16-week Open Randomized Comparative Effectiveness Trial of Lamotrigine vs. Fluoxetine for Bipolar Depression: Pharmacogenomic and Biomarker Predictors of Response.

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IRB Protocol

Title: 16-week Open Randomized Comparative Effectiveness Trial of Lamotrigine vs. Fluoxetine for Bipolar Depression: Pharmacogenomic and Biomarker Predictors of Response.
Short title: The FLAME Study
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ABSTRACT:

This study is part of the Mayo Clinic Bipolar Biobank and Biomarker Development (3BD) Program. This project is supported by the Marriott Family and Foundation.

Depression is the predominant prevailing mood state of bipolar disorder and it is associated with substantial morbidity and mortality. However, in comparison to acute mania, bipolar depression is understudied both from the standpoint of its pathophysiology as well as clinical trials and treatment development. Given the lack of evidence-based guidelines, clinicians and participants enter a treatment phase with little guidance.

The FLAME Study is a 16-week, open randomized comparative effectiveness trial evaluating genomic predictors and biomarkers of response and adverse events to treatment with lamotrigine (n=200) and fluoxetine (n=200) for bipolar I, II and schizoaffective bipolar depressed participants. Participants age 18-65; will be invited to take part of this study. Participating sites include: Mayo Clinic, Rochester and Austin and Lindner Center of HOPE in Mason, Ohio.

It is known that functionally significant genetic polymorphisms of pharmacokinetic and pharmacodynamic pathways can influence individual differences in repose to specific medications. We propose to evaluate the contribution of these pharmacogenomic variations to lamotrigine and fluoxetine treatment response and adverse events. We will correlate clinical phenotypes of response and adverse events to treatment with genotype and haplotype variations of drug metabolism, neurotransmitter biosynthesis, (metabolism, storage, release, reuptake), receptor and intracellular signaling-that have been previously implicated to either lamotrigine or fluoxetine drug mechanism of action. These initial steps will be complemented with genome-wide analysis (GWA), pathway analysis and other candidate gene studies.

Based on our results we aim to develop a translational treatment algorithm of bipolar depression that may help individualized treatment for bipolar depression. This algorithm for participants could potentially increase the likelihood of successful treatment interventions, deliver the “right treatment, for the right participant at the right time”, and decrease the number of ineffective treatments and/or risk for serious adverse events.

AIMS:

1. To evaluate the association between genotype and haplotype variations in candidate genes involved in pharmacodynamic and pharmacokinetic pathways of treatment response to lamotrigine (n=200) or fluoxetine (n=200) in bipolar depression. In addition, we aim to perform exploratory analyses of single-nucleotide polymorphisms (SNPs) obtained from GWA studies with drug response phenotypes.

2. To evaluate the association between genotype and haplotype variations in candidate genes involved in pharmacodynamic and pharmacokinetic pathways of drug adverse events to treatment [rash emergence and antidepressant induced mania (AIM+) to lamotrigine (n=200) or fluoxetine (n=200) respectively in bipolar depression. In addition, we aim to perform exploratory analyses of single-nucleotide polymorphisms (SNPs) obtained from GWA studies with drug adverse events phenotypes.

3. To identify blood and brain metabolites associated with treatment response to lamotrigine (n=200) and fluoxetine (n=200) in bipolar depression.

Hypothesis:

1. Treatment response to lamotrigine (n=200) or fluoxetine (n=200) in comparison to non-response, will be associated with different allele variation (either at a genotypic, haplotype or interaction level) in pharmacodynamic/pharmacokinetic functionally related genes.

2. Development of adverse event (rash or AIM+) while undergoing treatment with, lamotrigine (n=200) or fluoxetine (n=200) in comparison to no adverse event, will be associated with different allele variation (either at a genotypic, haplotype or interaction level) in pharmacodynamic/pharmacokinetic functionally related genes.

3. Treatment response to lamotrigine (n=200) or fluoxetine (n=200) in comparison to non-response, will be associated with biomarker expression (epigenetic and metabolomic/proteomic) in functionally related biological systems.

RESEARCH GOALS PERTINENT TO INDIVIDUALIZED MEDICINE:

The FLAME Study will be developed by a collaborative network to facilitate pharmacogenomic studies utilizing state-of-the-art research technology and phenotyping. The identification of pharmacogenomic predictors of treatment response could provide greater selectivity to treatment recommendations. Today, there are multiple medications from multiple classes (lithium, anticonvulsant mood stabilizers, antipsychotics, antidepressants) that are available to clinicians to treat the illness. However, there are no clear guidelines for choice of mood stabilizer, sequencing, or use in combination; pharmacogenomically-based treatment algorithms would enhance outcome and reduce ineffective or suboptimal treatment trials. The identification of genetic variations associated with treatment response or adverse events to treatment could lead to selective preventive interventions (i.e. individual-specific guidelines when or when not to prescribe lamotrigine or an SSRI antidepressant).

INTRODUCTION

Bipolar disorder is a medical illness with substantial morbidity and mortality characterized by episodic recurrent mania and major depression (Kraepelin, 1921). Bipolar disorder is associated with poor functional recovery (Tohen et al., 2000; Gitlin et al., 1995; Robb et al., 1997), substantial economic compromise (Murray and Lopez, 1997; Begley et al., 2001; Simon and Unutzer, 1999), premature mortality and morbidity with suicide rates approaching 20% (Tondo et al., 1999).

Lithium carbonate (LiCO3), which has been available for more than 30 years, is the bipolar pharmacopoeia gold standard and has unequivocally reduced personal illness, morbidity, mortality, and the costs of providing health care to participants with bipolar disorder (Goodwin and Jamison, 1990; Reifman and Wyatt, 1980). However, in recent years it has become increasingly clear that depressive symptoms of bipolar disorder have been underappreciated and understudied and may not respond optimally to lithium or other conventional treatments.

Bipolar depression is the prevailing predominant mood state of the illness. In general, episodes of depression are longer and more frequent than mania (Judd et al., 2002; Post et al., 2003; Frye, 2011), are the main driving force to work disability and are predictive of future illness burden (Coryell et al., 1998; Frye NEJM, 2011 and Calabresse et al., 2001). Finally, the field is increasingly aware of the prevalence of subsyndromal symptoms of depression and their association with functional disability and prodrome to relapse (Altshuler et al., 2002, Frye et al., 2007).

Despite the high prevalence rate of depressive symptoms, bipolar depression treatment has not been studied to the same degree as acute mania. In fact, as reviewed by Frye (NEJM, 2011), olanzapine-fluoxetine combination (Symbyax®),quetiapine (Seroquel®) monotherapy, and lurasidone (Latuda®) monotherapy and adjunctive are the only FDA-approved treatments for bipolar depression. Even more so, recent clinical trials found in bipolar

depression (armodafinil and agomelatine) have failed to separate from placebo, further emphasizing unmet need (Frye 2015, Yatham 2015).

Given the dearth of FDA approved treatments for bipolar depression, antidepressants are invariably prescribed; up to 49.8% of participants initiate treatment with an antidepressant (Baldessarini et al., 2007). In addition, in a meta-analysis of bipolar depression treatment with antidepressants vs. placebo or active comparison (n=3113), Sidor and MacQueen (2011, 2012) found the pooled treatment estimates for antidepressants to be ([RR]=1.17, 95% CI, 0.88-1.57; p=0.28) for clinical response and of (RR=1.14, 95% CI, 0.90-1.45; p=0.28) for clinical remission. While the AIM+ drugs vs. placebo in this same metaanalysis did not show significant risk with antidepressant therapy, clinical trial populations do not translate to community practice groups where estimates has been reported up to 30% (Frye et al. 2015).The contrast may be in part due to the difference between the studied populations: highly selected clinical-trial population less likely to switch vs. more naturalistic studies that include people with higher switching risks.

Lamotrigine: efficacy and safety as treatment of bipolar depression

Lamotrigine is currently FDA-approved for the maintenance phase of bipolar I disorder (Calabrese et al., 2003) in adults and for epilepsy in in participants ≥ 2 years of age. There are additional controlled studies by Van der Loos et al. (2009), Frye et al. (2000a), Obrocea et al. (2002), Calabrese et al. (1999) and a meta-analysis by Geddes et al. (2009). Three open-label trails by Biederman et al. (2010), Chang et al. (2006) and Pavuluri et al. (2009), suggest that lamotrigine may be an effective treatment of pediatric bipolar disorder, with a significant improvement in depressive symptoms. However, double-blind studies are needed to evaluate the efficacy of lamotrigine in bipolar depression of the pediatric population.

In contrast to all other FDA-approved mood stabilizers, lamotrigine appears to be more effective in the acute phase of depression of bipolar disorder. Glutamate has been extensively implicated in bipolar depression, with a complex interaction that involves the glutamate-glutamine and intracellular signaling pathways that result in alterations of gene expression; consequently affecting, synaptic efficacy and neuroplasticity (Zarate, in review, 2010). Together with other medications, lamotrigine acts as a glutamate modulating agent (see below "Lamotrigine pharmacogenomics") representing a significant advance in our treatment options for treating mood disorders.

Most current practice guidelines (i.e. APA, Expert Consensus, Texas Medication Algorithm, German Guideline on Diagnosis and Treatment of Bipolar Disorders) rank lamotrigine as a first-line treatment for acute bipolar depression. This is based on both evidence-based data such as work at NIMH (Frye et al., 2000a), the first industry sponsored trial (Calabrese et al., 1999), and now almost a decade of experience by psychiatrists in the community treating participants with bipolar disorder. The reason that lamotrigine does not have an indication for the acute phase of depression, in contrast to its maintenance indication, is that the 5 industry sponsored studies, with the exception of the 1999 Calabrese trial, have been largely negative or have been judged to be failed studies. However, it has generally been agreed upon that the 5 individual studies were underpowered and may have been compromised by a large placebo response rate. Beyond common sources of variability (i.e. severity of illness and BPI/BPII heterogeneity), the greatest source of variability may likely relate to the fact that the acute depression registration program took more than 10 years to complete. A selection bias as to the characteristics of participants enrolled in these latter negative or failed trials may be relevant.

Nonetheless, a recent meta-analysis by Geddes and colleagues, has addressed the inadequate power issue of the individual studies (Geddes et al., 2009). A meta-analysis of the full ITT trial populations (see clinical trial register for lamotrigine at GSK.com) was conducted with estimates of the relative risks of response (50% reduction in depression rating scale) and remission (<12 on MADRS) using the Mantel-Haenszel fixed effect. Bipolar depressed participants treated with lamotrigine were more likely to respond to treatment than those treated with placebo on both HAM-D (RR=1.27, 95% CI=1.09-1.47, Heterogeneity $\chi 2$=1.80 (df=4) p=0.772, Test of RR=1: z=3.13 p=0.002) and MADRS (RR=1.22, 95% CI=1.06-1.41, Heterogeneity $\chi 2$=3.12 (df = 4) p=0.538, test of RR=1: z=2.80 p=0.005). Remission rates were higher on MADRS (pooled RR=1.21, 95% CI=1.03-1.42; Heterogeneity $\chi 2$=5.86 (df=4) p=0.210 Test of RR=1: z=2.30 p=0.021), but not on the Ham-D (Figure 1). Thus, with a larger sample size, the data is suggestive of an acute antidepressant response with lamotrigine. More

recently, a placebo-controlled add-on study of lamotrigine to lithium for acute bipolar depression was positive (Van der Loos et al., 2009).

Anti-epileptic drugs such as lamotrigine have type A and type B adverse drug reactions. “Type A” are neurologic toxicity symptoms that are dose-related, predictable and thus relatively preventable with close supervision (Hung, 2010). On the other hand “type B” adverse drug reactions are idiosyncratic and to date difficult to predict; cutaneous adverse reactions are among the later. Severe cutaneous reactions namely, Stevens Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN); incidence in lamotrigine treatment has been estimated in 1.8 per 10,000 person-year (Mockenhaupt et al., 2005). Although dose titration has reduced largely the incidence of cutaneous adverse events associated to lamotrigine (Messenheimer et al., 1998) the lack of predictiveness of cutaneous adverse reactions is still a major limitation in the prescription of this drug.

Lamotrigine pharmacogenomics

Most of lamotrigine pharmacogenomic studies that have been developed to date, sought to understand the emergence of adverse events, especially cutaneous adverse reactions (Lopez et al., 2012). Lamotrigine is an aromatic antiepileptic drug and together with the rest of this group, has been associated to cutaneous adverse events (see above). Since the association of carbamazepine induced SJS/TEN to HLA-B*1502 in Han Chinese (Chung et al., 2004) many studies have investigated different polymorphisms of the HLA gene family in association to aromatic antiepileptic drugs, including lamotrigine and cutaneous adverse reactions; most of those studies were performed in Han Chinese populations. However, European descendants have been recently investigated; Lonjou and collaborators (2008) reported an association of HLA-B*38 with an extreme phenotype of SJS/TEN, additionally, a second study found an association between HLA-A*A6801 and a broader phenotype of adverse cutaneous reactions (Kazeem et al., 2009). Finally, the only GWAs to our knowledge to investigate cutaneous adverse events in lamotrigine treatment for epilepsy, did not find associations for imputated genes of the HLA family (McCormack et al. 2012). It is of note, in comparison to this study; the majority of studies regarding adverse reactions to lamotrigine have been performed in participants with epilepsy, making their interpolation to bipolar depression rather questionable; different genetic risks are hypothesized for both conditions making the study of bipolar depressed participants necessary.

Lamotrigine is metabolized by glucuronidation UDP glucuronosyltransferase coded by the UGT gene family. A study in Turkish population found an association of UGT1A4 wild-type, P24ZT and L48V polymorphisms and changes in lamotrigine blood levels (Gulcebi et al., 2011). Other studies regarding UGT polymorphisms in association to lamotrigine metabolism have been performed in cell culture; polymorphisms in UGT1A4, UGT1A1 and UGT2B7 have demonstrated altered lamotrigine pharmacokinetics (Lopez et al, 2012).

The mechanisms of action by which lamotrigine exerts its antidepressant effects are multiple. These studies are of very different design and have involved different brain regions, focusing sometimes on models of neurodegeneration not specific to bipolar disorder. However, to date the following mechanisms relative to antidepressant and mood-stabilizing action have been described: 1) voltage-dependent sodium channel inhibition and secondarily preventing Glu exocytosis (Leach et al., 1986, Cheung et al., 1992); 2) blockade of N and L-type voltage-dependent calcium channels in cerebrocortical nerve terminals attenuating depolarization-induced cytoplasmic calcium increase, additionally inhibiting 4-aminopyridine-induced Glu exocytosis (Wang et al., 2001; Jefferson et al. 2005); 3) modulating glutamate at the synapse level as evidenced by lamotrigine-associated hippocampal microdialsalate Glu reduction (Ahmad et al., 2005), 4) post-synaptic AMPA receptor modulation as evidenced by hippocampal AMPA subunits GluR1/2 lamotrigine-associated upregulation (both time- and dose-dependent) similar to riluzole and in contrast to antimanic agent divalproex (Du et al., 2007), 5) glutamate enzymatic regulation as evidenced by a lamotrigine-associated increase in hippocampal GAD1 glutamate decarboxylase converting Gln to GABA shunting away from Glu production (Hassel et al., 2001). 6) Lamotrigine has also been shown to be neuroprotective in several models (hippocampal ischemic (Crumrine et al., 1997)), malonate (Henshaw et al., 1994), and 3-nitropropionic acid (3-NP)) of Glu-mediated excitotoxic neuronal degeneration. In the latter study by Lee et al. (Lee et al., 2000), it was suggested that higher doses of lamotrigine may be more neuroprotective, as measured by MR spectroscopic NAA/Cr and T2 mapping of 3-NP lesions, than the NMDA antagonist MK-801; and 7) increasing levels of acetylated histone H3 and H4, enhancing B-cell lymphoma 2 (Bcl-2) mRNA expression and protein levels; the later has anti-apoptotic activity and thus neuroprotective action (Leng et al., 2012) synergistic with valproate and lithium. These mechanisms have been

addressed scarcely in pharmacogenomic studies. For example, several polymorphisms of the SCNA gene family, that codes the alpha subunit of voltage-dependent sodium channels, have been studied in association to lamotrigine efficacy on voltage-dependent sodium channel inhibition in vitro; lamotrigine efficacy varied significantly between rs3812718 polymorphisms (Thompson et al., 2011). Perlis and collaborators (2010) performed a genotyping study focusing on monoamine pathway, histamine, glucocorticoid and melanocortin receptor related genes and treatment response in a self-reported Caucasian population treated with either olanzapine/fluoxetine or lamotrigine; the authors did not find any associations between treatment response and the studied polymorphisms. Our study will contribute to widen the study of genes related to adverse events, metabolism and mechanisms of action of lamotrigine.

Fluoxetine: efficacy and safety as treatment of bipolar depression

Fluoxetine is currently FDA-approved for the acute treatment and maintenance of unipolar depression in participants ≥ 8 years of age. In 1987 it became the first SSRI to obtain an FDA approval for clinical practice; its sustained effectiveness and low side-effect profile, overdose safety and dosing practicality, make it a safe and effective treatment in psychiatry to date (Wong, 2013). As part of a commercialized component of olanzapine-fluoxetine combination, is one of the two FDA-approved treatments for bipolar depression (see above). In an 8-week, randomized, double-blind study (Tohen et al., 2003), comparing the combination of olanzapine-fluoxetine (n=86) with olanzapine alone (n=377) and placebo (n=370); the combination was associated with the highest response rate (56%), compared to olanzapine alone (39%) (P=.006; OR, 2.00; 95% CI, 1.23-3.26) and placebo (30%) (P<.001; OR, 2.92; 95% CI, 1.79-4.80). Brown et al. (2006) compared olanzapine-fluoxetine combination to lamotrigine and did not find differences in response between the combination (69%) and lamotrigine (60%) (p=0.73).

Fluoxetine efficacy as monotherapy treatment for bipolar depression was investigated by Cohn and colleagues (1989) in a 6-week randomized, double-blind, study comparing fluoxetine (n=30), imipramine (n=30) and placebo (n=29) as monotherapy or as add-on treatment to lithium (n=11, n=5 and n=6, respectively). Using the Hamilton Rating Scale for Depression (HAM-D) >50% reduction in total score as response measure, they found significant differences between groups: 86% fluoxetine, 57% imipramine and 38% with placebo (p<0.05, RR not reported). In the same study (n=0, 0%) of participants developed AIM+ with fluoxetine compared to imipramine (6.7%) and placebo (3.4%). Amsterdam and Schults (2010a) performed a one-arm open-label 14-week study of fluoxetine monotherapy (n=167) in bipolar II depressed participants. There were 88 responder participants (59.5%; CI, 51.1%–67.4%) and 86 remitter participants (58.1%; CI, 49.7%–66.2%). Six participants (4.1%) developed hypomania. Following the previous study, (Amsterdam and Shults, 2010b) a double blind randomized controlled study of fluoxetine vs. lithium monotherapy or placebo, in maintenance of participants that had previously responded to fluoxetine monotherapy was performed. The estimated relapse hazard for lithium monotherapy (n=26) was 2.5 times higher than fluoxetine (n=28). There was no significant difference in mean time to relapse. Furthermore, although reviews have found antidepressants to be equally or superior in effectiveness when compared to mood stabilizers (Salvi et al., 2008); randomized, double blind, placebo controlled studies (Sacks et al., 2007) and recent meta-analysis (Sidor and MacQueen, 2011) have found a limited value on antidepressant as an add-on or monotherapy treatment for bipolar depression.

In terms of risk, antidepressant agents have been associated with the emergence of mania in participants with bipolar disorder; this infrequent recurrence can often be a devastating consequence of attempting to ameliorate depressive symptoms. It is generally thought that all antidepressants have this potential and, therefore, may worsen the acute and overall course of bipolar disorder. It is generally thought that newer antidepressants, as compared with tricyclic antidepressants (TCAs), have a lower propensity to induce mania in participants treated for bipolar depression. Pooled data from several clinical trials reported a manic switch in 9 of 242 (3.7%) participants with bipolar depression treated with selective serotonin reuptake inhibitors (SSRIs), 14 of 125 (11.2%) treated with TCAs (P<0.01 vs. SSRIs), and 2 of 48 (4.2%) treated with placebo (Peet, 1994). Fluoxetine switch rate varied substantially between 0% (Cohn et al., 1989) and 50% (Amsterdam and Schults, 2005) in monotherapy studies for bipolar depression. It is clear from the limited and heterogeneous studies performed to date, that further investigation is needed to determine the efficacy and safety of fluoxetine in the treatment of bipolar depression.

Fluoxetine pharmacogenomics

The SSRI fluoxetine will be studied from a pharmacogenomic perspective, taking advantage of strengths which already exist within the Mayo Department of Psychiatry and Psychology in antidepressant pharmacogenetics as well as the long history of contributions by the Mayo PGRN to the pharmacogenomics of monoamine neurotransmitter metabolism. The 3BD Clinical Program will employ genetic association studies of genes encoding proteins in both pharmacokinetic and pharmacodynamic "pathways". Homogeneous well characterized phenotypes of drug response, adverse events, symptom and comorbidity will be analyzed.

Pharmacogenetic variation in cytochrome P450 (CYP) isoforms can result in large individual variations of the activities of these enzymes and, as a result, large differences in the pharmacokinetics of drugs such as SSRI antidepressants that are metabolized by these CYP isoforms. The widely prescribed SSRI, fluoxetine, is a racemate and has a half-life of approximately of 4 to 16 days; its mechanism of action is mainly through SSRI; although noradrenergic and dopaminergic action has been suggested, they seem of minimal or no clinical effect (Schatzberg & Nemeroff, 2009). Fluoxetine is a substrate of CYP2C9 and CYP2D6 (predominantly of the last) and it inhibits CYP2C19, CYP2D6 and CYP3A4. Since all 4 of these CYP isoforms display genetic polymorphisms, it is not surprising that it has been reported that the oral clearance of fluoxetine in CYP2D6 "poor metabolizers" is significantly less than in extensive metabolizers (Preskorn, 2003), with side effects raising to clinical concern (Sallee et al., 2003). Furthermore, fluoxetine is a P glycoprotein, a transport protein involved with many drugs, inhibitor (Marzolini et al., 2004). Human P glycoprotein, like the CYPs, displays common, functionally significant genetic polymorphisms (Marzolini et al., 2004).

In addition to the sources of possible pharmacokinetic pharmacogenomic variation mentioned above, pharmacodynamic factors are also a possible source for pharmacogenomic variation in response to these agents. Fluoxetine is an inhibitor of 5-HT reuptake, with the majority of its effect – at least in experimental animals – exerted as a result of its influence on serotoninergic neurotransmission. A recent meta-analysis (GENDEP, MARS and STAR*D Investigators, 2013) of the pharmacogenomics of treatment response, included the 3 largest pharmacogenomic studies of SSRIs found only genotypic trends; meanwhile, an analysis restricted to individuals treated with SSRIs citalopram (STAR*D) or escitalopram (GENDEP) identified an intergenic region on chromosome 5 associated with early improvement after 2 weeks of treatment. The authors concluded that in order to find stronger genetic associations of treatment response, larger case numbers and homogeneous phenotyping is needed and our study will address both necessities. The 3BD Clinical Program will contribute with a relevant number of participants with a detailed and homogeneous phenotypification (of treatment response, adverse events to treatment, correlation with specific symptoms/symptom change and comorbidity).

Antidepressant-induced mania (AIM+) in bipolar depression is still one of the main concerns when prescribing antidepressants; understanding the genetic risk factors associated with AIM+ would improve clinical care.. Sidor and MacQueen (2009) pooled treatment estimates for 1000 participants and although could not demonstrate a greater risk of AIM+ with antidepressants in general, when they performed analysis by groups of medication, found a risk on switching to mania of 43% for tricyclic antidepressants, 15% for venlafaxine, 7% for SSRIs and 5% for bupropion. Several studies of the potential association between AIM+ and variation in the serotonin transporter gene (SLC6A4) have been completed, focusing primarily on the length polymorphism in the promoter region, known as 5HTTLPR. A meta-analysis performed by our group found no significant evidence of association of the 5HTTLPR long/short variant with risk of AIM (Biernacka et al., 2012) but also identified significant methodological limitations in these studies such as lack of ancestry report, heterogeneity in the selected phenotype, lack of covariate analysis and environmental factors

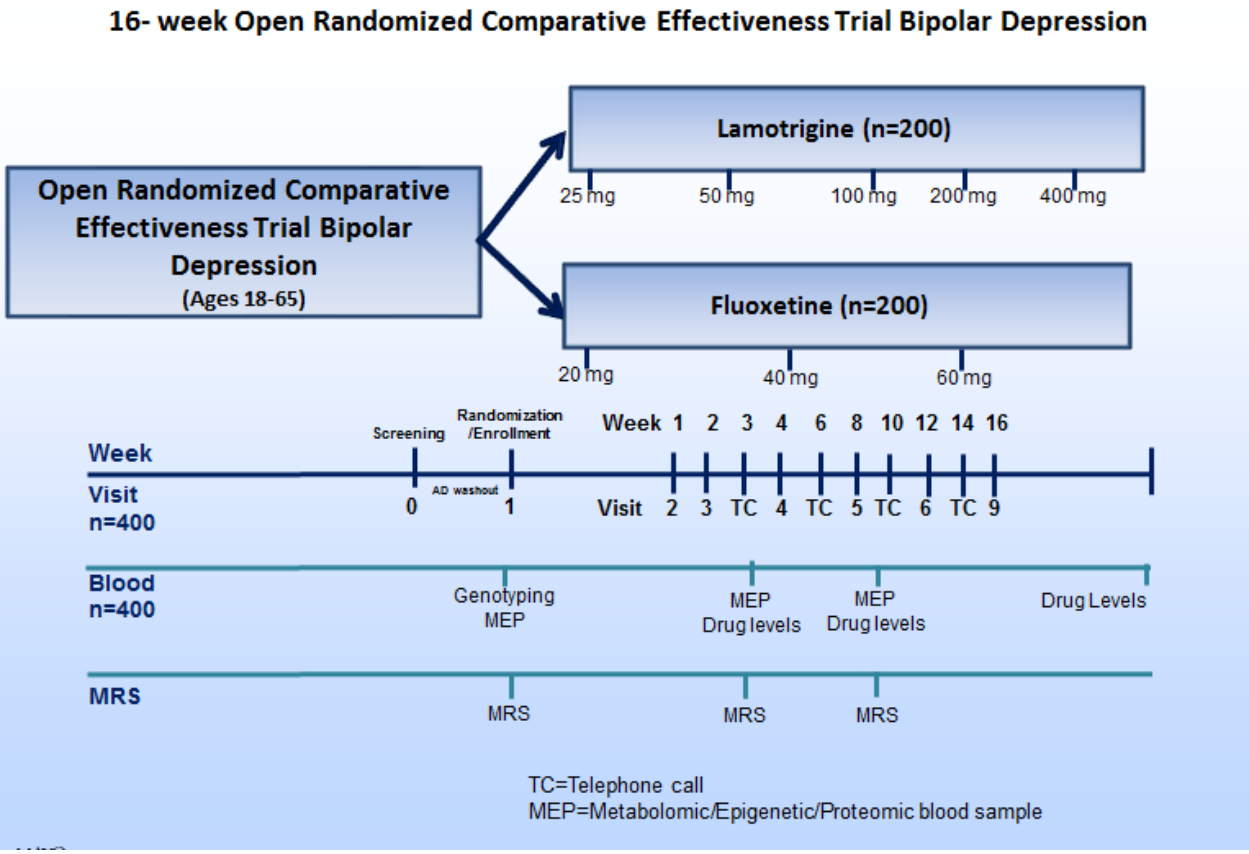
Identifying clinical and genetic risk factors associated with antidepressant-induced mania (AIM) may improve individualized treatment strategies for bipolar depression. From 2009 to 2012, bipolar depressed patients, confirmed by DSM-IV-TR–structured interview, were screened for AIM. An AIM+ case was defined as a manic/hypomanic episode within 60 days of starting or changing dose of antidepressant, while an AIM– control was defined as an adequate (≥ 60 days) exposure to an antidepressant with no associated manic/hypomanic episode. 591 subjects (205 AIM+ and 386 AIM–) exposed to an antidepressant and a subset of 545 subjects (191 AIM+ and 354 AIM–) treated with a selective serotonin reuptake inhibitor (SSRI) or serotonin-norepinephrine reuptake inhibitor (SNRI) were used to evaluate the association of AIM with phenotypic clinical risk factors previously published. 295 white subjects (113 AIM+ cases, 182 AIM–controls) were genotyped for 3 SLC6A4 variants: the 5-HTTLPR, single nucleotide polymorphism (SNP) rs25531, and the intron 2 variable number of

tandem repeats (VNTR). Tests of association with AIM were performed for each polymorphism and the haplotype. The only clinical risk factors associated with AIM in the overall and the SSRI + SNRI analysis was bipolar I subtype. The S allele of 5-HTTLPR was not significantly associated with AIM; however, a meta-analysis combining this sample with 5 prior studies provided marginal evidence of association (P = .059). The L-A-10 haplotype was associated with a reduced risk of AIM (P = .012). Narrowly defined, AIM appears to be at greatest risk for bipolar I patients. Our haplotype analysis of SLC6A4 suggests that future pharmacogenetic studies should not only focus on the SLC6A4 promotor variation but also investigate the role of other variants in the gene (Frye et al. 2015)

SCHEMATIC DESIGN OF THE STUDY:

The FLAME Study is a 16 week, open randomized clinical trial, evaluating the pharmacogenomic predictors of response to treatment and adverse events, of treatment with fluoxetine (n=200) and lamotrigine (n=200), for bipolar I, II, and bipolar schizoaffective depressed adults (18 to 65). Participants will be recruited over a 5-year period.

Figure 1. Timeline



Biomarkers that predict response or adverse events to treatment; and biomarker targets of response or adverse events to treatment will also be studied.

Description of Recruitment Methods:

How will participants be identified?
Potential participants will be identified through routine clinical appointments, advertising, and existing lists/databases of individuals interested in being contacted about participation in research studies.
The recruitment of this study will be based in Mayo Clinic, Rochester and Lindner Center of HOPE. Mayo Clinic in Rochester will coordinate the recruitment, data collection and analysis. For Mayo Clinic, Rochester, the main recruiting sources will be the Depression Center (Ge2A), Mood Disorders Unit (Ge3W), Adult Psychiatric Acute Care Unit (Ge2E), Primary Psychiatry and Psychology (GeMW), and Tertiary Psychiatry and Psychology (Mayo W11). Also, Mayo Midwest sites including Austin, Albert Lea, and Eau Claire will be considered for referring patients to Mayo Clinic Rochester. We will also be utilizing the Research Match (www.researchmatch.org) registry in order to obtain information about bipolar individuals who are interested in participating in research as an additional recruitment resource. Additionally, this study will be listed in www.clinicaltrials.gov.

How will participants be contacted?
The participants will be contacted by the Principal Investigator or study staff. Potential participants may be informed of the FLAME study during a routine clinical appointment. If a potential participant is currently hospitalized, permission to approach the individual will be obtained from the consultant providing psychiatric care to that individual. If a participant indicates interest in learning more about the study, a study Principal Investigator, Co-Investigator(s), or Coordinator will either talk with the participant in person or over the telephone to determine interest and eligibility. Potential participants may also come across one of the recruitment materials (listed below) and contact a Study Coordinator or Investigator directly to learn more about the project.

Potential participants identified from lists/databases of individuals interested in being contacted about future research studies they may qualify for may either be contacted via telephone (with an IRB-approved telephone script) or letter mailing (with an IRB-approved contact letter). The recruitment materials for this study include advertising posters and brochures posted in participant and employee waiting areas, physician referrals, and online listing in Mayo's clinical trials website and classifieds. The research team may also request to retrieve diagnosis data from medical records at the Mayo Clinic and Lindner Center of HOPE to compile a list of participants with bipolar disorder type I or II. An invitation to participate letter will be sent to the list of participants. Eligibility will be determined by the principal investigator or a psychiatrist who is a co-investigator by interview with the subject and by consultation of subject's medical record.

Participant Population:
Number of participants (total, each subgroup including anticipated dropouts, screen failures, nonvisible scan data) 1000 total, n=200 lamotrigine participants, n=200 fluoxetine participants.

Site	Anticipated Sample Collection
Mayo Clinic (Rochester and Austin)	400
Lindner Center of HOPE	100

Inclusion Criteria:
1. Adult participants, age 18-65.
2. Outpatients or inpatients with a diagnosis of bipolar I, II or schizoaffective bipolar disorder, depressed phase, non-psychotic, (DSM-IV criteria, SCID Module A and D confirmed).

3. At least mild symptom severity of depression as defined by the Clinical Global Impression for Bipolar Disorder (CGI-BP, Spearing et al. 1997) >2
4. Antimanic mood stabilization treatment. Bipolar I participants must be on conventional mood stabilizing treatment [lithium, divalproex or valproate, or an atypical antipsychotic]. Participants with Schizoaffective Bipolar Disorder must be on an atypical antipsychotic. Participants with bipolar II disorder may pursue the FLAME Study as monotherapy.
5. Negative urine pregnancy test.
6. Participants not planning pregnancy in the near future (6 months).
7. Negative urine toxicology screen (except cannabis).
8. No evidence of clinically significant laboratory screening tests (complete blood count (CBC); electrolytes; thyroid stimulating hormone (TSH); creatinine/BUN, AST/ALT). Clinical laboratory evaluation within the last three months is acceptable.

Exclusion Criteria:

1. Inability or unwilling to provide informed consent.
2. Inability to understand English.
3. Actively suicidal participants at screening or enrollment visit defined as a response of 3 or 4 on question 4 of the Bipolar Inventory of Symptoms Scale (BISS).
4. Active delusions or hallucinations defined as a score of 3 or 4 on the BISS question 40 (persecutory ideas) or 41 (delusions or hallucinations).
5. Impaired insight as defined as a score of 3 or 4 on BISS question 42 (insight).
6. Hypomania defined by a BISS manic subscore of ≥15.
7. Actively suicidal patients as defined by any of the following responses to questions from the C-SSRS: ”Yes” response to questions 4, “Yes” response to question 5, ”Yes” response to questions 1, 2 or 3 with scores of 4 or 5 in any of the intensity of ideation items within the last week. Axis I or II comorbidity that by referring mental health professional and/or study psychiatrist is primary need of treatment (This will be assessed by the site principal investigator, who has >10 years clinical experience with this population. Hospital discharge summaries and outpatient medical records may be reviewed (i.e. adequate trials of mood stabilizing treatments with minimal to no response, prominent self-injurious behavior in the absence of significant mood symptomatology, or atypical cycle patterns) to make this decision.
8. Pregnant participants.
9. Unwilling or unable to taper any current antidepressant therapy.
10. Participants currently breastfeeding.
11. Female not practicing a reliable form of birth control (condom, IUD, depo injection)
12. Due to lamotrigine pharmacokinetics, female subjects wishing to commence oral contraceptive therapy (OCT) within 3 months of enrollment date or anticipate discontinuing OCT during study (stable oral contraceptive therapy exception).
13. History of active substance abuse disorder within the last 3 months (other than caffeine or cannabis).
14. Participants with medical contraindications that preclude lamotrigine or fluoxetine treatment.
15. History of severe adverse reaction to lamotrigine and/or fluoxetine.
16. Current carbamazepine or oxcarbazepine treatment
17. Unstable active medical illness.

Step-by-Step Schedule

Enrollment and Study Visits

Pre-screening procedures:

A potential participant will meet with a member of the study team (PI, Co-I, Study Coordinator, or Research Protocol Specialist) to discuss details of the study and to answer any questions a potential participant may have. A telephone script will be used for this purpose and for telephone calls described during the visits.

If a potential participant agrees to participate in the Study, they will then sign the IRB-approved informed consent form.

Once informed consent is obtained, the participant will complete the Comprehension Assessment (CA) to ensure they understand what their role in the clinical trial is. The study team member completing the consent process with the participant will review the responses provided by the participant. Any incorrect responses will be reviewed in detail until the participant understands the nature of the statement -- these reviews will be documented next to the appropriate items on the CA. The completed CA will be stored with the original copy of the participant's informed consent form. The participant will be given a copy of the consent and the CA. Some research activities may occur after the initial participation process described above and the participant will be asked for their permission to be contacted in the future to obtain additional information for the study. These activities include annual computer linkages to a participant's medical record to ensure accuracy of the biobank database, requests to complete additional questionnaires, and requests for additional blood samples. All participants reserve the right to deny any requests for additional survey completion or sample collection.

Screening visit

Once informed consent is accepted and signed, the patient will taper off their antidepressants for two weeks and come back for the baseline visit, or if they are not on any antidepressants, the patient will complete the baseline visit the same day as the screening visit. The baseline visit will include an initial structural diagnostic interview will be completed by a member of the study team (PI, Co-I, Study Coordinator, or Research Protocol Specialist) using the SCID, Module A, D, E, and F to confirm the diagnosis of bipolar I, II or bipolar schizoaffective disorder, current depressive episode according to DSM-IV criteria. Additional rating scales and assessments will include:
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a. Bipolar Inventory of Symptoms Scale (BISS)
b. Clinical Global Impression (CGI-BP),
c. Actigraph
d. WHO-5
e. Bipolar Biobank Participant Questionnaire (BiB-PQ)*,
f. Bipolar Biobank Clinical Questionnaire (BiB-CQ)*,
g. Moodcheck
h. Columbia-Suicide Severity Rating Scale (C-SSRS) Assessing for the past 7 days

If a participant was previously enrolled in the 3BD study(IRB 08-00874 Mayo Clinic Individualized Medicine Biobank for Bipolar Disorder), the clinical questionnaire and patient questionnaire will be repeated if the participant was enrolled to 3BD after 6 months from the screening visit or if clinical judgement suggest that there were significant changes in the participants clinical history.

Participants who wish only to participate in the FLAME study can consent to limit their biospecimens for the pharmacogenomics and biomarker research studies stated in this protocol (and destroyed afterwards) or be available for future studies.

Participants will be asked additionally to participate in biomarker epigenetic and metabolomic/proteomic studies; for which they will be asked to donate two additional blood samples at week 2 and 4 of the comparative effectiveness trial.

If time is a concern, the participant will be allowed to take the BiB-PQ with them to complete it; the participant will be instructed to return the BiB-PQ either via mail or in person.

Selected participants may be asked to have their SCID interview audio/videotaped. These participants would be selected by the investigator and would sign a separate consent allowing permission to be taped. This tape would only be identified with the participant's study identifier and kept in a locked file. The purpose of these tapes would be to be used for inter rater reliability and quality assurance across the multi-site project.

If a participant fulfils all inclusion and exclusion criteria and he/she is not on an antidepressant at the screening visit, can continue directly to the enrollment visit and be randomized.

If the participant was on an antidepressant at the time of the screening visit, he/she is willing to discontinue it and it would be clinically reasonable to stop it, then the antidepressant will be tapered off in 1 week. The participant will have to be at least 7 days completely off the antidepressant before randomization. Then, the total number of days from screening to enrollment for these participants will be 14 days.

Patients enrolling in FLAME will also be given the option to participate in a separate study called 1H-MR Spectroscopy Before and After Treatment with Lamotrigine or Fluoxetine in Bipolar Depression (IRB 13-003660). This study is designed to only recruit subjects who participate in FLAME. This study will be conducted in tandem with FLAME.

Enrollment/baseline/randomization visit (could be at the same day of screening visit)

After consent is obtained and eligibility has been confirmed (inclusion/exclusion criteria; SCID, questionnaire ratings and blood drawn), RAVE® randomization case report form (CRF) will be submitted. Randomization will be done using blocking (blocks of 6 participants), and by study site with RAVE® (License) randomization tool. Block randomization will be utilized to assure balance between the 2 treatment arms
BPI disorder participants, who are randomized to fluoxetine and were not receiving any mood stabilizers at the enrollment visit, will be started on a conventional mood stabilizer just after randomization. These participants should be on a mood stabilizer for at least 7 days before starting study medication.

For participants who were tapered off their antidepressant medication, the eligibility criteria will be reassessed at the enrollment/baseline visit, using the BISS, C-SSRS will be performed at all study visits after screening visit and will be used to assess any changes since last study visit, and CGI-BP scales. SCID, CQ and PQ will not be repeated.

After week 16 of the study, a blood draw will be performed to evaluate medication blood-levels (see Biospecimen section below).

Psychometrics Testing:

Structured Clinical Interview for DSM-IV (SCID) (Michael et al., 2002) SC rated; the SCID will be administered to all participants for screening purposes for entry into the study. This is a structured interview that allows for making diagnoses in accordance with DSM-IV. The interview takes approximately 30 minutes, depending on the pathology of the subject. If a SCID has been completed by study team within 1 year prior to enrollment and no change has occurred in mood/condition of participant, the previous SCID administered can be used as part of this study instead of duplication the diagnostic interview.

Bipolar Inventory of Symptoms Scale (BISS) (Bowden et al. 2007, Gonzale et al. 2008,). The Bipolar Inventory of Symptoms Scale (BISS), a 44-item scale designed to encompass the spectrum of behavioral disturbances in Bipolar Disorder. The BISS total score and depression and mania subscales were compared to the Young Mania Rating Scale (YMRS), the Montgomery Asberg Depression Rating Scale (MADRS) and the Global Assessment of Functioning Scale (GAF). The BISS has adequate reliability, concurrent validity and is capable of discriminating between bipolar subtypes. It also provides a comprehensive scale to assess discrete behavioral components of Bipolar Disorder.
Clinical Global Impression Scale for Bipolar Illness (CGI-BP) (Spearing et al., 1997) Clinician Rated; The CGI as modified for bipolar illness allows for rating of degree of clinical improvement in depression, mania, and overall illness. Moreover, it addresses many of the specific criticisms leveled at the CGI relating to the types of ratings, technical and scaling problems, definition of time domain of the rating, and confounding of clinical response with tolerability and side effects. Index II of this instrument assess "change from preceding phase" of bipolar illness, rates of 1 (very much improved) or 2 (much improved) in the mania, depression and overall bipolar illness ratings will be considered the outcome measure of treatment response. Part "E" of the CGI-BD will be used to record side effects reported by the participant as well as their severity.

Mood Check Application (Utley 2015)
MoodCheck™ is a mobile application with the purpose of mood and medication monitoring designed for patients with depression or bipolar disorder. The platform is designed to engage patients in managing/quantifying symptoms and monitoring change associated with treatment intervention.
The app prompts users to enter their mood each day at a preset time that can be changed by the user for a "push notification" or alarm. Data takes less than 30 seconds to input, making it simple for patients to use. App users can automate reports and graphs to be sent to their mental health provider, allowing the provider and clinical team to analyze how the patient feels their medication dosage or medication brand is impacting their mood, while allowing a tracking mechanism for clinical trials, research studies, and overall patient care success.

WHO-5 Wellbeing index (Wu 2014) (Bech 1998)
The WHO-5 Well-being Index is a short, self-administered questionnaire covering 5 positively worded items, which are related to positive mood (good spirits, relaxation), vitality (being active and waking up fresh and rested), and general interests (being interested in things), and has proved to be a reliable measure of emotional functioning. Each of the five items is rated on a 6-point Likert scale from 0 (= not present) to 5 (= constantly present). The lower the total score is, the more severe the depression, poor physical health, and psychological health are. The higher the total score, the better the physical and psychological health. An answered score of 1 or 0 on any of these items means that it may be helpful to consult with a counseling professional. A score of 13 or lower suggests further investigation into possible symptoms of depression.

Columbia-Suicide Severity Rating Scale (C-SSRS), (Posner et al. 2008) Clinician Rated; 21 -item interviewer administered rating scale that measures current intensity of suicidal ideation and will be measured at baseline at each visit.

Actigraph ™
100 subjects (50 from Mayo Clinic and 50 from Lindner Center of HOPE) who are enrolled in this study will be asked to participate in a sub-study using Actigraphs (ActiGraph wGT3X-BT) to measure circadian activity and sleep patterns for a period of 16 weeks. The subject's decision to participate in this sub-study is completely voluntary and does not affect participation in the FLAME study.

Study visits are specified in Table 1.

Table 1. Study visits.

		Bipolar I or II Depressed Participants										
	Screening	Enrollment	Week 1	Week 2	Week 3	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14	Week 16
Procedure		V*1	V 2	V3	Telephone Call	V4	Telephone Call	V5	Telephone Call	V6	Telephone Call	V7
Consent	X											
Vital Signs		X	X	X		X		X		X		X
Drug Initiation		X										
Blood Draw		X		X		X						X
Urine pregnancy		X										
Toxicology Urine		X										
BISS	X	X	X	X		X		X		X		X
CGI-BP†		X	X	X		X		X		X		X
C-SSRS	X	X	X	X		X		X		X		X
MoodCheck		x	x	x	x	x	x	x	x	x	x	x
WHO-5		X										X
Compliance medication count			X	X		X		X		X		X
Actigraph		X										X
Treatment Adherence Form			X	X		X		X		X		X

*Visit (V). ** Only participants who accept to participate in biomarker epigenetic and metabolomic/proteomic studies. †Drug adverse events to treatment for pharmacogenomics phenotype are also recorded in the CGI-BP.
The visit window will be +/- 7 days from the scheduled date,

Study Medication

1. The study PI or Co-I will be in charge of medication administration, dosage and changes at baseline and follow-up visits:
2. Bipolar I, Bipolar II, or schizoaffective disorder depressed
3. Dosing for adults as monotherapy or on lithium or antipsychotic: LTG 25 mg daily x 2 weeks, 50 mg daily x 2 weeks, 100 mg daily x 2 weeks, 200 mg (100 mg bid)) x 4 weeks. If patient still has at least mild depressive symptoms (CGI ≥ 3) the dose can be increased to 300 mg daily for 2 weeks and 400 mg for 4 weeks. Dose will be held for treatment response and can be reduced for side effect.
4. Fluoxetine dosing: 20mg for month 1, 40mg for month 2, and if still depressed (CGI ≥ 3) 60mg for month 3 and 4. Lower doses of fluoxetine will be prescribed for those with side effects. For known 2D6 Poor Metabolizers, fluoxetine will not be dosed > 40mg.
5. Valproate Lamotrigine Titration: 25mg (half tablet) daily for week 1 and week 2, 25mg daily for week 3 and week 4, 50mg daily for week 5-6, 100mg daily for week 7-10, and 100mg daily for week 11-16.

Fluoxetine:
Given the dearth of FDA approved treatments for bipolar depression, antidepressants (FDA approved for unipolar major depression) are invariably prescribed; up to 49.8% of bipolar patients have reported initial treatment with an antidepressant (Baldessarini et al., 2007). In combination with an atypical antipsychotic (olanzapine), fluoxetine has been FDA approved for the treatment of bipolar depression. It is the only antidepressant combined with a mood-stabilizer that is FDA approved for bipolar depression. In the study by Tohen et al (2003), olanzapine plus fluoxetine combination (n=86) was associated with a significant reduction of depressive symptoms in comparison to olanzapine monotherapy (n=370) and placebo (n=377). In a study by Cohn et al (1989), fluoxetine monotherapy (n=30) was reported to be more efficacious than imipramine (n=30) and placebo (n=29) in patients with bipolar depression; furthermore, there were no cases of antidepressant induced mania among fluoxetine treated patients. Finally, in two studies by Amsterdam et al (2005, 2010), fluoxetine monotherapy (n=37 and n=28) was reported to have equal efficacy and no increased risk of antidepressant induced mania compared to lithium (n=26) or placebo (n=27), in the acute and prophylactic treatment of bipolar II depression.
There are no comparative effectiveness trials of fluoxetine in unipolar depression vs bipolar depression, however the evidence shows efficacy and safety of fluoxetine for bipolar depression,

Lamotrigine:
Lamotrigine is FDA approved for the maintenance phase or relapse prevention of bipolar I disorder and there is increasing impression of efficacy in acute bipolar depression. Geddes et al (2009) reported that bipolar depressed participants treated with lamotrigine were more likely to respond to treatment than those treated with placebo on both HAM-D (RR=1.27, 95% CI=1.09-1.47, Heterogeneity $\chi 2$=1.80 (df=4) p=0.772, Test of RR=1: z=3.13 p=0.002) and MADRS (RR=1.22, 95% CI=1.06-1.41, Heterogeneity $\chi 2$=3.12 (df = 4) p=0.538, test of RR=1: z=2.80 p=0.005). In a study by van der Loos et al (2009; n=124), the use of lamotrigine as an adjunctive treatment for patients already on lithium treatment was shown to significantly decrease depressive symptoms compared to placebo. Regarding safety of lamotrigine in bipolar depression, no AEs had greater than 5% frequency in the lamotrigine-treated groups, and no AE occurred at least twice as frequently in the lamotrigine-treated groups (n=1256) as in the placebo groups", as shown in a recent meta-analysis (Seo HJ et al, 2011). In the same study, it was shown that nonfatal serious AEs were observed in 6.6% of the lamotrigine treatment group and 7.2% of the placebo group. In general, lamotrigine has been considered a safe medication when used as a treatment for bipolar depression. The PI of this protocol has an ongoing IRB approved protocol looking at imaging markers of lamotrigine response which did not require an IND exception.

Risk mitigation plans:
As there will be 3 adverse events of special interest for this study (skin rash, antidepressant-induced mania and suicidal ideation), to mitigate those risks there will be a special monitoring and tracking of those events. At each visit, patients will be assessed for treatment-emergent manic symptoms using the BISS scale, for suicidality using the BISS and C-SSRS scales also and all study clinician will assess for skin rash at each visit. In addition, for populations were the risk of antidepressant-induced mania has been shown to be higher than placebo (bipolar I disorder), patients who are randomized to fluoxetine will be required to stay on a mood-stabilizer during the course of the study to decrease the likelihood of a treatment-emergent mania. A Data Safety and Monitoring Board will review safety data every 6 months.

Date and time of last dose of study medication will be gathered at the 1, 2, 4, 8, 12, and 16 week visits. To ensure compliance with the protocol, subject will bring in their medications at each visit for medication counts. The importance of medication compliance will also be discussed with the subject. To study medication compliance and pharmacokinetic pharmacogenomics blood levels of medication will be measured at week 16.

If participants are on current medications and are otherwise candidates for the study, their primary clinician will be contacted as to the relative merits of enrolling in the study. If it is clinically indicated (i.e. medicines are not helping optimally), the subject will be tapered off all psychotropic medications.

2. Adjunctive and concurrent medications. Adjunctive treatments are those used to manage transient associated symptoms (e.g., insomnia) or transient or longer-term medication side effects (e.g., sexual dysfunction). Adjunctive treatments that are not used to treat depression may be taken during the study. However, drugs in the exclusion criteria for this study may not be used as adjunctive therapies. Participants can participate in the study while receiving concurrent medications for general medical conditions as long as there is no contraindication to their use while taking fluoxetine.

3. Drug efficacy phenotype evaluation. The change in CGI-BP will constitute the major research outcome measure used to assess drug response phenotype because it is widely used in psychiatric research, making it possible to perform comparisons with other studies; it also provides a continuous phenotype of response to treatment. However, the BISS assessment will be administered at each research visit (week 0,enrollment week1,,2,4,,8,12, and 16),and the results of these scales will also be examined and analyzed as more specific phenotypes by item and continuous phenotypes of response of depressive and emergence of manic/hypomanic symptoms. The instruments will be assessed by a SC assisted by the PI/CoPI.

5. Drug adverse event to treatment phenotype evaluation. Drug side effects will be recorded as expressed by the participant.

6. Gene selection. Gene pathways will be selected for inclusion in the genotyping studies based on the characteristics of the probe drugs', lamotrigine or fluoxetine, as explained above in the introduction. Genes encoding proteins that metabolize or transport lamotrigine/fluoxetine or participate in CNS glutamate, calcium or monoamine neurotransmitter systems (especially 5-HT neurotransmission) are candidates for variation that might influence drug response. Genes encoding 5-HT, glutamate, catecholamine and calcium biosynthetic, metabolic, storage, reuptake, receptor and intracellular signaling proteins, plus lamotrigine/fluoxetine metabolizing and transport proteins will be potentially chosen for genotyping. These gene pathways are and will be identified as described in preceding and future pharmacogenomic literature. The genomic and pharmacogenomics study of bipolar disorder and its medication is continuously being enriched; the gene selection of the present study will evolve to reflect what is deemed scientifically relevant and innovative by current literature.

Early discontinuation

1. At each research visit, the BISS, and overall clinical impression (CGI-BP) will be completed. Early discontinuation will be defined as any of the
following: 1) hospitalization for depression, mania, or suicidality; 2) worsening of depression (CGI-BP change from preceding phase of much or very much worse); 3) treatment emergent mania (BISS mania score>20 and/or CGI-BP change from preceding phase of much or very much worse); 4) Actively suicidal patients as defined by a response score of 3 or 4 on question 4 in the Bipolar Inventory of Symptoms Scale (Thinks of suicide/death several times a day in depth, or has made specific plans, or attempted suicide) or actively suicidal patients as defined by any of the following responses to questions from the C-SSRS: "Yes" response to questions 4, "Yes" response to question 5, "Yes" response to questions 1, 2 or 3 with scores of 4 or 5 in any of the intensity of ideation items, 5) serious cutaneous adverse reaction meeting at least one of the following criteria: ≥20% body surface involvement, systemic involvement (fever, swollen glands, blood or liver function abnormalities), mucosal or ocular involvement, or hospitalization, 6) intolerable side effects or 7) other administrative criteria as determined by the research team. The dose of lamotrigine or fluoxetine will be discontinued immediately.

2. Special attention will be given to participants who develop suicidal ideation. Participants will be closely monitored at visits and also contacted by telephone to ensure medication compliance and participant safety. The SC will be supervised up by the study psychiatrist and principal investigator and will defer to the physician's judgment as needed. The SC will be trained to notify the RC at the time of the clinical visits or the phone interviews if the subject endorses any suicidal ideation or plans. All questionnaires that involve questions related to depression and/or harm to self or others will be reviewed within 1 working day of receipt from the subject. If a subject endorses suicidality with any of the following: Active suicidal ideation as determined by the investigator. If a subject responds positively to questions regarding the harm of self and/or others, the research team will immediately contact the subject in person or by telephone to discuss their responses. If the subject continues to endorse *suicidal ideation,* the investigator will immediately refer subject to the nearest emergency room and/or psychiatric admission on a voluntary or if need be, involuntary basis. If subject denies suicidal feelings and immediate referral to the emergency room deemed un-necessary, the subject will be recommended to get a referral for appropriate psychiatric consultation from the primary provider. Investigators will notify the primary provider about the need for such referral. If the subject does not have a primary provider, the research consultant will provide a referral for psychiatric consultation. The participant will be asked to provide contact information, including an emergency contact. In an emergency (as determined by the PI) the study team will reach out to the emergency contact if the participant cannot be contacted.

Remuneration: Participants will be provided study drugs for free and free parking when they come to any of the study visits or study related procedure.: The participant will be paid $50.00 at the first visit; last visit: $50.00.

Tracking and Retention

In order to achieve the aims of the study, significant effort will be made to insure high levels of retention throughout the study. Procedures that could be used to support high retention rates will include: (1) collecting names, addresses, and phone numbers of relatives and friends who are likely to know the contact information of the participants; (2) contacting participants with personalized letters and cards; (3) setting up a password protected electronic database with names, addresses, phone numbers, and projected times of proposed contacts for all participants; and (4) maintaining phone contact with participants throughout the study and reminding participants in advance of study appointments. If a participant misses a visit or a telephone call, this must be rescheduled within a week. If 3 consecutive visits are missed, the participant will be dropped from the study.

Management of Serious Adverse Events (SAE)

As mentioned in the prior sections, study participants will be screened for adverse events and serious adverse events by the study team. Regarding SAEs, we will utilize standard FDA classification and forms for SAEs handling and reporting. In general, SAEs will be defined as any event that:

- results in death
- is life threatening
- requires hospitalization (or a prolongation of hospitalization in already hospitalized participants)
- results in a persistent or significant disability or incapacity
- are congenital anomalies or birth defects
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention.

For the purpose of this study, treatment-emergent mania (as defined previously), treatment-emergent skin rash and treatment-emergent-worsening suicidality (as defined previously) will be treated as SAEs and then will follow the same procedures as any SAE with regards to reporting timelines.

Mayo Clinic's IRB defines an unanticipated problem/event involving risk to subjects or others (UPIRTSO) as any problem or event that was 1) serious; 2) unanticipated; AND 3) at least possibly related to the research procedures. The Investigator shall assess whether a problem/event may be an Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSOs). The Investigator will report UPIRTSOs as follows: If the UPIRTSO Occurs at Mayo Clinic or a site for which the Mayo Clinic IRB serves as the IRB of Record the principle investigator will submit to the IRB by completing the UPIRTSO form as soon as possible, but no later than 5 working days after the Investigator first learns of the problem/event. If the UPIRTSO occurs at a Non-Mayo site, the study team will submit to the IRB at time of continuing review as well as submit a modification to the protocol if other monitoring bodies (e.g. IRB at the site where the UPIRTSO occurred, or the Data Safety Monitoring Board) require changes to the protocol as a result of the non-Mayo Clinic problem/event. Non-UPIRTSOs will be reported at the time of continuing review. The PI will submit a brief narrative summary describing the nature, type, and frequency of events that have occurred since the last progress report. Additionally, the PI will continually monitor severity and frequency of non-UPIRTSO events throughout the course of the study and consider whether any changes caused such events to be considered UPIRTSOs. The external site PI will be in charge of reporting the SAE to the local IRB in less than 5 business days according to each IRB policy. The external PI will report the UPIRTSO/SAE to the Mayo PI.

Table 4. Summary of biospecimen collection in the FLAME Study.

	Baseline*	Week 2	Week 4	Week 16	Total volume in study
Laboratory tests	15 ml	-	-	-	-
Genomic/Biomarker	55 ml	55 ml	55 ml	-	-
Drug levels	-	10 ml	10 ml	10 ml	-
Total volume per visit	70 ml	65 ml	65 ml	10 ml	210 ml

Biospecimen

1. Baseline: Venipuncture will be performed using standard techniques. A total of 70 mL blood sample will be collected at baseline, taking into account laboratory tests and genetic studies.
The samples are detailed as follows:

1. a. Laboratory tests: A total of 15 mL will be collected for laboratory tests. One 10-mL EDTA tube (CBC, 10 mL total sample) and into one 10 mL no-additive serum tube (electrolyte panel, TSH, AST/ALT and pregnancy test; 5 mL total sample).

2. b. Biomarkers: Within the same informed consent for the FLAME Study, participants will be additionally asked to participate in biomarker epigenetic and metabolomic/proteomic studies. These two additional blood samples will be drawn at week 2 and 4 of the comparative effectiveness trial. Approximately, two blood samples of 55 mL each, with the same characteristics of blood collected in the 3BD Disease Risk Program (IRB-08-008794) will be collected from each participant that accepts to participate in the biomarker studies (see table 5)

2. Subsequent visits: Biomarker and drug levels

2. a. Drug levels: At week 2, 4 and 16, a total of 10 mL will be collected for blood drug level of fluoxetine and its metabolite norfluoxetine (5 mL); and lamotrigine (5 mL), in one 10 mL no-additive serum tube.

2. A total of 210 mL blood sample will be collected throughout the study for these participants, taking into account laboratory tests, blood drug levels, genetic and biomarker studies as summarized in Table 4.

3. Urine Pregnancy and Toxicology testing may not need repeating if the subject is screened and enrolled as an inpatient and these labs were already performed.

Table 5. Biomarker and Genetic Breakdown

Sample Type	Number of Tubes	Total Volume	Aliquots
Blood No Additive (10mL) Red top	2	20 mL	8 mL serum: 4 in 1 ml 6 in .5 5 in .2 12 ml RBC destroyed

EDTA (10mL) Lavender top	2	20 mL	4 ml DNA 125 ug 8 ml plasma: 4 in 1 ml 6 in .5 5 in .2 Buffy coats (WBCs) x 3: proteins (signaling proteins and enzymes) and DNA 125 ug Total DNA: 250 ug
Sodium Heparin (10mL)	1	10 mL	Cryopreserved PBMCs x 3

PAXgene Blood RNA tube (2.5ml) Clear	2	5 mL	Extract 1 tube (6 ug RNA) to calculate RIN number (quality of RNA) Freeze 1 tube RIN number
Total	7 tubes	55 mL	

* See 1.b. in this section. If the participant did not enroll in the 3BD Disease Risk Program (IRB-08-008794), or did so more than a month before enrolling the FLAME Study; a separated blood draw of 55 mL, with the same characteristics, will be collected as baseline sample (the total blood collection for this participant would be of 245 ml).

If the participant only consented to this study, his/her samples will be destroyed at the end of the study. Blood samples will be stored for the duration of the FLAME Study including all data and sample analysis.

Statistical Considerations

Endpoints:
There are two primary endpoints for the 3BD Clinical Program namely treatment response and adverse events. Detailed information on possible contributing factors (i.e. age, gender, previous treatment history, alcohol abuse or dependence comorbidity, seasonal and/or circadian rhythm, preceding stressful event, etc) will be evaluated.

Data Analysis:
We will investigate association between the primary endpoints a set of SNPS in the gene candidates and/or GWA studies to evaluate a greater number of SNPs and bioinformatics tools to perform pathway analysis. Primary analyses will evaluate the association between treatment response and adverse events phenotypes and SNPs in candidate genes by comparing genotype frequencies between the treatment responder and non-responder groups and the presence/absence of adverse events, using logistic regression, assuming a log-additive model for

allele effects. Logistic regression will enable multivariate analyses, and in particular to assess genetic effects on treatment response and adverse events, while adjusting for other possible risk factors, and modeling gene-environment interactions. Covariates modeled in the analysis will include age of onset of bipolar disorder, and presence or absence of circadian rhythm liability, concomitant medication, alcohol abuse or dependence comorbidity (lifetime), stressful events (defined as presence or absence of any stressful event in the last 12 months and as a score that will summarize the number of stressful events in the prior 12 months), binge eating, body mass index measures and other relevant phenotypes.

A large majority of the Mayo Clinic Rochester participant population is white, non-Hispanic. However, due to the greater ethnic diversity of participants at the other sites, we anticipate that approximately 25% of our sample may come from other racial or ethnic groups. Therefore, all of our genetic analyses will be adjusted for self-reported ethnicity and secondary analyses will be undertaken within ethnically homogeneous subgroups.

Power Statement:
Assuming a response rate of 50% (i.e. 150 treatment responders and 150 treatment non-responders), and assuming that 1000 independent SNPs will be evaluated and thus a significance level of 5*10-5 will be used for analysis, this study will have 80% power to detect odds ratios of 2.90 for SNPs with minor allele frequency of 0.2, or odds ratios of 2.35 for SNPs with minor allele frequency of 2.35.

However, there is a lack of consistency in results from prior studies of association of different genotypes (and GWA studies) in depression response to medication. There are two critical factors that influence the result of pharmacogenomics and genetic-translational studies in general; 1. The number of recruited participants,and 2. A well characterized phenotype (Hennekam and Biesecker 2012)). Recent meta-analysis on pharmacogenomics of antidepressants have identified the necessity for more homogeneous phenotypes needed for stronger associations (GENDEP, MARS and STAR*D Investigators, 2013). Using publically available data from the GAIN consortium, our group has demonstrated that the number of participants needed to find genetic associations in complex phenotypes, when they are homogeneous, can be reduced considerably (unpublished data; Winham, 2013). This study will focus on a strict homogeneous and highly reliable phenotypic classification of participants, both for treatment response and adverse events. In addition, unlike rigorous traditional clinical trials, pharmacogenomics trials benefit from a more “real practice setting” in the sense of including participants with comorbidity and non-ideal treatment panoramas like ours (Mrazek and Lerman, 2011).

We propose to use a pharmacogenomic approach to study the contribution of genetic variation in fluoxetine pharmacokinetic and pharmacodynamic pathways to variation in drug response to lamotrigine and fluoxetine in bipolar depression. The genes in each of the pathways to be studied will be tested, not merely for individual SNPs, but rather by selecting htSNPs for intragene haplotypes for genes that encode proteins which participate in these pathways.

Human Safety Aspects Risks: PROTECTION OF HUMAN PARTICIPANTS

1. Risks to the Participants
1a. Human Participants Involvement & Characteristics: The project will involve individuals with a DSM-IV diagnosis of bipolar disorder type I or II depressive episode. All participants will be aged 18-65 years and will be in relatively good physical health with no unstable medical conditions.

1b. Sources of Materials: Psychiatric diagnoses, psychiatric history, and other data collected from study participants will be obtained using standardized interviews and self-report measures. In addition, medical records may be obtained. This will be obtained specifically for research purposes. For laboratory analyses, samples of blood and urine will be obtained from participants for research purposes.

1c. Potential Risks: The risk of participating in a clinical interview is no greater than routine clinical care (i.e., minimal risk).Participating in mood interviews. The material covered is routine and consists of questions that they would be asked by the psychiatrists under normal clinical conditions. Standardized instruments will be administered. Participants are asked to answer questions on a voluntary basis and are told that uncomfortable questions do not have to be answered. Some questions that will be asked during the study interviews or questionnaires may make study participants uncomfortable. Participants have the right to choose not to answer any questions that are uncomfortable to them.

The risks of having blood drawn include pain, bruising, or, rarely, infections at the site of needle stick. These risks are no different than with any routine clinical blood draw.

There is the possibility that a participant's personal information could accidentally be disclosed. It is possible that this information could potentially be used to discriminate against a person, including socially or for insurance or employment purposes. The Bipolar Biobank will utilize safeguards such as de identifying data, using secure servers for databases, using locked storage for any hard-copy information, and privacy training to help ensure that a participant's information is not disclosed.

Researchers may determine that certain research findings may be valuable to participants. The risks to participants of learning the results of genetic testing can include emotional upset, changes in relationships, and/or insurance or employment discrimination. The Bipolar Biobank Managing Group and Phenotypic Concordance Committee will work with the Mayo Clinic Biospecimen Trust Oversight Group (BTOG) to determine if test results should be offered to participants. If results are offer red, participants will have the option to decline learning the information. If results are offered, it may be necessary for the participant to meet with a health care professional, such as a psychiatrist or genetic counselor.

Possible Risks for study medications
Fluoxetine is FDA approved for unipolar depression in adult and pediatric populations. Double-blind, randomized controlled studies in monotherapy (Cohn et al., 1989) and as a combination with olanzapine (Tohen et al., 2003; Brown et al., 2006) have found it effective for the treatment of bipolar depression. All participants will be informed of all known side effects. The most commonly observed side effects of fluoxetine are lightheadedness, fainting, dizziness, confusion, hallucinations, rhinitis, dry mouth, tremor, nausea, ejaculation disorder (primarily ejaculatory delay), impotence, fatigue, diarrhea, sleep disorders (somnolence), and sweating. The FDA has issued a black box warning for fluoxetine regarding antidepressants increased the risk compared to placebo of suicidal thinking and behavior (suicidality) in children, adolescents, and young adults in short-term studies of major depressive disorder and other psychiatric disorders.

For bipolar depressed participants participating in the open lamotrigine trial, lamotrigine is FDA approved for the maintenance phase of bipolar disorder. Although we are using the FDA bipolar drug in a different phase than its original indication, there is placebo-controlled data showing efficacy in the phase that we are studying (Calabrese et al., 1999; Frye, M.A. et al., 2000).

Most side effects of lamotrigine are mild and go away without stopping the drug. The most frequent side effects include dizziness, muscular incoordination, drowsiness, headache, insomnia, double-vision, blurred vision, bradycardia (slow heart rate), nausea, vomiting. If any of these side effects are experienced, the subject must not drive a motor vehicle until these symptoms have stopped. Approximately 16% of participants receiving lamotrigine for Bipolar Disorder in premarketing trials discontinued therapy because of an adverse experience – most commonly due to rash (5%) and mania/hypomania/mixed mood adverse events (2%).

Based on older guidelines for dosing of lamotrigine, there were some serious side effects, which in clinical trials of bipolar and other mood disorders occurred in approximately 0.08% (0.8 per 1,000) of adults taking lamotrigine as initial monotherapy and 0.13% (1.3 per 1,000) in adults receiving lamotrigine as adjunctive therapy. These side effects can be serious and potentially life-threatening. These are mainly side effects related to the skin, and include Stevens Johnson Syndrome (a severe rash around the eyes, mouth, genital, anal, and other skin areas), toxic epidermal necrolysis (severed blistering and sloughing of the skin), angioedema (swelling under the skin), and hypersensitivity syndrome (an allergy-like reaction with fever, swollen glands, rash, blood or liver problems, and facial swelling). Almost all of the life-threatening rashes have occurred within 2 to 8 weeks after starting to take lamotrigine. However, there have been a few people who developed these rashes months after starting lamotrigine. The only known factor that has been identified to predict the risk of the occurrence or severity of lamotrigine associated rash is age; pediatric participants are at greater risk than adults. Three factors have been suggested as potential causes for an increased risk of serious rash: 1) starting at too high of a dose, 2) increasing the dose too quickly, and 3) coadministration with valproate. The risk is increased to 1 out of every 100 adults who take a combination of lamotrigine and divalproex sodium. In worldwide post-marketing experience, rare cases of rash-related death have been reported, although the numbers are too few to estimate a risk. However, these deaths occurred when using the old dose recommendations. New data would suggest that with more conservative guidelines, as noted in the Physician's Desk Reference and as being followed in this study, this risk is reduced.
Non-serious rashes also occur when taking lamotrigine; however, it is not possible to know which rashes will become severe. Thus any rash, skin discoloration, blister, or mouth sore should be reported immediately. In general, lamotrigine should be discontinued at the first sign of rash, unless the rash is clearly not drug-related. Even if one stops taking lamotrigine, a rash could become severe or permanently disabling or disfiguring. In extremely rare cases, multiorgan failure has been observed in 2

out of 3,796 participants. In clinical trials with bipolar disorder participants, 2 participants experienced seizures shortly after abrupt withdrawal of lamotrigine. Though there were confounding factors that may have contributed to the occurrence of those seizures, it is important that lamotrigine should not be abruptly discontinued. Unless safety concerns warrant otherwise, the dose of lamotrigine should be tapered over at least a period of 2 weeks. Overdoses of up to 15 g have been reported, some of which were fatal. Overdose can result in ataxia, nystagmus, increased seizures, decreased consciousness level, coma, and intraventricular conduction delay. A recent findings based on FDA's review of adverse event reports submitted to the agency from December 1994 (when the drug was approved) through November 2009 indicate that Lamictal may pose a rare but increased risk associated with aseptic meningitis. A total of 40 cases of aseptic meningitis occurring in pediatric and adult participants taking lamotrigine were identified. The symptoms present in these cases included headache, fever, nausea, vomiting, nuchal rigidity, rash, photophobia and myalgias. These symptoms occurred 1 to 42 days after starting Lamictal. There was one reported death, although the death was not thought to be the result of aseptic meningitis. In most cases, symptoms resolved after lamotrigine was discontinued. Fifteen of these cases reported a rapid return of symptoms following re-initiation of Lamictal; symptoms recurred within 30 minutes to 24 hours following re-initiation of lamotrigine. Symptoms were frequently more severe after re-exposure. Although this is currently not a black box warning, this new rare but serious side effect will be included on an updated black box warning insert for lamotrigine. The research data for this study will be shared as part of the NIH Data Share policy. All participants will be identified by a unique enrollment identification number that will maintain their anonymity. All shared data will be coded by this I.D. All personal information will be kept separate and locked in a file only accessible by the Principal Investigators.
The above research pose greater than minimal risk to the subject, but are reasonable in relation to the anticipated benefits to better understanding of the illness. There is no guarantee that the study medication will improve a participant's condition. Based on prior research, we anticipate that those taking lamotrigine will experience improvements. Overall participation will provide useful scientific information about the study medication and its benefits for future participants with bipolar disorder complicated by alcohol abuse or dependence. Alternative treatments are available for bipolar depression and include the following medications: Lithium, divalproex sodium (Depakote®) carbamazepine (Tegretol®), atypical antipsychotics, and antidepressants. Electroconvulsive therapy is a non-medication alternative mood treatment.

Protection Against Risk: Participants will be assured that they do not need to answer any question on the mood or functional scales that which they do not wish to address. Participants will be fully informed of the risks of the medication and strongly advised to notify the doctor immediately if any symptoms of serious side effects (rash, aseptic meningitis or other hypersensitivities, antidepressant induced mania, serotoninergic syndrome) are noticed. The physician will discuss the symptoms with the participant. These symptoms are also detailed in the informed consent form. Subject will be cautioned to keep medication out of reach of children or others of limited capacity to read or understand because it may cause serious side effects and possible death. Participants will be carefully monitored throughout the study for either intolerable mood effects or medication side effects, and the doctor will discontinue a subject from the study if his/her psychiatric condition worsens, and will follow-up closely with other recommendations for treatment.

During the course of treatment with lamotrigine, participants will be closely monitored for any serious adverse cutaneous reactions (as mentioned above in drop-out section), rashes, aseptic meningitis, or hypersensitivities. Fluoxetine participants will be monitored closely for AIM+ or appearance of suicidal ideation/planning. If a subject notices any such symptoms, he/she will immediately report to the study doctor.

If the subject is a woman able to become pregnant, she will not participate in this research study unless she has a negative urine pregnancy test, is not breast-feeding, and is using an approved method of birth control. The following are considered to be approved methods of birth control: 1) intrauterine device (IUD), 2) barrier protection, 3) a contraceptive implantation system (Norplant), 4) oral contraceptive pills, 5) a surgically sterile partner, or 6) abstinence. Female participants will be instructed to inform the principal investigator if they have any reason to suspect pregnancy, if their circumstances have changed and there is now a risk of becoming pregnant, or they have stopped using an approved form of birth control. If a subject becomes pregnant, intends to start breast-feeding, or starts to breast-feed, she must notify the study doctor, because the effects of study medication on unborn or nursing

children are unknown. In the case of pregnancy, the study doctor can contact the Lamotrigine Pregnancy Registry for information by calling (800)336-2176.
Male subject will be required to use birth control methods.

Stopping Rules for Efficacy and Safety

In the case of withdrawal from the study, a subject will continue to be monitored even after he/she has discontinued study medications. If at the end of the study the subject is transferred to the care of a different psychiatrist, the study doctor will fully inform the new treating psychiatrist of the details of the subject's study involvement in order to provide continuity of care and for the subject's health and safety. Information regarding study participants will not leave the institution in any form that would identify an individual participant. Participants will be identified by code only. All files will be kept within locked cabinets or access-restricted computer networks within the institution. A Certificate of Confidentiality will be obtained to protect participant information. All procedures will comply with the Health Insurance Portability and Accountability Act (HIPAA).

A participant may withdraw their consent at any time. To have their specimen and information removed from the Bipolar Biobank, they must do so in writing. Withdrawal from the Bipolar Biobank will not affect any studies already conducted or those which have already received sample and/or information from the Bipolar Biobank.

Inclusion of Minorities
We recognize the importance of inclusion of minorities in medical research. The influence of ethnicity on the representation of specific polymorphisms in different ethnic and racial groups and their effects on response to medications is important. Participants of all ethnic and racial categories will be included. If results of this study clarify the role of polymorphisms in the genes that will be studied for treatment response, studies could be designed to address the possible role of these polymorphisms in minority groups. Collaboration with other research facilities would be required in order to achieve this goal, given the demographic characteristics of southeastern Minnesota.

Individual Participant Stopping Rules (if applicable): A subject has the right to withdraw consent at any point during the study. The investigator reserves the right to discontinue a subject's participation in this study at any time.

DSMB: Data & Safety Monitoring Plan (DSMP)
Per protocol guidelines, study participants must meet all inclusion criteria and none of the exclusion criteria. All criteria will be reviewed by the study coordinator and PI prior to commencement of study drug. The PI and study coordinator will meet on a weekly basis.

1. **Subject Safety**
Safety monitoring will be undertaken by a committee integrated by psychiatrists outside of this protocol consisting of: Yonas E. Geda M.D. Chair, Mayo Clinic Arizona; Robert Post, M.D., George Washington University and Director, Bipolar Collaborative Network; and Anne Duffy, M.D., FRCPC, University of Calgary. The DSMB will meet twice per year by phone with Dr. Mark Frye and Dr. Joanna Biernacka. The DSMB Board will review enrollment numbers, demographics, serious adverse events, CGI outcomes, and publications based on biobank data. There is no blind so there is no need for unbinding outcome data.

2. **Data Integrity**
RAVE® platform will be utilized for data collection by all centers. Data integrity will be managed by the project manager working with the study coordinator and supervised by the PI, in coordination with RAVE®.

3. **Subject Privacy**
Participants will be identified by a deidentified code only. All files will be kept within locked cabinets or access-restricted computer networks within the institution. A Certificate of Confidentiality will be obtained to protect participant information. All procedures will comply with the Health Insurance Portability and Accountability Act (HIPAA).

4. **Data Confidentiality**
The research data for this study will be shared as part of the NIH Data Share policy. All participants will be identified by a unique enrollment identification number that will maintain their anonymity. All shared data will be coded by this I.D. All personal information will be kept separate and locked in a file only accessible by the Principal Investigators. There will be no participant identifiers in the subject casebook.

5. **Product Accountability**
Medication will be stored with the Mayo Clinic Pharmacy. All prescriptions will be signed by a study doctor and picked up by a member of the study team.

6. **Study Coordination**
This study will be overseen by a Principal Investigator (PI), co-investigators, project manager, and a designated study coordinator. Every week, the PI, project manager, and the study coordinator will review the following information in detail at the study meeting:
1) Subject recruitment rate
2) Subject drop-out and the reasons for drop-out
3) Side effects and mood ratings per subject visit
4) Subject compliance with the protocol
5) Study coordinator questions or concerns

Questionnaires that ask about Depression (if applicable)
Included in study? Yes X No
If "Yes", state the plan of management for participants with possible depression:
There are no known risks to the participants participating in mood interviews. The material covered is based on standard clinical care and consists of questions that they would be asked by the psychiatrists under normal clinical care. Standardized instruments will be administered. Participants are asked to answer questions on a voluntary basis and are told that uncomfortable questions do not have to be answered.
All questionnaires that involve questions related to depression and/or harm to self or others will be reviewed within 1 working day of receipt from the subject. If a subject responds positively to questions regarding depression, the PI or other psychiatrist will contact the subject in person, by telephone, or by letter to inform the subject of their results and to offer a referral for appropriate consultation. If a subject endorses suicidality or responds positively to questions regarding the harm of self and/or others, the PI or another psychiatrist who is part of the study will be contacted to evaluate what clinical treatment should be recommended. At no time will the subject's relatives be contacted. This is due to the nature of confidential research results.

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