9 only for participants crossing over to an alternative treatment.
- ‡ Evaluation Labs: Liver Function Tests (LFTs), Blood Urea Nitrogen (BUN)/Creatinine, Complete Blood Count (CBC), Thyroid Stimulating Hormone (TSH), Lipid Panel, C-reactive protein test, Urine Pregnancy Test, Urine Drug Screen
- *** 66mL (about 13.5 teaspoons) of blood will be collected at baseline and 20-49 mL (about 4-10 teaspoons) at subsequent visits for biological sample storage and processing.
- **** The C-SSRS will be completed only for participants scoring positive on a suicide item of the CHRT.
- ‡ The WASI will be completed once and may be done between evaluation and study week 2.

All week 8 or 16 ratings will be obtained, if possible, at endpoint if a participant exits the study early.

Altman Self-Rating Mania Scale (ASRM)
 Anger Attacks Questionnaire (AAQ)
 Antidepressant Treatment History Questionnaire (ATRQ)
 Body Mass Index (BMI)
 Childhood Trauma Questionnaire (CTQ)
 Clinical Global Improvement scale (CGI)
 Columbia Suicide Severity Rating (C-SSR)
 Concise Associated Symptoms Tracking (CAST)
 Concise Health Risk Tracking (CHRT)
 Edinburgh Handedness Inventory (EHI)
 Family History Screen (FHS)
 Frequency, Intensity, Burden of Side Effects Rating scale (FIBSER)
 Mood and Anxiety Symptom Questionnaire (MASQ-30 item)
 Mood Disorders Questionnaire (MDQ)
 NEO-Five Factor Inventory (NEO-FFI)
 Pain Frequency, Intensity, and Burden Scale (PFIB)
 Patient Adherence Questionnaire (PAQ)
 Physical Examination (PE)
 Quick Inventory of Depressive Symptomatology – Self Report (QIDS-SR)
 Self-Administered Comorbidity Questionnaire (SCQ)
 Sexual Functioning Inventory (SFI)
 Snaith-Hamilton pleasure scale (SHAPS)
 Social Adjustment Scale (SAS) short form
 Spielberger State Anxiety Inventory (SSAI)
 Standardized Assessment of Personality – Abbreviated Scale (SAPAS)
 Structured Clinical Interview for DSM-IV (The SCID-I)
 Structured Interview Guide for the Hamilton Rating Scale for Depression (HRSD) – 32 item (SIGH-D₃₂)
 Treatment Emergent Signs and Symptoms (TESS)
 Visual Analogue of Mood Scales (VAMS)
 Wechsler Abbreviated Scale of Intelligence (WASI)

4.4. Electrophysiology Procedures

Eligible participants will undergo electrophysiology measures at baseline and week 1. Electrophysiology measures include both EEG acquisition and behavioral phenotyping. Procedures for each of these measures are outlined below. For further details, please refer to the EEG and Behavioral Tasks Procedures Manual, respectively.

4.4.1. EEG Procedures

The EEG markers we are interested in studying can be divided into three groups. The first acquisition participants will undergo will be resting state EEG. Specifically, resting EEG is measured during four, 2 minute periods, half the time with the patients' eyes open, and the other half with the patients eyes closed. The second EEG measure we are interested in assessing is specifically localizing theta activity using a technique called Low Resolution Electromagnetic Tomography (LORETA). Finally, patients will have headphones placed, and will be presented with 1000Hz tones at 5 different intensity levels. The loudness dependency of auditory evoked potentials (LDAEP) will be measured during this time. The total time we estimate for completion of the EEG collection at each visit is approximately 1-1.5 hours (some variability will occur with lead placement across sites). The EEG workgroup, chaired by Drs. Bruder and Pizzagalli, will ensure that quality control is maintained across sites and that adequate training and standardization of measures is obtained prior to study related activities commencing.

4.4.2. Behavioral Phenotyping Procedures

We will be administering five distinct tasks to eligible study participants, to assess four domains: 1) psychomotor slowing, 2) cognitive control, 3) working memory, and 4) reward responsiveness. To assess these domains we plan to administer the following tasks: psychomotor slowing, 1) Choice RT task (~5 min) and 2) word fluency task (~5 min); cognitive control, the Flanker task (~10 min); working memory, the A not B task (~10 min); and reward responsiveness, the Probabilistic Reward task (~10 min). Of note, the Probabilistic Reward task includes a monetary incentive of up to \$16.20 for participants, which will be paid in cash to the participant at the time of testing. This task measures the subject's ability to modify behavior in response to rewards. On each trial, the subject sees a cartoon face with a short or long mouth. The task is to indicate whether a short or long mouth was presented by pressing one of two buttons. Critically, the size difference between the short and long mouths is very small, and correct responses of one type (e.g., short mouth) are followed by monetary rewards three times more frequently than correct responses of the other type (e.g., long mouth). The primary dependent measure is response bias: the degree to which the subject preferentially chooses the response that is more frequently rewarded (in this example, short mouth vs. long mouth). Other dependent measures include RT and a measure of the subject's ability to discriminate between the mouth sizes. For more information, see Pizzagalli et al. (2005).^a In the consent form, participants are told that their performance will determine a monetary reward after the task, with some – but not all – correct responses rewarded, for a total of up to \$16.20. The total time we estimate for completion of all tasks at each study visit is about 1 hour. All tasks will be presented using Eprime software, under standardized procedures across sites. The Behavioral Phenotyping workgroup, chaired by Dr. Pizzagalli, will ensure that quality control is maintained across sites and that adequate training and standardization of measures is obtained prior to study related activities commencing.

4.5. Neuroimaging Procedures

Eligible participants will undergo neuroimaging at baseline and week 1 (DTI will be obtained only at baseline). Neuroimaging includes both functional MRI (fMRI) and structural acquisition. A brief description of procedures for each of these is outlined below; for further details, please refer to the Neuroimaging Procedures Manual. In total we estimate that approximately 90 minutes (80 minutes at week 1, b/c no DTI) will be spent in the scanner per visit. In addition, site research coordinators will spend about 15-20 minutes preparing the subject. The Imaging workgroup, chaired by Drs. Phillips, Parsey, and Buckner, will ensure that quality control is maintained

^a Pizzagalli et al. (2005). *Biol Psychiatry*, 57, 319-327.

across sites and that adequate training and standardization of imaging paradigms is obtained prior to study related activities commencing.

4.5.1. fMRI Procedures

Functional imaging will be acquired in two states: 1) while undergoing challenge tasks and 2) at resting state. The two challenge tasks employed as part of this study include the emotional conflict task (~14 min), and a reward task (~8 min). The non-task images acquired at rest include both low frequency BOLD fluctuations (LFBF – 2x6 min), and arterial spin labeling (ASL – 6 min). Of note, the reward task includes a monetary incentive of up to \$3.00 for participants, which will be paid in cash to the participant at the time of testing. This task is a slow event-related card-guessing game that allows examination of neural activity during anticipation and receipt of monetary reward feedback. Each trial is a possible win, where outcome is either win or no-change (disappointment), or a possible loss, where outcome is either loss or no-change (relief). Trials are presented in pseudorandom order with predetermined outcomes. In the consent form, participants are told that their performance will determine a monetary reward after the scan, with \$1 for each win and 50 cents deducted for each loss, for a total of up to \$3.00.

4.5.2. Structural Imaging Procedures

Structural Imaging will be acquired via Diffusion Tensor Imaging (DTI – 10 min) and 3D High Resolution MPRAGE (~7 min).

5. Procedures: Collection of Blood for DNA, mRNA, and Plasma Protein Biomarker Analysis

5.1. Overview:

Since the primary endpoint for this study is to discover biomarkers and biosignatures of treatment outcome for patients with MDD, it is imperative that all potential biomarkers are collected. As such, subjects will not have the option to participate in the DNA, mRNA, and plasma protein biomarker sub-study. Additionally, some of the samples collected may be sent and analyzed at UTSW to assess study drug levels. An overview of the blood collection is provided below, for further details please refer to the Blood and Biological Specimens Procedure Manual.

5.2. Sample Collection and Processing:

As part of this study, every patient enrolled in EMBARC will be asked to provide blood samples at baseline, week 1, week 4, week 8, week 12, and week 16. Participants who are crossed over to an alternate treatment in Stage 2 will be asked to provide additional blood samples at week 9. The samples to assess for DNA, mRNA, and plasma protein biomarkers. will be sent to the Rutgers University Cell & DNA Repository (RUCDR), as part of the National Institute of Mental Health (NIMH) Human Genetics Initiative, where lymphocytes from each patient will be immortalized. The repository will store, catalogue, and distribute the samples to approved and qualified scientists for the conduct of biomedical research of depression and other health disorders.

5.3. Consent

Prior to any study procedures being conducted, at the time of study screening, prospective subjects will be provided with information such as the purpose, procedures, possible risks/benefits, compensation for injury, record retention, and contact information for study-related questions. Because of the different medications to be used in the feasibility study and the efficacy study, separate consents will be used for each. The investigator, sub-investigators, and/or the study coordinator will obtain informed consent. Study staff will explain language in the consent pertaining to all study procedures, including blood draws and genetic analyses, to the potential subjects and allow them time to ask questions and read the consent in the presence of study staff. Subjects will also be asked to sign an authorization for the use and disclosure of health information for research purposes (i.e., Health Insurance Portability and Accountability Act - HIPAA authorization). This form describes how information about the patient and their health will be used and shared by the researchers when they participate in the research study. Copies of both the informed consent and HIPAA authorization will be given to the patients, and the originals retained and kept securely at the study site.

5.4. De-identifying Samples

Blood samples will be de-identified, removing any identifying information (e.g., name, date of birth) that could be linked to the patient's identity prior to sending for analysis. Therefore, only the identified investigators can learn/discover the names of subjects. Genetic, mRNA, and protein analyses will be carried out on data that have been de-identified, as is the customary approach according to good clinical practice.

6. Sources of Research Material

We conducted extensive literature reviews and discussed intensively the selection of assessment and measurement tools. In a large practical trial, there is always a tension between the desire to measure as many parameters as possible and the need to streamline measurements to minimize participant and Study Clinician burden. In striking the proposed balance, we chose to be parsimonious whenever feasible.

Clinical, Demographic, and Prior Treatment History: Structured Clinical Interview for DSM-IV: The SCID-I; the Antidepressant Treatment Response Questionnaire (ATHQ); Standardised Assessment of Personality – Abbreviated Scale (SAPAS)⁶⁴ the NEO-Five Factor Inventory (NEO-FFI⁶⁵).

Symptom Measures: 1) the Quick Inventory of Depressive Symptomatology – Self Report (QIDS-SR); 2) the Structured Interview Guide for the Hamilton Rating Scale for Depression (HRSD) – 32 item (SIGH-D₃₂)^{1, 47, 66}; 3) the Concise Associated Symptoms Tracking (CAST); 4) the Columbia Suicide Severity Rating (C-SSR-C)⁶⁷; 5) the Concise Health Risk Tracking (CHRT); 6) the Treatment Emergent Signs and Symptoms (TESS) form; 7) the Childhood Trauma Questionnaire⁶⁸ (CTQ); 8) Self-Administered Comorbidity Questionnaire (SCQ)⁶⁹; 9) the Family History Screen (FHS)⁷⁰; 10) the Clinical Global Improvement scale (CGI); 11) The Altman Self-Rating Mania Scale (ASRM)⁷²; 12) the Mood Disorders Questionnaire (MDQ)⁷³; 13) the Sexual Functioning Inventory (SFI)⁷⁴; 14) Frequency, Intensity, Burden of Side Effects Rating scale (FIBSER)⁶²; 15) Social Adjustment Scale (SAS)⁷⁵; 16) the self-rated Anger Attacks Questionnaire (AAQ)^{76, 77}; 17) the Patient Adherence Questionnaire (PAQ); 18) the Mood and Anxiety Symptom Questionnaire (MASQ-30 item); 19) the Snaith-Hamilton pleasure scale (SHAPS); 20) Spielberger State Anxiety Inventory (SSAI); 21) the Visual Analogue of Mood Scales (VAMS); 22) the Wechsler Abbreviated Scale of Intelligence (WASI); and 23) the Pain Frequency, Intensity, and Burden Scale (PFIB).

Neuroimaging paradigms: fMRI measures of activity and functional connectivity to two tasks (an implicit emotion processing and regulation task assessing amygdala-anterior cingulate gyrus [ACC] circuitry, and a reward processing task assessing ventral striatal-orbitomedial prefrontal cortex [OMPFC] circuitry); resting state intracerebral connectivity; pulsed arterial spin labeling (PASL: a measure of regional cerebral blood flow); diffusion tensor imaging; and structural MRI measures of cortical thickness.

Electrophysiology: Pretreatment alpha and theta EEG power, and source localization measures of theta activity in the rostral ACC; Loudness dependency of auditory evoked potentials (LDAEP).

Behavioral Phenotyping: Psychomotor slowing (as measured during reaction time, word fluency, implicit emotion processing or regulation and reward learning tasks), cognitive control (as measured by interference and post-error adjustments), working memory, and reward conditioned learning.

Additional Biomaterials being collected and stored: Whole Blood collected in EDTA (purple-top), ACD (yellow-top), and PAXGene tubes; Plasma aliquots isolated from whole blood.

7. Inclusion and Exclusion Criteria

Nationally, a minimum of 400 subjects, 200 per group, will enter the study and be randomized to one of the two treatments. This trial will be conducted according to the FDA guidelines and the Declaration of Helsinki.

7.1. Inclusion Criteria

- Adults, age 18-65

- Written informed consent obtained
- Outpatients with a current primary diagnosis of nonpsychotic chronic or recurrent MDD or dysthymic disorder per the SCID-I
- Age of onset of first episode of MDD or dysthymia < 30 years
- QIDS-SR score of ≥ 14 at Screening Visit and Randomization (Baseline) Visit
- No failed antidepressant trials of adequate dose and duration, as defined by the MGH-ATRQ, in the current episode
- Agrees to, and is eligible for, all biomarkers procedures (EEG/psychological testing, MRI, and blood draws)

7.2. Exclusion Criteria

- History of inadequate response (to trials at adequate dose for adequate duration) or poor tolerability to sertraline (SERT) or bupropion (BUP)
- Pregnant or breastfeeding
- Plan to become pregnant over the ensuing 12 months following study entry or are sexually active and not using adequate contraception
- History (lifetime) of psychotic depressive, schizophrenic, bipolar (I, II, or NOS), schizoaffective, or other Axis I psychotic disorders
- Meeting DSM-IV criteria for substance dependence in the last 6 months, except for nicotine, or substance abuse in the last 2 months
- Require immediate hospitalization for psychiatric disorder
- Have an unstable general medical condition (GMC) that will likely require hospitalization or to be deemed terminal (life expectancy < 6 months after study entry)
- Require medications for their GMCs that contraindicate any study medication
- Clinically significant evaluation laboratory abnormalities
- Have epilepsy or other conditions requiring an anticonvulsant
- Receiving or have received during the index episode vagus nerve stimulation, ECT, or rTMS, or other somatic antidepressant treatments
- Currently taking any of the following exclusionary medications: antipsychotic medications, anticonvulsant medications, mood stabilizers, central nervous system stimulants, daily use of benzodiazepines or hypnotics, or antidepressant medication used for the treatment of depression or other purposes such as smoking cessation, since these agents may interfere with the testing of the major hypotheses under study. Nonexcluded concomitant medications are acceptable as long as their clinician determines that antidepressant treatment is safe and appropriate.
- Significant liver disease that would contraindicate any study medication
- Taking thyroid medication for hypothyroidism may be included only if they have been stable on the thyroid medication for 3 months
- Using agents that are potential augmenting agents (e.g., T₃ in the absence of thyroid disease, SAMe, St. John's Wort, lithium, buspirone, Omega 3 fatty acids)
- Therapy that is depression specific, such as CBT or Interpersonal Psychotherapy of Depression (IPT) is not allowed during participation (participants can participate if they are receiving psychotherapy that is not targeting the symptoms of depression, such as supportive therapy, marital therapy).
- Subjects must be fluent in English and have the capacity to understand the nature of the study and sign the written informed consent since non-English speaking personnel are not available for this study, and the research instruments are not yet translated and validated in other languages.
- Currently actively suicidal or considered a high suicide risk
- Are currently enrolled in another study, and participation in that study contraindicates participation in the EMBARC study.
- Baseline ECG QT_c interval of > 460 msec (For Feasibility Sample only)
- Requiring concomitant medications known to increase the QT_c interval (For Feasibility Sample only)

- Any reason not listed herein yet, determined by the site PI, medical personnel, or designee that constitutes good clinical practice and that would in the opinion of the site PI, medical personnel, or designee make participation in the study hazardous.

Note: Participants ineligible for Stage 2 treatment because of medical contraindications to bupropion (e.g., history of seizures, eating disorders, etc.) will be study eligible but will not be eligible for bupropion treatment in Stage 2.

7.3. Concomitant Treatment for Human Subjects Protection

Treatments for Depression: Only protocol medication treatments for depression are allowed. Other treatments that may have antidepressant effects are not allowed as they may confound our efforts to compare the Stage 1 and 2 antidepressant effects of the protocol treatments.

7.4. Treatment for General Medical Conditions (GMCs):

Any treatment for any GMC is allowed. SERT is an inhibitor of the cytochrome 2D6, 3A3/4, and 2C19 isoenzyme systems, and BUP is a moderate inhibitor of the 2D6 isoenzyme system. Study clinicians will be trained to recognize nonprotocol medications (e.g., Type 1C antiarrhythmics, beta blockers, etc.) for which serum levels or dose adjustments may be needed for these patients.

7.5. Treatments for Antidepressant Medication Side Effects:

Medications to treat antidepressant medication side effects are allowed so as to mimic practice and to increase retention. These side effects may be treated based on clinician judgment. The most common side effects are likely to be anxiety/agitation, insomnia, and sexual dysfunction. The following are suggested remedies for these side effects, but others are allowed within the bounds of the protocol.

- Anxiety/Agitation.
Treatment emergent anxiety/agitation may be treated by watchful waiting/support, protocol medication dose adjustment, or lorazepam 0.5-2.0mg/day.
- Insomnia.
Insomnia may be treated by watchful waiting/support, protocol medication dose adjustment, or zolpidem 5-10 mg qhs, or a benzodiazepine hypnotic (e.g., temazepam 15-30 mg qhs) at clinician discretion.
- Sexual dysfunction.
Phosphodiesterase (PDE) inhibitors (e.g., sildenafil 50-100 mg prn; tadalafil 5-20mg prn) may be used to treat treatment-emergent sexual dysfunction as clinically dictated.

8. Recruitment Methods and Consenting Process

Potential subjects will be screened at each Clinical Site (CS) using the site's standard procedure (which may vary across sites). All Clinical Sites, however, will use the study standard inclusion/exclusion criteria for enrollment. No participants will be excluded on the basis of race or ethnicity. All subjects will provide written informed consent before study participation. If the study clinician believes a patient may be appropriate for the EMBARC study based on the study clinician's judgment, the Clinical Research Coordinator (CRC) or Study Psychiatrist will explain the protocol and obtain written informed consent before proceeding. The Clinical Research Coordinators are trained in how to obtain informed consent. The Institutional Review Boards at the NCC (University of Texas Southwestern Medical Center), each participating site, and the Columbia University/Stony Brook University DMC will approve the study protocol and all consent and study procedures. In addition, if needed, IRBs at all relevant Clinical Sites will approve the study protocol and all study and consent procedures. Each CS will provide a specific recruitment plan for NCC approval prior to study initiation. The recruitment strategies employed by each CS will be customized to take into account the clinic flow,

characteristics of the patient population seen, and the culture of each clinical site. The Site Director will routinely monitor recruitment and modify strategies as needed to meet enrollment goals.

In addition to the presence of the CRC in the clinic and the use of a depression screening tool, recruitment flyers and posters will also be developed according to IRB guidelines for display in the clinic waiting areas and exam rooms. These will raise awareness of the availability of the EMBARC study as a potential resource for care and will encourage patient self-referral. The Site Director will also educate the clinic support staff and participating physicians about the study on an ongoing basis throughout the recruitment phase, providing updates of study progress to keep clinician interest high.

8.1. Local Recruitment

At the study screening visit subjects will sign a written informed consent document prior to any study-related procedures being conducted. Prospective subjects will be provided with information such as the purpose, procedures, possible risks/benefits, alternative treatments, compensation for injury, record retention, and contact information for study-related questions. Study staff will explain the consent to the potential subject and allow them time to ask questions and read the consent in the presence of study staff. Subjects will also be asked to sign an authorization for the use and disclosure of health information for research purposes (i.e., Health Insurance Portability and Accountability Act - HIPAA authorization). This form describes how information about the patient and their health will be used and shared by the researchers when they participate in the research study. Copies of the informed consent/HIPAA authorization will be given to the subjects and the originals retained and kept securely at the study site.

8.2. Methods to Enhance Participant Adherence to Research Procedures.

Participants will receive monetary remuneration for their participation in the trial to compensate for their time and effort. Essential to the study is appropriate support for participants' time and effort contributing to the study. Additional non-monetary incentives to reward participation may be developed on a site-specific basis. Recommendations for specific amounts are subject to change per IRB guidelines and other local considerations. In order to receive maximum compensation, all study attendance requirements must be met.

We will post a bimonthly (every 2 months) publicly available study update on the study Web site that Clinical Site Directors and CRCs can download for posting or distribution in the clinics. This update will include tips to CRCs and patients on how to enhance retention and adherence. At the end of year 1, certificates will be provided (at no cost to the study) to recognize those CRCs and Clinical Sites with the highest enrollment, retention, and treatment adherence numbers.

Participants may withdraw from the study at any time. However, participants who withdraw from the study early may be asked to continue to complete study assessments (if they are willing).

9. Potential Risks

Risks commonly associated with taking FDA approved antidepressant medications (either sertraline or citalopram in Stage 1, and bupropion XL in Stage 2) will be fully described in the patient informed consent document, as will the risks associated with being randomized to placebo. Similarly, all risks associated with fMRI, EEG, behavioral phenotyping and blood draws will be described in the informed consent and relayed to patients at the time of consent. While, patients and clinicians are masked to medication type and dose, dose adjustments will be guided by specific assessment of the magnitude and types of side effects at each clinic visit, and downward titration (as well as slower upward titrations) is allowed based on tolerability and efficacy. Human subject risk related to administration of medication in this study will be minimized since participants are exposed to either placebo or FDA-approved medications that are prescribed in routine practice and for time-periods that are consistent with recommend treatment practices. Additionally, all participants will have frequent visits providing close supervision and monitoring of symptom severity and side effect burden. This heightened

level of monitoring will allow patients and/or physicians the opportunity to make appropriate treatment decisions that safeguard patient well-being.

Patients will also be made aware that a slight risk for psychological discomfort exists when assessing depression and clinical/behavioral phenotyping. There may be some anxiety or discomfort associated with the blood draw. This may include minor pain of the needle stick, some risk of slight bleeding, bruising, and, rarely, infection and fainting. MRI scanning is associated with panic attacks or distress in some individuals. It also poses risks to subjects who have metal in or on their body, so patients with known or possibly ferromagnetic metal in their bodies that cannot be removed will not be scanned. Patients may find the sound generated by the MRI during the scan unpleasant or uncomfortably loud. EEG procedures may cause some discomfort due to electrode placement. Additionally, there may be inconvenience associated with the use of conductive gel which will come into contact with patient's hair.

In terms of alternative treatments, patients may choose not to be involved and still receive treatment by their current medical provider, or patients may initially consent to participate and later decide to withdraw consent for participation in the study and still receive treatment by their current medical provider.

Patients randomized to placebo may experience a worsening of symptoms during the study (including suicidal ideation, and worsening depression). If, at any time during the study the patient or study doctor feels that worsening symptoms may be hazardous or harmful to the patient's safety, he/she may be exited from the study, at the discretion of the site's study psychiatrist.

10. Subject Safety and Data Monitoring

Clinical interviews are conducted by trained and experienced clinicians who will be able to reassure subjects if they are distressed. Subjects will be provided counseling if necessary. During all procedures, adequate rest periods are provided so that subjects are not fatigued or stressed during the testing. Standard, appropriate measures will be used to minimize risk of infection during venipuncture. MRI-related risks such the presence of internal and external ferromagnetic metal objects in or on the patient will be handled by 2 layers of screening, first by the CRC, and secondly by MRI personnel. Additionally, internal (ear plugs) and external ear protection will be utilized to reduce discomfort during the MRI. Patients will be provided adequate facilities to clean up after the EEG.

10.1. Suicide Procedures

There are two types of contacts during which time increased suicidal risk can be identified: 1) at the time of study visits, and 2) during phone assessments or a phone call. Prior to study implementation, each Regional Center will provide to the Principal Investigator a copy of risk management procedures for this study for clinic and phone-based participant contacts based on their selected clinic's specific crisis management requirements. All Regional Centers' risk management procedures will be reviewed and approved by the Operations Committee.

Risk management procedures typically include the following: Participants who report risk of self-harm at any point of contact will be thoroughly evaluated by the CRC for intent, plan and means for suicide. If the participant is in the clinic, the physician will be immediately notified to further assess the participant. If the participant is deemed an acute risk for suicide, the physician will direct the measures necessary to get the participant to safety, using clinic specific crisis management procedures. If the CRC identifies that the participant may be at acute risk during a phone assessment, a complete evaluation of the situation is necessary. For example, if the participant indicates thoughts of suicide/death several times a day in depth on the QIDS-C₁₆, the study team will obtain information about the participant's intent, plan, and means at hand for suicide, as well as determine, as appropriate, the location of the participant and whether anyone else is with the person. Measures to keep the participant safe, including the option of calling 911 for police assistance will

be implemented as based on the assessment of risk, and consistent with the clinic's crisis management procedures.

Study staff will report to emergency treatment providers any information relevant to treatment decision making, including any evidence of suicidality. All serious adverse events will be reported to the Principal Investigator, EMBARC Safety Officer, and NIMH (via the NCC) within 24 hours of the occurrence. Notice will also be provided to the DSMB and reported to RC IRBs per institutional policy.

CRCs will be trained prior to study initiation in the identification and assessment of suicidal risk based on both participant responses to study instruments, as well as in-depth questioning of participants about current suicidality in either setting. Training will include case scenarios. There will be ongoing opportunities to discuss safety-related procedures and individual participant cases in semimonthly CRCs led by the NCC and study Safety Officer.

Effective treatment, ongoing follow-up and evaluation, and 24-hour telephone coverage will be available to participants to minimize the risk of suicide. When necessary, hospitalization is available. Physician, CRC, and Investigator phone numbers are provided, and participants are encouraged to call should they need additional assistance beyond scheduled appointments. The clinicians and CRCs maintain close contact with participants and reschedule appointments as needed. Either the participant or the physician may discontinue the participant's study participation at any time should the participant's symptoms worsen or if the participant simply desires to withdraw. Use of these procedures minimizes risk.

At each visit, the participant will complete a self-report form indicating any side effects to the study treatment. The clinician side-effect report will be completed as well. Side effects will also be evaluated by the study physician. All information will be used by the study physician, along with any other pertinent medical information the participant reports. Depression symptoms will be monitored at each visit, with the risk for suicide thoroughly assessed. All data forms completed by the participant will be reviewed for content by the CRC before the participant leaves the clinic. This will ensure that nothing is overlooked in the evaluation process, including any indication of suicidality. All data collected at each visit are reviewed by the CRC and study physician so that monitoring of study data for any risk consistently occurs in real time throughout the study.

At any point when the CRC learns that the patient is at risk for suicide, the Suicide Risk Management procedure for that clinical site is to be implemented.

The Safety Officer is available 24 hours a day/7 days a week to respond to reports of serious adverse events (SAEs) or any other safety issues (214-648-0181). The NCC PI will be back up to the Safety Officer and is also available 24 hours a day/7 days a week (214-648-0181).

Following resolution of the acute suicidal status, the patient will be evaluated for continuation in the study or withdrawal, as appropriate. This will be based on the current need for best clinical care.

In the event that a patient indicates suicidal ideation with intent or a plan, the CRC or clinician initially made aware of the situation will take the following steps:

- Assess patient's location and access to the means for suicide.
- Determine if the patient is alone or has supportive family or friends with him.
- Contact family members/significant friends using the contact information provided by patient at study entry.
- If family is contacted, alert them to the urgency and need to limit the patient's access to the means of suicide. Review plan with family should patient need urgent care to prevent a suicide attempt.

Whether the patient presents in clinic or calls in expressing active suicidality, the clinician or CRC will instruct the patient to go to the nearest emergency room. If family or friends are not available to take the patient to the emergency room, or if the patient refuses, the clinician/CRC may call 911 and have the patient escorted involuntarily. In situations where patients have made initial contact with the CRC and not the study clinician, the CRC will notify the treating clinician of the event as soon as possible. Clinic-specific suicide risk procedures

will be followed. The Site Director will also be notified of the event and disposition of the patient. A copy of the clinic-specific suicide procedure for each clinic involved in the EMBARC will be submitted and kept on file at the NCC.

10.2. Safety Measures

The Columbia Suicide Severity Rating (C-SSR-C)⁶⁷; Concise Health Risk Tracking (CHRT); Concise Associated Symptom Tracking (CAST).

10.3. Development, Implementation and Oversight of Safety Procedures

CRCs and study physicians provide a net of safety procedures to identify suicidal risk. As in any clinical setting, the clinician treating each patient may be the first to identify that the patient is at risk for suicide during a contact. Study clinicians may hospitalize patients, increase visit frequency, change treatments within the protocol, or remove the patient from the study if indicated. The CRC also evaluates the patient at each visit, has an ongoing relationship, and passes any indication of risk immediately to the study physician or clinician for action.

10.4. Serious Medication Side Effects/Other Adverse Events

A patient reporting a serious medication side effect or other serious adverse event will be directed to go to the nearest emergency room. Events judged by the study clinician or CRC to be less urgent may require a same-day clinic appointment or more frequent study visits to monitor.

10.5. Data Collection for Serious Adverse Events.

A general description of a serious adverse event (SAE) is an anticipated or unanticipated event involving risk to study participants. The reporting of SAEs will be responsibility of each Clinical Site. The SAE procedures for EMBARC will be similar to those procedures used in other studies, such as CO-MED. The procedure will be developed so that SAEs are processed within 24 hours and evaluated to determine whether or not the SAEs meets expedited reporting criteria to the Food and Drug Administration (FDA). For EMBARC, the procedure would be initiated when a Clinical Site investigator enters a SAE form into the EMBARC database. The system would then automatically sends e-mail notification (with Participant ID and event date, and a link to the SAE report describing the event) and a SAE Summary form to the Safety Officer at the NCC who is responsible for processing SAEs and if decided upon in advance, representatives of the NIMH. The Safety Officer completes a series of questions via the web-based system. Using these data, an algorithm determines whether the SAE meets the requirements for expedited reporting to the FDA. Serious adverse events that meet the expedited FDA reporting criteria will also be sent to the DSMB. Depending upon the review, the files are moved to the appropriate area of the EMBARC web site (Expedited SAE or Nonexpedited SAE), and e-mail notifications are sent to the Clinical Site and NIMH. A full report of SAEs is provided in the DSMB reports that are to be submitted at least three times per year, based on current NIMH policies.

10.6. Subject Burden

Participants will be given frequent breaks to lessen the burden of the research visits.

10.7. Data and Safety Monitoring Plan

EMBARC will be monitored by the National Institute of Mental Health (NIMH) DSMB. To ensure the ethical conduct of the trial, the DSMB will carry out an independent review of the protocol and consent documents and will review adverse events and outcome data (as needed) to determine if any study procedures should be altered or stopped based on indications of clinical benefit or harm to trial participants attributable to the interventions under evaluation. The Board will also review plans for (and results of) interim analyses. The purpose of this process is to assess participant safety issues, the adequacy and integrity of accumulating data, and the study's capability to test the hypotheses. The protocol will be reviewed before subject enrollment can commence, and the DSMB will have decisional authority to stop or modify the trial.

10.7.1. Functions of the Data and Safety Monitoring Board (DSMB)

Board members will review and approve, disapprove, defer, or suggest modifications or revisions to the protocol and consent documents. This process is intended to assure both scientific integrity and adherence to relevant human subject protection policies.

The Board monitors adverse event reports to determine whether their nature, frequency, and severity are in accord with expectations. In the case of unanticipated serious adverse events, the DSMB can decide upon appropriate actions. These may include introducing new monitoring tests or safeguards, altering inclusion or exclusion criteria, determining that part or all of a trial be suspended or discontinued, and/or that the wording of consent documents be revised.

Review of the ongoing study will focus on the following issues:

- status of the study
- problems with accrual and follow-up
- demographic data
- compliance issues
- toxicity issues and adverse events
- documentation of endpoints
- data quality issues
- data-based protocol modification issues
- other study-specific relevant information.

Board members will assess data management activities. In the monitoring of ongoing trials, the DSMB may vote for continuation, suspension (pending further information), or stopping part or all of a study. The DSMB may request reports separately for each of the multiple centers participating in the clinical trial whenever warranted. The Board may ask to review data relevant to quality control, e.g., from sites with missing or late data submissions or with high dropout or low accrual rates. The DSMB will review requests for interim analyses and approve, disapprove, ask for additional information, or defer decisions.

The Board may also monitor data regarding efficacy. To do this, the DSMB will receive a summary with outcome measures in a "semi-blinded" form. That is, a DSMB can review data (in closed session) indicating certain outcome events for each treatment group (across sites) identified as "group A", "group B", etc. based upon the study design. When differences in results between the groups appear to be clinically significant, a DSMB may request unblinding and/or interim analysis. In such circumstances, the Board will decide whether the clinical trial should continue with or without further enrollment of new subjects, or be modified in some way.

The full DSMB may not need to consider requests for minor changes in previously approved NIMH-sponsored protocols. Such changes may involve inclusion or exclusion criteria, or other study design revisions based on protocol refinement and experience of the investigators conducting the study. The Chair of the relevant DSMB can review requests for approval of relatively minor changes. If the Chair approves such changes, then they can be implemented by the investigators. On the other hand, if the proposed changes appear to be potentially problematic, they will be referred to the full DSMB for a more formal review.

In summary, the Institute's DSMB will assure that NIMH-sponsored clinical trials initially meet, and continue to maintain, high standards. The DSMB will periodically review accumulated data (on an annual basis at minimum) to monitor adherence to the protocol and data quality standards. The Board will monitor for patient safety and evidence of serious adverse events on a more frequent basis (typically at each meeting). In trials where interim analyses are justified in the original design or considered by the Board to be necessary to decide whether the trial should be continued, modified, or terminated, these analyses will be carefully reviewed. The Boards will also review substantive modifications to the trial proposed by the Institute and/or investigators, prior to their implementation.

10.7.2. DSMB Membership and Conflict of Interest

DSMB members are appointed by the NIMH Director, and will have no direct involvement in the study or conflict of interest (COI) with the research teams conducting the trials. Membership reflects the disciplines and medical specialties necessary to interpret the safety of, and data from, these trials. These disciplines include the fields of biostatistics, medical/research ethics, clinical trials research design, clinical diagnosis and treatment of mental disorders, and consumer/advocate perspectives.

Board members will serve for three-year terms. Ad hoc (non-voting) appointments may be made for specific reviews or as circumstances require. The DSMB's Scientific Administrator and other NIMH staff (representing the Institute Director) may serve as ex officio (non-voting) members. Ex-officio members may attend DSMB meetings, and will be the only other individuals (besides regular and ad hoc members) privy to unblinded or interim analysis data.

Full voting Board members will not be NIMH employees (with the possible exceptions of one NIMH IRP employee on each DSMB), nor will they have involvement with the clinical trial. Members will be recused if a COI, or appearance of conflict, exists based on their involvement in research addressed in the protocol under review. This independence is an essential characteristic of the DSMB. If a Board member is involved in the research project under consideration and/or may co-author a paper on the results of the trial, works in the same department as a study investigator, or is in a position to affect the study other than via the DSMB, that member is presumed to have a COI. Board members participating in discussions regarding specific studies must have no financial interest related to outcomes of such studies reviewed by the Boards. Individuals invited to serve on a DSMB will disclose in writing to the Scientific Administrator any potential conflicts of interest, actual or implied by appearance (e.g., financial or scientific). Members with a COI will be recused from evaluative discussions, formulation of decisions, and voting. Prior to reviewing any clinical trial protocol or data, DSMB members will also sign a confidentiality statement promising not to disclose any data, deliberations, or decisions of the DSMB.

10.7.3. DSMB Meeting Schedule and Format

The DSMB will be called to meet by the Scientific Administrator three times per year (generally in February, June, and October). Such meetings may be assembled in-person, or conducted via teleconference or mail/e-mail correspondence, as determined by the Chair and Scientific Administrator. In-person meeting(s) will typically be held in the Washington D.C. area. Any quarterly meeting may be postponed or canceled by the Scientific Administrator or DSMB Chair (e.g., in the event that there are insufficient data or protocols to justify a Board meeting, lack of a quorum, or some unforeseen event).

On occasion, the DSMB may be asked to review accumulating clinical data (e.g., serious and unanticipated adverse events related to the study) that warrant the Board's immediate discussion. In such circumstances, a special DSMB (or subcommittee) meeting can be conducted either in person, by email, or teleconference. The Chair and the Scientific Administrator will determine meeting logistics based upon clinical urgency and the availability of DSMB members.

DSMB meetings may include closed sessions, but appropriate Institute Staff representatives from trials being discussed will be available for DSMB consultation and questions prior to the Board's critiques and votes. Trial representatives may include the study's GPO, data management statisticians, and/or study investigators (as appropriate). Closed sessions will be restricted to voting and ad hoc members (who have not been recused), the Scientific Administrator, and NIMH staff as appropriate. During open or closed sessions, the Chair will review the issues and, as necessary, put each to a vote. While consensus is desirable, a decision will be considered reached with a majority vote (with 40% or more of the voting members participating as a minimum quorum). If two or more members disagree with the majority decision, they shall document their concerns in a minority opinion statement to become part of the DSMB report.

Meetings conducted via mail or e-mail may not be able to use the dual (open and closed) session approach. Rather, Board members might review the accumulating data from each clinical trial and submit evaluative comments (assessments and recommendations) directly to the DSMB Scientific Administrator. This individual will tabulate the submissions and present the members' decisions to the Chair. Following the Chair's approval, these decisions will be forwarded to the NIMH Director by the Scientific Administrator.

10.7.4. DSMB Reporting

NIMH staff will draft a summary report after each meeting. The draft report will be reviewed and edited by those Board members and staff who attended, prior to issuance of the final report. Draft reports of closed sessions may not be distributed to program staff unless circumstances warrant such. The Scientific Administrator will submit the final reports to the NIMH Director within one month of each meeting. The principal investigator (of each clinical trial under review) will then be informed of the Board's decisions. The results of interim analyses and closed session discussions will not be disseminated unless the Institute Director concludes that extraordinary circumstances warrant their release.

The summary report will include the DSMB conclusions and decisions (including formal minority opinions) regarding the appropriateness of commencing, continuing, or terminating the trial (or phase of the study) under review. When a termination decision has been made by a DSMB, this will be conveyed expeditiously to the NIMH Director and relevant staff. In such circumstances, the report must explicitly state the reason(s) for discontinuation of all or part of a study.

10.7.5. Data Reporting to DSMB

The Data Center will submit statistical reports to the Scientific Administrator three weeks prior to the scheduled meeting. These will include data up to and including 6 weeks prior to the reporting deadline (except for unanticipated Serious Adverse Events, which will be reported within 14 days; 7 if life-threatening).

Data from clinical trials with blinded treatment assignments will be presented without breaking the treatment codes, and will use letter or other codes instead. In some circumstances, however, identification of treatment groups may be necessary for meaningful discussion and interpretation of results. In situations when a DSMB requests that data be unblinded, the treatment codes will be provided but will only be revealed to those voting and ex officio members of the Board in attendance, and relevant NIMH staff. Only by majority vote of a DSMB will such data be unblinded.

The data to be reviewed will include the following:

- screening and baseline data
- outcome data, as needed
- safety and adverse event data
- accrual status, including actual enrollment compared to projections
- number of subjects by assessment point
- drop-out and termination information (number, reason, and assessment point)
- any other data that will help in assessing the clinical trial

Data will be reported by site as well as for the whole study sample. The sample size and missing/incomplete data must be clearly indicated on each report. For any data measured on a scale, the data should be presented in a format that identifies outliers rather than just by means and standard deviations. Study analyses of variables of interest will be prepared when requested by a DSMB.

The reports presented to DSMB members will be kept confidential. The details of the analyses (i.e., who was unblinded, results, number of analyses, and decisions) will be documented in the Summary Report to the NIMH Director and relevant staff.

10.7.6. Interim Analyses and Stopping Rules

Any plan for interim analyses will be added to the protocol as an amendment, and will be based on the principle that the result (the difference between treatment groups) would be clinically or biologically significant. Sufficient power will be documented in terms of percentages and significance levels (including both p-values and confidence levels). The DSMB review will evaluate whether an interim analysis is appropriate based on power, safety, and/or scientific reasons. Results of interim analyses that show substantial differences in efficacy or adverse events, or demonstrate “futility” of continuing a study, will be expected to result in a decision to modify or terminate parts or all of the protocol.

The Board will consider any stopping rules outlined in the protocol of the clinical trial under review. As the study progresses, the Board can modify or reconsider the stopping rules. For specific variables of interest, the DSMB may pre-specify confidence intervals or statistical differences to be detected for the next review. A DSMB may define stopping rules that differ from those in the protocol should safety considerations or accumulating data so warrant. Final decisions on stopping all or parts of a study will be made by the DSMB.

The factors that will determine if a clinical trial is to be terminated include the stopping rules, interim analyses, evidence that unanticipated adverse events are study-related, and the frequency and severity of study-related adverse events.

10.7.7. Release of Interim Analyses and DSMB Deliberations

Release of results of interim analyses and deliberations of the DSMB to anyone outside the Boards will be carefully controlled in order to avoid the possible introduction of bias or confidentiality problems. Release of interim results could lead to impaired accrual, individual unblinding, and premature termination of a trial. Decisions regarding release of the DSMB’s interim analysis data will be made by the NIMH Director (and staff as appropriate).

All requests for results of interim analyses must be made in writing and submitted to the NIMH Director for consideration and consultation with the Board as needed. Such requests may be referred to the Board to make decisions for or against. These decisions will take into account the impact on volunteers in the trial and the scientific integrity of the ongoing trial, reason for the request, the extent of critical endpoint data or unblinding requested, constraints on dissemination beyond the original requestor, and workload considerations based on the priority of the analyses.

10.7.8. DSMB Definitions

- **Adverse Event:** Any untoward behavioral or biomedical occurrence in a research participant, which does not necessarily have to have a causal relationship with the interventions or other aspects of the study. An adverse event can therefore be any unfavorable and unintended: sign (including an abnormal laboratory finding, for example); symptom (including new or exacerbated delusions or hallucinations, depression, cognitive impairment, etc); behavioral problem (suicidality, agitation/aggression, threats, etc); or disease. Such adverse events are temporally associated with the research participation, whether or not considered related to the study.
- **Serious Adverse Event:** An adverse event (see above) that results in any of the following outcomes: death, a life threatening experience, inpatient hospitalization (or prolongation thereof), a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important behavioral or biomedical events that may not be lethal, life threatening, or require hospitalization, may still be considered a serious adverse event (SAE) when, based upon appropriate clinical judgment, they may require behavioral, medical or surgical intervention to prevent one of the serious outcomes listed in this definition.
- **Unexpected:** Any adverse experience, the specificity or severity of which is not consistent with the risks information described in the protocol or consent documents, and is not considered due to the underlying disorder or condition.
- **Related to (or Associated with) the Study:** There is a reasonable possibility that the experience may have been caused by the interventions or other aspects of research participation. Causality assessment includes,

for example, assessment of temporal relationships, association with (or lack of association with) an underlying disorder or condition, presence (or absence) of a more likely cause, plausibility, etc.

10.7.9. Monitoring of Safety Data by the DSMB

Safety information for this study will be reported to the DSMB in an unblinded manner. A statistical penalty will not be assessed for the ongoing unblinded review of safety by the DSMB.

- Range of safety reporting to the DSMB. It is considered necessary for the purpose of monitoring the safety of the study that the DSMB review not only adverse events (AEs) and serious adverse events (SAEs), but also other data that may reflect differences in safety between treatment groups. This includes treatment retention rates, reasons for drop-out, and laboratory values reflecting potential toxicity.
- Serious Adverse Events. Expedited review will occur for all events meeting the FDA definition of Serious Adverse Events (SAEs) – i.e., any fatal event, immediately life-threatening event, permanently or substantially disabling event, event requiring or prolonging inpatient hospitalization, or any congenital anomaly. This also includes any event that a study investigator or the DSMB judges to impose a significant hazard, contraindication, side effect, or precaution. *For purposes of this study, all SAEs will be required to be reported to the DSMB, regardless of any judgment of their relatedness to the study treatment.* All relevant information will be reported to the DSMB for each SAE including information about the event and its outcome, dosing history of all study drugs and treatments, concomitant medications, the subject's medical history and current conditions, and all relevant laboratory data. Notification by e-mail, and fax transmittal of all related study forms shall be made to the DSMB within 2 days of the occurrence of any SAE. Information will be reviewed and a determination made of whether there was any possible relevance to the study. Additional reporting to the IRBs will be done within 24 hours of the SAE; reporting to NIH and FDA will be made according to their respective regulations governing SAE reporting.
- Non-Serious Adverse Events. At periodic intervals, the DSMB will be provided with summaries of the numbers and rates of adverse events by treatment group. These reports will include types of events, severity, and treatment phase. Data on individual non-serious adverse events is not expected to be needed for this review.
- Other Safety-Related Reports. At 4-month intervals throughout the study, the DSMB also receives unblinded summary reports of treatment retention and reasons for dropout by treatment arm and study phase from the Data Management Center (DMC).
- Study stopping rules. If at any time during the course of the study, the DSMB judges that risk to subjects outweighs the potential benefits, the DSMB shall have the discretion and responsibility to recommend that the study be terminated. Stopping rules for the trial could include stopping because of a significant number of injuries or illnesses that could be attributed to participation in the intervention, inability to recruit and measure the required number of participants to conduct the primary outcomes analyses, and poor intervention quality and delivery.

10.7.10. Monitoring of Data Quality by the DSMB

At least annually during the study, the DSMB receives a report on data quality and completeness from the DMC. At a minimum, this will include an overview of the progress of patient intake and retention; summary reports describing patient compliance with visits, evaluations, and dosing as described in the protocol; and a summary of the completeness and quality of key data elements needed to characterize patients, their dosing, and their primary and secondary outcomes. These reports will be used by the DSMB to evaluate the capacity of the data capture and processing to support scientifically valid analyses.

10.7.11. DSMB Reports to NIMH

After each DSMB meeting, the DSMB provides a summary report of its findings regarding safety and quality based on data received to that point in the study. This report includes a summary of all safety findings, as well as an assessment of protocol compliance and data quality. Any recommendations to improve patient safety, protocol adherence, or data quality are made in the annual DSMB report. A copy of the annual DSMB report is sent to the local IRBs along with the annual renewal report.

10.8 Emergency Unblinding Procedures

Each clinical site will have an identified non-treatment study clinician who will be able to unblind participants at their site. The study safety officer will also be able to unblind participants at any site. Unblinding will be accomplished by logging into the StudyTRAX using the site unblinding login ID and password provided by the Data Center. All logins using the unblinding login will be tracked within StudyTRAX. The Data Center will be notified of the unblinding login immediately, and the patient record accessed will be flagged to indicate unblinding has occurred.

11. Procedures to Maintain Confidentiality

All efforts will be made to protect patient confidentiality. A Certificate of Confidentiality will be obtained. Participants are supplying information that is catalogued and analyzed, and thereby subject to possible confidentiality risks. Participant-specific data will be coded with a subject number. Only individuals at the care settings will be able to link this number to the participant's name.

11.1. Biological specimens

Biological specimens will be collected at the sites and shipped to centralized facilities (Rutgers University Cell & DNA Repository (RUCDR)) for processing and banking. A tracking system that has been used in other studies, including STAR*D and CO-MED, will be used to ensure that the shipments are received by the centralized facilities. The system was designed to ensure that the confidentiality of study subjects is maintained, to the point where the research staff at the centralized facility is unaware of the site from which the sample originated. Randomly generated ID numbers, independent of the EMBARC study ID for the participant will be assigned by RUCDR.

11.2. Clinical, MRI, EEG, and Behavioral Data

A similar, but slightly different system will be used for clinical, MRI, EEG, and behavioral data. All data will be sent to the DMC from the clinical sites using appropriate software. Prior to storing any data in the EMBARC database, it will be stripped of any identifying information, including the subject name, date of birth, etc. Once the data are at the DMC, the DMC will transfer the de-identified data to the appropriate facilities for evaluation. A log will be generated summarizing the results of the transactions.

11.3. Data Sharing

To maximize the scientific knowledge to be gained from this project, de-identified data from all subjects will be made available to qualified researchers external to this project for additional analysis. The data made available will include clinical, behavioral and biological data collected in the study, as well as biological specimens as described above. The NIMH will utilize the RUCDR to store biological specimens and the database of Genotypes and Phenotypes (dbGAP) or other NIMH-designated repositories to store clinical, behavioral, and other biological data (i.e., EEG, MRI). Data access will be limited to qualified researchers at institutions with appropriate protections for human subjects, as defined by federal regulations, in place.

Details about the data sharing plan are available in the Data Sharing agreement.

12. Potential Benefits

The study participants have an opportunity to directly benefit from the care and treatment of their disorder. The efficacy of medication treatment algorithms to produce greater success in the treatment of comorbid depression has already been established in previous research. It is also known to improve outcomes in real-world treatment settings over usual care treatment. All subjects in the trial, including those randomized to placebo in Stage 1, have the opportunity to receive active treatment should they remain in the trial and their

symptom severity necessitate it. In addition, the active medications used in the study are FDA-approved. As such, the potential risks of the study are largely those experienced by any patient undergoing antidepressant therapy.

Despite early progress in the development of antidepressant treatments and the understanding of neurobiology, the last 20-plus years have not provided any significant improvements in the number of patients achieving remission with any given treatment. Current research methods have not been sufficient to develop individualized treatment strategies in part due to a lack of the true understanding of the heterogeneity of the disorder. Rather than wait for a comprehensive understanding of this neurobiology, the next step proposed in this study will not only provide insight into what treatments are best for which individual but may also assist in the understanding of the neurobiology.

This study is the first step in the development of biosignatures. Biosignatures can assist depression treatment in two ways, selection of optimal treatments for specific patients at the onset of treatment (biosignatures that act as moderators) and prediction of the ultimate efficacy of treatment based on biological changes early in treatment (biosignatures that act as mediators). Both approaches can improve the outcome and efficiency of treatment. We will identify biosignatures for SERT, BUP and PBO that will emerge from the following clinical and biological markers: clinical features including early life trauma, anxious depression and other illness characteristics, imaging, qEEG, behavioral phenotyping and other biological specimens (i.e., genetics, epigenetics, immune markers and neurotrophins). The Gene x Environment interactions (early life trauma, resilience, illness characteristics, qEEG markers, neural circuitry, cognitive/behavioral phenotypes) and the resulting impact on brain function and structure will provide the means to tailor treatments to individuals. The findings from this approach will lead to the development of a differential depression treatment response index (DTRI) that will produce a clinically meaningful process that is aimed at replacing the current trial and error method to antidepressant treatment selection. The development of the DTRI is akin to the Framingham approach to identify cardiovascular risk factors, and is designed to guide individualized treatment decisions in clinical practice by identifying clinically meaningful variation in biological and clinical factors among individuals. A personalized treatment strategy for patients with MDD will have the greatest potential for identifying treatment approaches that eliminate the need for multiple steps to achieve full remission for a given patient, while maximizing the number of patients who can be effectively treated while they remain in treatment.

13. Statistics

13.1. Power

The overarching purpose of this study is discovery, rather than confirming pre-existing theories and testing hypotheses. Therefore, the emphasis is on estimation, rather than p-values for hypotheses testing. The primary goal of the study is to investigate a broad range of biological markers and their interactions to be used as moderators/mediators of treatment response. For this reason, sample size determination and power for hypotheses testing regarding any given marker or series of markers are not provided.

13.2. Planned Analyses

Assessment of individual moderators/mediators: The first set of analyses will test an a priori list of individual variables for status as moderators and mediators. These analyses will be conducted using mixed effects models as follows:

$$Y_{it} = \beta_0 + \beta_1 trt_i + \beta_2 t + \beta_{12} t * trt_i + \beta_3 X_i + \beta_{13} trt_i * X_i + \beta_{23} t * X_i + \beta_4 t * trt_i * X_i + error_{it},$$

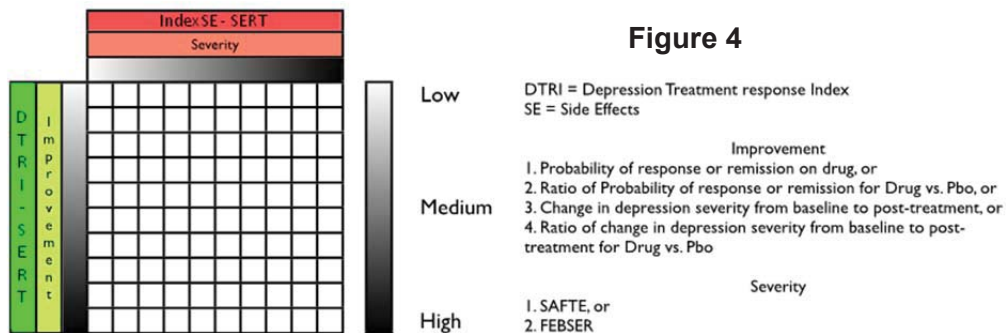
where Y_{it} indicates a depression severity measure for subject i at time t , trt_i is an indicator for the treatment for subject i (for stage 1 data it would be an indicator for SERT (vs. placebo)) and X_i is the value of the variable to be considered as a candidate for a moderator or mediator of treatment outcome for subject i . The term $error_{it}$ consists of random effects for subjects' individual slopes and intercepts and a random error term with mean zero. A significant β_4 term would identify X as a moderator or mediator. HRSD₁₇ total score will be used as a depression severity measure. Other measures (i.e., response, remission) may also be used.

Developing DTRI and improvement/side effects matrix: Statistical methods currently under development will be employed to identify combinations of variables and interactions among variables that best moderate treatment outcome. We will also consider inclusion of mediators in the combination of variables. The depression treatment response indices (DTRI) will be associated with levels of improvement expected with SERT treatment. Improvement might be measured in several different ways (e.g., absolute improvement from baseline, or improvement on the drug, relative to improvement on placebo). The sample will be randomly divided into developmental and validation samples (for example, 2/3 developmental, 1/3 validation). The DTRI will be created using only the developmental sample then the DTRI will be assessed by applying it to the validation sample.

The side effect indices Index-SE will be associated with the severity of side effects expected with the specific treatment. Severity of the side effects can be measured by FIBSER, SAFTEE or a combination of them.

In practice, the indices can be constructed in 2 ways. In the first approach points can be assigned for different ranges of the variables used in the selected model and their combinations; the points then can be summed and the sum will constitute the index. The pros of this approach is the simplicity of computation, allowing doctors and patients to understand how different factors affect patients' probability to respond to a given treatment. The Farmingham Cardiac Risk score is constructed in this way. We call this a *points-counting* method. The second approach is directly based on the best model and, for given values of the moderators/mediators in this model, the linear combination of main effects and interactions is computed (on the background by a computer). We call this approach a *black-box* method since clinicians and patients do not directly see how the index is constructed, rather they are provided only with the final index. Pro of this method is accuracy of the index (for example, continuous moderators need not be categorized in order to determine the number of points added to the index, as in the first approach); con is the need to have software in clinicians' offices that would compute the index based on the values of the moderators/mediators; of course, an index calculator can be made available on line.

Prior to treatment, patients can be charted on a graph as the one depicted on Figure 4 below. Subjects falling in the lower left corner would be good candidates for treatment with SERT. Guidelines for treatment can be established based on the chart.



In addition to the development of the DTRI using the a-priori list of potential moderators/mediators as described above, post-hoc analyses will continue to be done to identify additional moderators/mediators.

Stage 2 data will also be analyzed. Although the design of the proposed study does not make possible the similar development of BUP depression response indices, it does allow us to begin the process of identifying baseline factors that may be related to outcome from treatment with BUP. Comparing the relationship between outcome from BUP treatment and baseline predictors for subjects unsuccessfully treated with SERT in Stage 1 vs. the relationship between the same baseline predictor and outcome for placebo treated subjects in Stage 1, may suggest potential moderators of BUP treatment response. While the two groups are not exactly comparable, this comparison may give an indication for the baseline characteristics that are related to outcome with BUP treatment among failures to treatment with SERT. It may also yield results that can provide useful

leads in future studies, which is a major goal of this project which aims to generate candidate moderator and mediator variables rather than to definitely test *a priori* hypotheses.

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