Cyclooxygenase-2-Inhibitor Combination Treatment for Bipolar Depression: Role of Inflammation and Kynurenine Pathway Biomarkers

ABSTRACT

A chronic sub-threshold pro-inflammatory status may interfere with the ability of an antidepressant to exert its full therapeutic action in bipolar disorder (BPD) patients whose depression has failed to respond or remit. Additionally, a pathological shunt in the kynurenine pathway may be involved in these delayed or diminished responses to antidepressant monotherapy.

We propose a placebo-controlled study to treat bipolar depressed patients not responsive to antidepressant monotherapy with the well-known antidepressant escitalopram in combination with the anti-inflammatory agent celecoxib. We hypothesize that combination treatment will result in augmented treatment responses. We also expect to see greater numbers of remitters in response to escitalopram + celecoxib combination therapy compared to escitalopram monotherapy.

BACKGROUND

Burden of Bipolar Depression

In bipolar disorder I and II, the depressive phase dominates over manic or hypomanic symptoms and most adversely affects the quality of life. In the natural course of bipolar I disorder (BPD), depressive symptoms occur in 31.9% of the weeks, and hypomanic/manic symptoms occur in 8.9% of the weeks the patient experiences symptoms (Judd et al, 2002). In bipolar II disorder, depressive symptoms occur in 50.3% of the weeks and hypomanic/manic symptoms in 1.3% of the weeks (Judd et al, 2003). Amongst 258 bipolar patients admitted from 1996 to 1999 to the Stanley Foundation Bipolar Network, 25% were ill with depressive symptoms averaging 214 days/year and 40% were ill with depressive symptoms averaging 120 days/year (Post et al, 2003a). Despite comprehensive pharmacologic treatment, mean time depressed was 3-fold higher than manic time. 62.8% of patients had 4 or more episodes per year. It was concluded that in bipolar patients' depressive symptoms pose a greater burden for treatment than mania (Post et al, 2003b). The suicide rate is highest in patients with bipolar disorder. It occurs most frequently after severe and recurrent depressive episodes (Tondo et al, 2003). Regarding response to antidepressant treatment, bipolar depressed patients generally respond more poorly than patients with major depression. In a study comparing response to antidepressant treatment of bipolar depressed patients versus that of patients with major depression, the non-response rate was 1.6 times higher in bipolar depressed patients. In another study, failure to respond to anti-depressant therapy was reported to be 3.4 times more common in bipolar depressed patients (Ghaemi et al, 2004). The reasons for the above observations are not clear. However, prompt diagnosis and specific therapeutic strategies are critical for the successful management of bipolar disorder.

Role of Inflammation

For decades our understanding of the pathophysiology of affective disorders and the development of antidepressant medications have been almost solely guided by the Monoamine Theory of Depression. While monoamine-based antidepressants have proven suitable as a mainstay treatment for most cases, their prolonged intervals to achieve symptom control, and the high percentage of non-remitters in both unipolar and bipolar depression have remained insurmountable attributes of these medications. New and innovative treatment strategies are therefore needed. Meanwhile, there has been an impressive accumulation of knowledge about pro-inflammatory compounds (e.g., cytokines, chemokines, C-reactive protein) contributing to the pathophysiology

of depression. This has led to the formulation of the Cytokine Theory of Depression (Ur et al, 1992; Dantzer et al 2008; Leonard and Song, 1996; Miller et al, 2009). There are numerous reports (Drexhage et al; Drexhage et al; Kim et al, 2007; Kim et al, 2002; Maes et al, 1995; Rapaport et al, 1999; Su et al, 2002; Tsai et al, 1999; Tsai et al, 2001) linking the immune system, in particular cytokines and C-reactive protein (CRP), to bipolar disorder. There is additional evidence that the persistent pro-inflammatory status found in depression may delay, diminish, and even thwart antidepressant response. Furthermore, a persistent pro-inflammatory status is likely increases the risk for depressed patients to develop serious and potentially life-threatening complications such cardiovascular and cerebrovascular disease.

There is an interaction between cytokines and serotonergic transmission involving the metabolism of tryptophan and modulation by the NMDA receptor. This interaction in the CNS occurs in part through the enzyme indoleamine 2,3-dioxygenase (IDO). IDO catabolizes tryptophan to kynurenine. Kynurenine metabolism has been implicated in the pathophysiology of depression, especially the chronicity of the disorder (Myint and Kim, 2003). The involvement of the NMDA receptor in manic relapse of patients with bipolar I disorder was first proposed by Hoekstra and colleagues (Hoekstra et al, 2006). The up-regulation of the initiating step of the kynurenine pathway has been demonstrated in postmortem anterior cingulate cortex from individuals with schizophrenia and bipolar disorder (Miller et al, 2006). A lower plasma tryptophan index, lower neuroprotective kynurenic acid and higher tryptophan breakdown has been observed in bipolar patients compared to controls (Myint et al, 2007a; Myint et al, 2007b). Our unpublished data also indicates that the kynurenine/tryptophan ratio in major depression may serve as an indicator of response to add-on treatment with the cyclooxygenase-2 inhibitor, celecoxib.

Pro-Inflammatory and Anti-Inflammatory Cytokines

Endothelial damage leads to the release of pro-inflammatory cytokines, which can induce thrombus formation, vascular occlusion, and have been implicated in the pathogenesis of atherosclerosis and cardiovascular disease (Koenig et al, 2001; McIntyre et al 2007; Piletz et al, 2000). Endothelial damage of the cerebral vasculature is postulated to contribute to the development of an entity referred to as Vascular Depression, which presents with many typical symptoms of depression. Studies have shown that pro-inflammatory cytokines are over-expressed in many stress-related disorders, including depression (Leonard et al, 1999; Tsao et al, 2006; von Kanel et al 2006).

Interestingly, depressed patients with or without cardiovascular disease have been shown to exhibit elevations in pro-inflammatory biomarkers (Dinan et al, 2009; Koenig et al, 2001; Meyer et al 2008). The pro-inflammatory cytokines TNFalpha, IL1beta, and IL6 have consistently been found to be elevated in the plasma of depressed subjects (Himmerich et al, 2006;Tsao et al, 2006; Tulugu et al, 2003), and elevated IL-6 concentrations have been associated with an increased prevalence of major depression (O'Brien et al, 2007). Interleukin-6 (IL-6) is secreted in response to stress, induces the release of other pro-inflammatory cytokines and has been implicated in the pathophysiology of MDD and in the effects of antidepressant treatment (Liciono et al, 1999). These observations have led to the postulation that depressive illness is a disease of inflammation in response to chronic psychological stress (Koenig et al, 2001).

Anti-Inflammatory Therapy in Depression: Existing Studies with Add-On Celecoxib

The presence of a pro-inflammatory state in bipolar disorder and the usefulness of anti-inflammatory treatment was the topic of a recent major review article (Berk et al, 2010). There have been 3 clinical studies describing the positive effects of various antidepressant agents with add-on celecoxib therapy. Each study independently concluded that administration of a COX inhibitor enhances the efficacy of the antidepressant. In some instances

non-responders remitted. All studies underscore the safety of celecoxib when used in doses up to 400 mg/day for 8 weeks.

Study No. 1: In a double-blind, randomized, placebo-controlled study, celecoxib + reboxetine was administered to MDD patients to determine if 6 weeks of combination therapy would result in improved outcomes as compared to reboxetine monotherapy. Celecoxib was titrated from 200 mg/d to 400mg/d over 3 days. Subjects receiving reboxetine + celecoxib showed a significantly greater antidepressant response by week-5 than subjects on reboxetine + placebo. Safety issues were assessed by self-reporting. No laboratory measurements were performed to assess hematologic or cardiovascular issues that might have arisen with drug combination. Mueller et al, 2006.

Study No. 2: In a double-blind, randomized, placebo-controlled study, add-on celecoxib or placebo was administered for 6 weeks to non-responsive bipolar patients experiencing depressive or mixed episodes while maintained on stable doses of multiple mood stabilizers, atypical antipsychotics, antidepressants, and benzodiazepines. A statistically significant improvement ($p= 0.03$) was noted in the first week of treatment with add-on celecoxib in those who completed treatment. This pilot study was limited by small numbers ($n=23$ for completers). The study did not measure drug levels or inflammation biomarkers. The overall finding was a better outcome in patients treated with celecoxib add-on. Nery et al, 2008.

Study No. 3: In a double-blind, randomized, placebo-controlled study, 40 acutely depressed MDD subjects were treated with fluoxetine + placebo or fluoxetine + celecoxib for 6 weeks. At 6 weeks of treatment, patients receiving combination therapy showed statistically more improvement than patients receiving monotherapy. The celecoxib add-on was well-tolerated in study patients. Plasma levels of fluoxetine were monitored and no significant drug-drug interactions in metabolism were noted. No inflammation biomarkers were measured. Akhondzadeh et al, 2009.

Using Celecoxib Instead of Another NSAID

There are more preliminary reports using CBX as an add-on treatment for depression than any other add-on NSAID strategy. Additionally, as a COX-2 inhibitor CBX lacks the substantive anti-platelet effects associated with other NSAIDs on the US market which are non-selective mixed COX-1/COX-2 inhibitors, such as aspirin (Riondino et al, 2008). Using CBX allows for reasonable confidence that changes (i.e., in biomarkers) could not result from indirect effects on platelets or coagulation. Furthermore, due to pharmacokinetic reasons the analgesic effects of CBX take effect slower than other NSAIDs. Having a 3-hour interval between medication ingestion and effect onset decreased the likeliness that a cue can be perceived by the patient, thereby breaking the blind.

In addition to these pharmacological issues, the use of CBX also rests on basic science findings linking COX-2 up-regulation to depression. Pro-inflammatory cytokines are known to activate the HPA axis and exert profound effects on central monoaminergic transmission (Besedovsky et al, 1991), and at least three of them (IL-1β, IL-6 and TNFα) are acted upon by COXs. While there are multiple isoforms of COXs, the main focus of the field has been on COX-2 because it is the isoform that is cytokine-induced through NFkappaB-dependent mode (Funakoshi-Tago et al, 2008). Stressors such as depression up-regulate transcription factor NFkappaB, which is mediator of pro-inflammatory cytokines in monocytes (Miller et al, 2009). Moreover, the predominant reaction of COX-2 is conversion of arachidonic acid to prostaglandin E2 (PGE2). Increased PGE2 in saliva, serum, and CSF of depressed patients has previously been described (Lieb et al, 1983; Calabrese et al, 1986). Since PGE2 stimulates IL-6 release, it seems likely that some of the changes observed in depression with IL-6 may be related to the COX-2 pathway.

The up-regulation of PGE2 has also been described in response to different physical, psychological, or mixed trauma experimental paradigms like social crowding, immobilization, or cold restraint stress in animals.

Furthermore, there is evidence that up-regulation of COX-2 can enhance glutamatergic neurotransmission. Sang (2007) demonstrated that PGE2 glyceryl ester (PGE2-G), a major COX-2 oxidative metabolite of 2-arachidonoyl-glycerol, enhances hippocampal glutamatergic synaptic transmission, inducing neuronal injury and death. This supports the fact that COX-2 has also been implicated in the pathogenesis of neurodegenerative disorders such as Parkinsonism, Multiple Sclerosis, Alzheimer's disease and traumatic brain injury. According to contemporary theories, depressive states are also neurodegenerative (Lieb et al, 1983; Calabrese et al, 1986; Yang et al, 2008). Taken together, there is a strong body of literature suggesting that COX-2 up-regulation in depression may pose a serious risk not only for the mood disorder itself, but also for the emergence of neurodegeneration at later time points in life.

Pro-Inflammatory Biomarkers: Anticipated Effect of Celecoxib Add-On
CBX inhibits COX-2 and its end-products (i.e. PGE2). Funakoshi-Tago et al. (2008) demonstrated that CBX significantly inhibits TNFα-induced NF-kappaB activation by preventing the nuclear translocation of the p65 NF-kappaB subunit. In a study of patients with osteoarthritis randomized to either CBX or aceclofenac, CBX potently inhibited PGE2 concentrations and down-regulated both COX-2 mRNA and protein expression at the synovial membrane (Alvarez-Soria et al, 2006). In a double-blind, placebo-controlled, crossover trial of CBX (200 mg BID) of patients with severe coronary artery disease undergoing stable background therapy with aspirin and statins, 2 weeks of treatment with CBX led to a marked reduction in two pro-inflammatory agents: CRP and oxidized LDL (Chenevard et al, 2003). Muller et al (2004) used add-on CBX (400 mg/d) with an antipsychotic, risperidone. Their study monitored soluble TNFα receptor levels before and during 5 weeks of treatment. CBX add-on led to significantly better-than-placebo responses in the total PANSS scores for schizophrenia improvement at weeks 2, 3, and 4 (Leonard et al, 1999). Finally, there are no major drug-drug interactions expected between CBX and ESC based on having common cytochrome P450 enzymes.

Pro-Inflammatory Biomarkers: Predictive Value with Respect to Antidepressant Response
Studies indicate that plasma levels of pro-inflammatory biomarkers could be predictive of treatment response or non-response. Lanquillon et al (2000) studied MDD inpatients before and after treatment with amitriptyline. Untreated MDD patients had overall higher levels of plasma CRP, TNFα, and IL6 than control patients. MDD patients fell into three groups during treatment: responders, non-responders, and drop-outs. Non-responders had the highest IL-6 values before treatment.

O'Brien et al (2007) compared patients at the end of a failed 6-week trial on an SSRI with patients who had previously failed an SSRI but were euthymic at time of blood drawing (due to switch to venlafaxine or SSRI augmentation with lithium). This study found SSRI-resistant patients have significantly higher levels of TNFα and IL6 compared to euthymic treatment responders.

Yoshimura et al (2009) compared TNFα and IL6 levels before and after treatment in antidepressant treatment responders and non-responders. High IL6 levels before and after treatment was indicative of non-responders, regardless of the agent used.

After 6 weeks of SSRI treatment, Dinan et al (2009) divided depressed patients into two groups: SSRI non-responders and SSRI-responders. Omega-3 fatty acid, eicosapentanoic acid (EPA) was shown to be a state marker. EPA competes with arachidonic acid (AA) as a substrate for cyclooxygenase-2 (COX-2). These studies

provide evidence that pro-inflammatory biomarkers may be clinically useful in predicting antidepressant response.

Genetic Testing
The association between depressive disease and serotonergic transmission is well established. Similarly, there is a compelling link between depression and risk for developing cardiovascular disease. Abnormalities of serotonergic mechanisms in depression have been described and reviewed (Leonard 2000 and Mendelson 2000) in the literature over the past decades. Studies have reported decreased platelet aggregation in response to serotonin, but Shimbo et al. (202) reported a significant increase in platelet reactivity to serotonin in depressed patients. Whyte et al. (2001) demonstrated that depressed patients with the serotonin-transporter-linked promoter region l/l genotype have increased platelet activation compared to both depressed patients without this genotype and non-depressed control subjects. These authors hypothesize that persons with this polymorphism are likely to experience higher mortality rates. More recent evidence shows that certain pro-inflammatory cytokines (e.g, TNFalpha, IL-6) reduce serotonergic transmission by inducing the enzyme indoleamine dioxygenase (IDO) and shunting tryptophan away from the synthesis of serotonin. Taken together, these findings strongly indicate that genotyping could be used to identify patients at greater risk of developing depression and depression-associated cardiovascular morbidity and mortality (White et al., 2001).

Salivary Cortisol Testing
Recent evidence suggests that a prolonged inflammatory response can lead to dysregulation of the hypothalamic-pituitary-adrenal axis, potentially due to pro-inflammatory modulators such as TNF-a and IL-6 (Straub et. al, 2011). HPA axis dysfunction has the potential to lead to an overall increase in secretion of cortisol and disruption of the well-established diurnal rhythm of cortisol, a glucocorticoid released in response to stress (Zunszain et. al, 2011). Evidence suggests that variation of the HPA axis and subsequent alterations in cortisol levels may be associated with the depressive phase in bipolar patients (Van der Werf-Eldering et. al, 2012). Obtaining cortisol through salivary collection has shown to be an effective, non-invasive method for evaluating the HPA axis, and research shows that there is a strong correlation between salivary and blood levels of cortisol (Marques et. al, 2010).

HYPOTHESES AND SPECIFIC AIMS

HYPOTHESIS: The pro-inflammatory state of BPD subjects can delay, diminish or even thwart antidepressant monotherapy. Combination therapy of escitalopram + celecoxib will result in improved treatment outcomes (faster action, augmented response, and/or higher remission rates) for treatment-resistant BPD subjects. Response to antidepressant treatment in BPD subjects will be proportional to the change in pro-inflammatory status before and after treatment.

> **SPECIFIC AIM #1:** Determine if combined pharmacotherapy of escitalopram + celecoxib will result in improved affective responses compared to escitalopram + placebo. Scores from the Short-Form McGill Pain Inventory will be used to control for variation in depression that could be attributed to the relief of physical pain resulting from administration of celecoxib.
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> **SPECIFIC AIM #2:** Determine whether combined pharmacotherapy of escitalopram + celecoxib causes any pharmacokinetic drug-drug interaction or poses safety/tolerability issues in terms of gastrointestinal bleeding or cardiovascular health. Clinical and laboratory assessments will be made throughout treatment and an adverse events inventory will be completed at each visit.

SPECIFIC AIM #3: Determine the blood concentration of pro-inflammatory biomarkers of all subjects before and after treatment. Determine whether there is a correlation between blood concentration of pro-inflammatory biomarkers and the psychiatric effectiveness of the combined treatment.

SPECIFIC AIM #4: Quantify the specific anti-inflammatory action of celecoxib by assaying for inhibition of PGE2. PGE2 concentrations will be measured from the blood of all subjects before and after treatment.

HYPOTHESIS: A chronic pro-inflammatory state leads to endothelial dysfunction, arterial stiffness, and dis-regulation of the autonomic nervous system with sustained sympathetic activation and diminished vagal tone.

SPECIFIC AIM #5: Determine whether endothelial dysfunction is present in BPD subjects and if this dysfunction will respond favorably to the combined treatment of escitalopram + celecoxib. The detection will be accomplished by means of by arterial pulse wave analysis, velocity and heart rate velocity.

HYPOTHESIS: A pathological shunt in the kynurenine pathway exists in BPD patients who fail to remit on antidepressant treatment. This shunt interferes with the ability of an antidepressant agent to exert its full therapeutic action.

SPECIFIC AIM #6: Determine the nature of the kynurenine pathway in all subjects before and after treatment.

HYPOTHSESIS: A genetic polymorphism identified in the 5-HT-transporter-linked promoter region may predispose subjects to depression and associated cardiovascular risk.

SPECIFIC AIM #7: Genotype the serotonin transporter length polymorphic region (5-HTTLPR) on all subjects to determine if the 5HTTLPR alleles associate with bipolar depression, inflammation biomarkers, heart rate variability and endothelial dysfunction.

STUDY ENDPOINTS

The primary endpoints pertain to the main hypothesized outcome: namely, a significantly better-than-placebo mood response for escitalopram + celecoxib that is not related to altered pain perception.

The HAM-D rating scale (17 items from the 21-item scale) will be the main measure of clinical improvement. Onset of response is defined as the day of treatment when a 30% decline in the initial (day 0) HAM-D score is recorded and is maintained for two consecutive weeks. Treatment response is defined as a 50% reduction in the initial HAM-D score occurs. Symptom remission is defined as a HAM-D score of 7 or less.

I. Primary Endpoint

We seek to establish whether the combination therapy of escitalopram + celecoxib will augment antidepressant efficacy compared to escitalopram therapy alone as demonstrated by:

- a significantly earlier group response (earlier decline in HAM-D scores)
- a more robust group improvement in HAM-D scores at 8 weeks of treatment
- more patients with symptom remission than in the escitalopram + placebo group at 8 weeks

II. Secondary Endpoint
We seek to determine whether there is a reduction in pro-inflammatory biomarkers at end of treatment in patients randomized to receive the combination treatment. Three key pro-inflammatory biomarkers (TNFalpha, hsCRP and VEGF) shown to be robustly elevated in depressed patients will be measured. Baseline values will be compared to age and sex matched controls from a normative dataset of healthy subjects. We will also attempt to subtype BPD patients by a median split into two groups (high and very high) according to their baseline biomarker levels.

III. Second Tier Exploratory Endpoints
Our "second tier endpoints" are exploratory in nature. Our study is not powered for them since existing data in the literature is insufficient to calculate an accurate sample size for these endpoints.

1. We will seek to establish whether a specific psychiatric symptom profile may be associated with the anticipated differential mood improvement in response to escitalopram + celecoxib or escitalopram + PBO. To that end, additional rating instruments have been included (over and above the HAM scales) and detailed assessments will be made of symptom profiles obtained from the rating instruments.

2. We will assess the safety and tolerability of the combination treatment by means of an adverse events symptom checklist and by relevant laboratory measures (e.g., CBC, PT, PTT).

3. Assessment of the efficacy of celecoxib by measuring PGE2 response to assess the extent of COX-2 inhibition.

4. An assessment of blood levels of escitalopram and celecoxib will tell us if these two agents interfere or promote each other's uptake and/or clearance.

STUDY DESIGN

This is a 10-week, randomized, double-blind, two-arm, placebo-controlled study. The study includes a screening visit, a 1-week washout phase, a 1-week placebo run-in phase and an 8-week flexible dose phase. A minimum of 70 subjects will be randomized. The randomization code will be created and kept by the pharmacist in sealed envelopes for each individual subject. The PI will have access to the randomization code through the pharmacist if there is a medical safety issue.

At the screening visit, subjects will undergo a psychiatric interview. The diagnosis of Bipolar Depression (BPD) will be established in this examination using portions of the MINI. The study will be explained to all subjects. If a subject meets inclusion criteria, he or she will be asked to review the consent form and agree to participate. Subjects who meet the screening criteria and sign the IRB-approved consent form will undergo comprehensive assessments in order to quantify depression and associated symptoms.
At the end of the screening visit, subjects will be instructed how to taper the antidepressant they have been taking over the course of one week. A one-week taper is sufficient for all antidepressant medications except fluoxetine. Subjects taking fluoxetine will undergo a two-week taper/wash-out. After the taper, all subjects will return for a second visit to be placed on a 1-week single-blind placebo run-in. At the second screening visit, the subject will provide a saliva sample prior to undergoing assessments. Subjects will be instructed how to take pills (escitalopram placebo 10mg/day and celecoxib placebo 400mg/day) until the next visit one week later. Due to the fact that a one-week placebo run-in may be insufficient for some patients to reliably establish whether or not they are placebo responders, there will be an option to extend the placebo run-in for one additional week, at

the discretion of the PI. No increased risks are anticipated with the extension of the placebo phase, as patients are evaluated weekly and in close telephone contact.

At the Baseline visit, subjects will be reassessed for depression and associated symptoms. If subjects continue to score 18 or higher on the 17-item HAM-D scale, they will be randomized to receive escitalopram + celecoxib, or escitalopram + placebo in an assignment ratio 1:1. Subjects will then go on to complete assessments at Week 1, Week 2, Week 4 and Week 8. If the subject scores below 18 on the 17-item HAM-D scale at Baseline, he or she will be terminated from the study and classified as a placebo responder. At each of these visits, subjects will arrive 30 minutes before their schedule appointment time, rest for 20 minutes in the exam room, and provide a saliva sample prior to undergoing any assessments. Terminated subjects will be offered conventional care at the same institution.

It is our desire to observe the diurnal nature of cortisol levels in saliva by measuring the patient's salivary cortisol levels at a time point prior to treatment and post treatment. At each of these two time points, we would collect saliva from the patient at five times during the day: upon awakening, approximately 30 minutes past awakening, before lunch, at approximately 3pm, and before bedtime. The patient would be required to do this at home and would then store the samples in their refrigerator until their next visit. Subjects will be provided with a log to record the exact time of saliva collection. We will be using salivettes purchased from Sarstedt Inc., and our Salivary Assay kits will be purchased from Salimetrics, LLC.

The overall study is powered for 90 subjects. We aim to randomize 70 subjects at LUMC. Approximately 30 subjects will be randomized by investigators at our sister site in Munich, Germany. At LUMC, 35 subjects will be randomized to each arm of the study. Subjects must complete 8 weeks of active medication to be considered study completers. We plan to enroll approximately 80 patients in anticipation of a 10% drop-out rate. The drop-out rate is based on our experience with our patient population over the past 5 years. If a subject drops out or is terminated after Week 6, their last observation will be carried forward in the analysis (LOCF). There are no healthy controls in this study because we already possess normative, untreated data for comparison.

SUBJECT SELECTION

I. Target population

We propose to study bipolar depressed subjects (BPD) whose depression has failed to remit following one or more adequate trials with an antidepressant, or who are experiencing a breakthrough depressive episode in spite of being maintained on a mood stabilizer and/or an atypical antipsychotic agent. Manic/hypomanic symptoms must have responded adequately to a mood stabilizer other than lithium. Subjects will be maintained on a mood stabilizer and/or atypical antipsychotic throughout the proposed study. Subjects who are maintained on lithium will not be included since there is a potential adverse interaction with celecoxib.

The following eligibility criteria are designed to select subjects for whom protocol treatment is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular subject. Re-screenings may be permitted at the discretion of the PI.

II. Inclusion Criteria

- Male or female of any race and 18 - 65 years of age at time of screening visit.
- Diagnosis of BPD I or II (DSM-IV-TR (296.52, 296.53, 296.89) without significant co-morbid secondary medical or psychiatric diagnoses.

- Depressive symptoms that failed to remit in response to antidepressant therapy OR current breakthrough depressive episode in spite of being maintained on a mood stabilizer and/or an atypical antipsychotic agent.
- Managed mania/hypomania with a mood stabilizer other than lithium, or an atypical antipsychotic.
- A minimum score of 18 on the Hamilton Depression Scale (HAM-D), 17-item.
- Willingness to institute lights-out at 11:00pm hours on the nights before blood drawings.

III. Exclusion Criteria

- Any abnormal findings on the physical exam, ECG, blood/urine or minor infections.
- Abnormal results on liver or bone marrow function as indicated by liver function or CBC with differential blood tests.
- Any pre-existing physical pain condition, including fibromyalgia.
- History of peptic ulcer complicated by perforation, hemorrhage, or obstruction; symptoms of peptic ulcer within 4 weeks of enrollment date.
- Substance abuse or dependence during the preceding 12 months.
- Clinically significant hypertension, anemia, liver disease, kidney disease, arthritis, diabetes, recurrent migraines, epilepsy, stroke, gum disease, autoimmune disease. Based on the judgment of the PI, a risk assessment should be done if a subject meets any of this clinical criteria but is otherwise suitable.
- Current use of lithium.
- Current use of a stimulant.
- Current use of any anticoagulant agents.
- Current use of nicotine-containing substances. Subjects who stop using nicotine-containing substances more than 1 month prior to assessment may be considered for the study.
- Certain steroids including use of hormonal birth control and any systemic or topical corticosteroids (hormone replacement therapy will be allowed).
- Unwillingness to refrain from H2 receptor antagonists, non-aspirin NSAIDs, ginko biloba, vitamin E or fish oils, or aspirin over 81 mg/day.
- Sensitivity or allergy to the study medications.
- Need to receive agents that are contraindicated in combination with celecoxib or escitalopram.
- Unwillingness to fast and abstain from caffeine on mornings of blood drawings.
- A sleep disorder other than insomnia or hypersomnia as a distinct symptom of MDD.
- Inability to commit to the follow-up visits between 8:30am and 12:00pm.

IV. Randomization Criteria

- Subjects must not have responded or remitted as measured by the HAM-D 17 after 1 week of treatment with single-blind placebo.

V. Criteria for Temporary Suspension of Study Participation Pending Clarification

- HAM-D 17 score of less than 18 at Baseline visit, indicative of a placebo responder.
- Subject attempts suicide or seriously deteriorates.
- Develops signs or symptoms suggestive of an ulcer or kidney disease or other serious illness.
- Develops elevated blood pressure, creatinine, potassium, or decreased hematocrit.
- Requires corticosteroids, warfarin, ticlopidine, or any anticoagulant.
- Takes more than 4 doses per week of any of the following: Vitamin E or fish oils (at doses > 600 IU/day), non-aspirin NSAIDs or aspirin (>81 mg/day).

VI. Criteria for Subject Termination

- Subject attempts suicide or seriously deteriorates; if so, appropriate care will be provided.
- Develops serious complications of an ulcer, such as gastrointestinal bleeding, perforation, or obstruction.
- Engages in behaviors or develops any condition that the PI deems makes it medically unsafe or inappropriate to remain in the study.

STUDY TREATMENTS AND COMPLIANCE

Subjects who meet the eligibility criteria will receive one week of single-blinded escitalopram placebo + single-blinded celecoxib placebo. Subjects who continue to meet eligibility at randomization will receive open-label escitalopram (beginning at 10mg/day) + double-blinded treatment of celecoxib (fixed at 400mg/day) or placebo. Escitalopram doses may be optimized based on efficacy and tolerability over the 8 weeks of active treatment. Subjects will be randomized according to a fixed assignment ratio of 1:1 (escitalopram + celecoxib: escitalopram + placebo). Assignment to groups is based on a pharmacy generated randomization code.

I. Compliance

To ensure compliance, participants be asked to return all unused medication at the following visit. The returned capsules will be counted. Additionally, blood concentrations of escitalopram and celecoxib will be determined at Baseline, Week 4 and Week 8.

II. Procurement of Compounds

Escitalopram and celecoxib will be purchased from the pharmaceutical companies that market them. In the US celecoxib is marketed by Pfizer and escitalopram is marketed by Forest Laboratories. The pharmacy will produce placebo identical in appearance to celecoxib and escitalopram.

STUDY PROCEDURES

Schedule of Events

Procedure	Screen 1	Screen 2	Baseline	Week 1	Week 2	Week 4	Week 8
Consent	X						
Assign Subject ID	X						
MINI	X						
HAM-D/HAM-A/MADRS	X*	X*	X	X	X	X	X
CGI/CSSRS/AdverseEvents/CSULGIE	X		X	X	X	X	X
Self-Assessment Packet			X	X	X	X	X
Dispense Study Medication		PBO	X	X	X	X	X
Physical Exam	X						X
Drug Screen and Pregnancy Test	X						X
Routine Clinical Labs	X						X
Research Blood Draw			X			X	X
CBC/PT/PTT Monitoring	X				X	X	X
Pulse Wave Analysis			X			X	X

Heart Rate Variability			X				X
Albuterol Challenge			X				X
Pulse Wave Velocity			X				X
Saliva Sampling		X	X	X	X	X	X

*HAM-D only.
Routine Clinical Labs include complete blood count, complete metabolic panel, lipid profile and thyroid function.

The following assessments are included in the self-assessment packet:

- Beck Depression Inventory (BDI)
- Beck Anxiety Inventory (BAI)
- State/Trait Anxiety Inventory (STAI)
- Spielberger Anger Expression Inventory (STAXI)
- International Physical Activity Questionnaire (I-PAQ)
- Quality of Life and Enjoyment Questionnaire (QLES-Q)
- The Short-Form McGill Pain Inventory (MPI)
- Perceived Stress Scale (PSS-14)

Subjects will be treated as outpatients for a total of ten weeks. Subjects must complete at least six weeks to be regarded as completers. If a subject chooses to withdraw from the study on or after six weeks of treatment, he or she will be asked to complete end-of-study assessments at that time. Those results will be carried forward for the purpose of data analysis.

Subjects will not receive any other type of therapeutic intervention (e.g., psychotherapy) during participation in the study. Such options may be offered to them at the conclusion of the study, if appropriate.

Subjects who complete the study and respond favorably will remain under the care of the LUMC physician, if they wish.

ASSESSMENTS

I. Psychometric Data Collection Instruments
Mini International Neuropsychiatric Interview (MINI)
Hamilton Rating Scales for Depression (HAM-D) and Anxiety (HAM-A)
Beck Depression Inventory (BDI); Beck Anxiety Inventory (BAI)
MADRS-Montgomery Asberg Depression Rating Scale (MADRS)
Columbia Suicide Severity Rating Scale (C-SSRS)
State/Trait Anxiety Inventory (STAI)
Spielberger Anger Expression Inventory (STAXI)
Inventory of Clinically Significant Upper and Lower GI events (CSULGIE)
Short-Form McGill Pain Inventory (MPI)
Perceived Stress Scale (PSS-14)
Quality of Life and Enjoyment Questionnaire (QLES-Q)
International Physical Activity Questionnaire (IPAQ)

II. Blood Sampling

Blood samples will be drawn at trough levels after instructing subjects to ensure a 12-hour interval after the last dose in the evening preceding the morning blood draw. Plasma, serum and buffy coat will be immediately separated and frozen at -80°C for future analysis.

Measurement of Plasma Pro-Inflammatory Biomarkers

These measures will be obtained from blood drawn at Baseline, Week 4 and Week 8 using the Randox Technologies "Evidence InvestigatorTM" system. This compact Biochip array system is comprised of a super-cooled Charge Coupled Device (CCD) camera and unique image processing software. This Biochip technology has been validated using blood samples from our preliminary studies. Several cytokines, chemokines and growth factors can be analyzed simultaneously using microliter volumes of plasma. The results produced by the Biochips are impressive and comparable to results obtained by individual ELISAs for each parameter. This assay will be completed in an LUMC lab.

Measurement of Serum Kynurenines and the Tryptophan Pathway

These measures will be obtained from blood drawn at Baseline, Week 4 and Week 8. The measurement of tryptophan and kynurenines has been established in the Psychoneuroimmunology (PNI) Laboratory of the Psychiatric Hospital of LMU, Munich using Ultra Performance Liquid Chromatography – Mass Spectrometry (UPLC-MS) methodology. The method has been standardized by the participation of 6 universities in the External Quality Assurance Scheme as a part of the EU collaboration project MOODINFLAME. The measured metabolites are: tryptophan, kynurenine, kynurenic acid, 3-hydroxykynurenine, 3-hydroxyanthranillic acid and quinolinic acid. This assay will be completed by collaborating investigators in Munich, Germany.

Measurement of Blood Levels of Celecoxib and Escitalopram

These measures will be obtained from blood drawn at Baseline, Week 4 and Week 8. Plasma concentrations of celecoxib and escitalopram will be measured using a solid phase HPLC method. This measurement will help the study team determine whether subjects are compliant, adequately dosed and if celecoxib changes escitalopram blood levels, or vice versa. Celecoxib levels will be assayed by collaborating investigators in Munich, Germany. Escitalopram levels will be assayed by collaborating investigators in Charleston, SC.

Measurement of COX-2 Inhibitory Level in Blood via PGE2 Activity

These measures will be obtained from blood drawn at Baseline, Week 4 and Week 8 using an EIA kit from Cayman Chemical Inc. This fast and reliable method uses LipoPolySaccharide (LPS) stimulation and a special Cremophor EL-EtOH vehicle to solubilize and assay PGE2. The method is rapid and eliminates COX-1 activity artifact using aspirin and an available thromboxane synthase inhibitor (TXBSI). This assay will be completed in an LUMC lab.

Monitoring of Possibility of Bleeding Diathesis

These measures will be obtained from blood drawn at Week 2, Week 4, and Week 8. Since the combination of an SSRI and a COX-2 inhibitor has the potential to induce bleeding diathesis, a complete blood count (CBC), prothrombin time (PT) and partial thromboplastin time (PTT) will be evaluated. Prothrombin Time and aPTT testing are performed on the Sysmex CA-7000 analyzer utilizing the scattered light detection method. The analyzer irradiates red light (660 nm) onto a mixture of blood plasma and reagent and detects the change in turbidity (when the fibrinogen is transformed into fibrin) as the change in scattered light. A curve is generated, plotting time against the amount of scattered light. This sample will be processed by LUMC Clinical Laboratories.

III. SphygmoCor® Assessments

All SphygmoCor® measures will be obtained using the non-invasive SphygmoCor® device (SCOR-Vx, AtCor Medical Pty, Ltd) equipped with the most recent software (v9.0, AtCor Medical, West Ryde, Australia). Brachial blood pressure will be recorded at the same session using a standard method.

Pulse Wave Analysis to Quantify Arterial Stiffness

Subjects will undergo the Pulse Wave Analysis at Baseline, Week 4 and Week 8. Pulse-wave analysis (PWA) is a non-invasive, FDA-approved technique that allows for the assessment of arterial stiffness. Pulse wave measurements have been established as prognostic markers of cardiovascular morbidity and mortality (Papaioannou et al, 2007; Weber et al, 2007). These wave indices may also serve as prognostic markers of response to therapy (Weber et al, 2007; Williams et al, 2006). A portable tonometric probe is positioned over the maximal arterial pulse of the artery under study. A minimum of twenty sequential pressure waveforms must be recorded. SphygmoCor® Software generates mean peripheral and corresponding central and aortic pressure waveforms using a validated transfer function. Intra- and inter- observer reproducibility of these measures has been established in various populations (Papaioannou et al, 2007; Wilkinson et al, 2002). Resting wave form will be recorded using standard PWA technique, as described by Nutt et al (2006) and Rybakowski et al (2006).

Albuterol Challenge to Assess Endothelial Function

Subjects will undergo the Albuterol Challenge at Baseline and Week 8 to assess endothelium-dependent vasodilation. Albuterol (2 x 200μg) will be given by inhalation with a spacer device. The subject will be asked to slowly inhale for 50 seconds, and then hold breath for 10 seconds. After a 30 second rest, the procedure will be repeated. Albuterol induces vasodilation by way of receptors on the endothelium through a nitric oxide pathway (Hayward et al, 2002; Wilkinson et al, 2002). Pulse Wave Analysis will be performed at 0, 5, 10 and 15 minutes after albuterol treatment. Responses to the Albuterol Challenge will be defined as the maximum possible difference between Baseline augmentation index and post-treatment augmentation index.

Pulse Wave Velocity

Subjects will undergo the Pulse Wave Velocity assessment at Baseline and Week 8. The assessment involves the measurement of pulse waves at the common carotid artery and on the femoral artery using the aforementioned probe. A 3-lead ECG will be used to simultaneously obtain heart rate values. ECG recording during measurements is necessary for synchronization of carotid and femoral pulse wave times. The distance from the sternal notch to the femoral location and from sternal notch to the carotid location are measured manually and entered into the program, transit time between carotid and femoral pressure waves is calculated and Pulse Wave Velocity is PWV computed by the software.

Heart Rate Variability

Subjects will undergo the Heart Rate Variability assessment at Baseline and Week 8. This technique allows for the assessment of sympathetic/parasympathetic imbalance in depression, which may be central to the development of cardiovascular disease secondary to depression. HRV is a natural physiological function of a healthy heart capable of responding and adapting to changing demands of life circumstances.

IV. Safety Assessments and Adverse Events Monitoring

A complete physical examination with clinical laboratory assessments will be performed at enrollment and termination. Bleeding diathesis monitoring will be completed with higher frequency, as outlined in the schedule of events. Any negative clinically significant changes from the entry examination will be recorded as adverse events.

Adverse event (AE) monitoring will formally occur after weeks 1, 2, 4, and 8 of treatment. Subjects will be asked to describe health concerns since the last visit. Suicidality will be assessed at each visit by direct questioning and the Columbia Suicide Severity Rating Scale (C-SSRS).

All adverse events will be monitored until stable regardless of when the event takes place. If the event occurs at the end of the study, it will be referred to the subject's primary care physician and/or psychiatrist for continued treatment and/or follow-up.

ADVERSE EVENTS REPORTING

All AEs will reported, regardless of whether the event seems related to the study drugs escitalopram and/or celecoxib. Study staff will also report AEs spontaneously reported by the patient and/or observed by the staff. Information that will be collected includes:

- A description of the event
- Start and stop date
- Action taken with regards to investigational product
- Outcome
- If the AE causes the patient to discontinue the study
- A statement reporting if the AE meets criteria for a Serious Adverse Event or not (see below)
- The investigator's assessment of the causal relationship between the event and the study drug
- Intensity of the AE
    - mild (awareness of sign or symptom, but easily tolerated)
    - moderate (discomfort sufficient to cause interference with normal activities)
    - severe (incapacitating, with inability to perform normal activities)

All serious adverse events (SAE) will be documented and reported to appropriate regulatory bodies. All adverse events will be summarized in a report at intervals requested by the IRB and the Data Safety Monitoring Board.

ETHICS AND EVALUATION OF RISKS

I. Consent Process Overview

Each subject will be presented the Informed Consent Form after meeting criteria for inclusion. Subjects will be asked to read the Informed Consent Form carefully. Subjects will be told about the effects of celecoxib and escitalopram. Subjects will be told that their participation in this study could benefit patients with BPD in the future. Subjects will also be informed of potential risks and discomforts. Any subject who appears incapable of providing informed consent (e.g., due to cognitive impairment) will not be enrolled. Subjects will be informed that they can decline to participate in the study without penalty or loss of treatment. Subjects will also be informed may withdraw from the study prior to analysis of their data. Following a thorough resolution of any questions, the subject will be asked to sign the consent form if he or she agrees to participate.

II. Evaluations of Benefits and Risks/Discomforts

Each subject will be given a medical and psychiatric evaluation. Subjects who receive active treatment may experience greater improvement or possible remission of their mood disorder, as well as improved biomarker

levels and arterial compliance as measured by PWA. To compensate for time spent and inconvenience experienced during the study, a predetermined compensation fee will be given.

Physical risks to subjects are minimal. Discomfort may result from the blood drawing. There may be bruising at the needle site. Certain individuals may feel light-headed during venipuncture. Venipuncture will be performed when the subjects are sitting or recumbent to avoid injury. Discomfort may be experienced due to tedious questionnaires and interviews.

Side effects listed in the Physician's Desk Reference for albuterol include hoarseness, headache, tremors, and palpitations. A hypersensitivity reaction to the drug has been reported in very rare cases. To avoid this risk, subjects will be asked if they have had previous exposure to albuterol, and if they displayed a hypersensitivity reaction. If any adverse reactions are noted after the first administration, the subject will receive no further exposure to the drug.

Discontinuation of subjects' current antidepressant medication is unlikely to lead to worsening of depression or suicidal thoughts because eligible participants are only those who are already depressed whose depression has not responded despite attempts to treat depressive symptoms with antidepressant medication. Subjects will be seen one week after beginning the wash-out phase and one week after beginning the placebo run-in, allowing study staff to frequently assess the subjects' mental status while not on any antidepressant therapy in the unlikely event that subjects begin to feel more depressed or suicidal. Subjects will also be encouraged to contact study staff throughout the duration of the study if they notice any changes in their mental or physical health. If in the opinion of the investigator the patient develops serious suicide risks, the patient will be withdrawn from the study and provided treatment as appropriate. Should this occur the subject will be responsible for the costs of treatment.

III. Adequacy of Protection Against Risks

The rights to privacy for participation in this research and safety of confidential information are paramount. These rights will firstly be protected through coding of data and storage of research records in a locked room. Records that contain identifiable subject information will be stored either in locked cabinets or on a locked computer in secure electronic files. These records will be kept confidential to the fullest extent permitted by law.

OTHER STUDY DRUG CONSIDERATIONS

I. Safety Issues of Celecoxib and SSRIs

There are potential risks associated with taking escitalopram. The most commonly reported side effects of escitalopram are dizziness, drowsiness, and light-headedness. Less commonly reported side effects include: headache, dry mouth, constipation, change in appetite, sweating, palpitations, upset stomach, abdominal pain, weight gain, dyslipidemia, insulin resistance, muscle twitching, and abnormal movements of the face. At the doses we are using, most of the side effects experienced by patients taking escitalopram should be mild to moderate and go away with continued treatment.

Celecoxib is the only coxib available in the US, but carries a FDA-mandated black-box warning for increased cardiovascular risk. The FDA issued this warning based on numerous observational studies and randomized clinical trials on cardiovascular risks associated with all COX-2 inhibitors, but the degree of this risk varies substantially among the different COX-2 inhibitors. Rofecoxib was removed from the market several years ago,

but celecoxib only received a black box warning. Celecoxib is still used widely because it appears at least as safe as other marketed NSAIDs. The full explanation of why celecoxib appears to be safe while other COX-2 inhibitors are not is an area of active research. A review of the literature shows this may relate to the fact that celecoxib possesses a number of artery-positive off-setting affinities compared to rofecoxib, a more pure COX-2 inhibitor. Whatever the reason, celecoxib appears safe at the dose regimen we will prescribe.

II. Co-Prescription of Celecoxib and Escitalopram at Loyola Medical Center in 2009

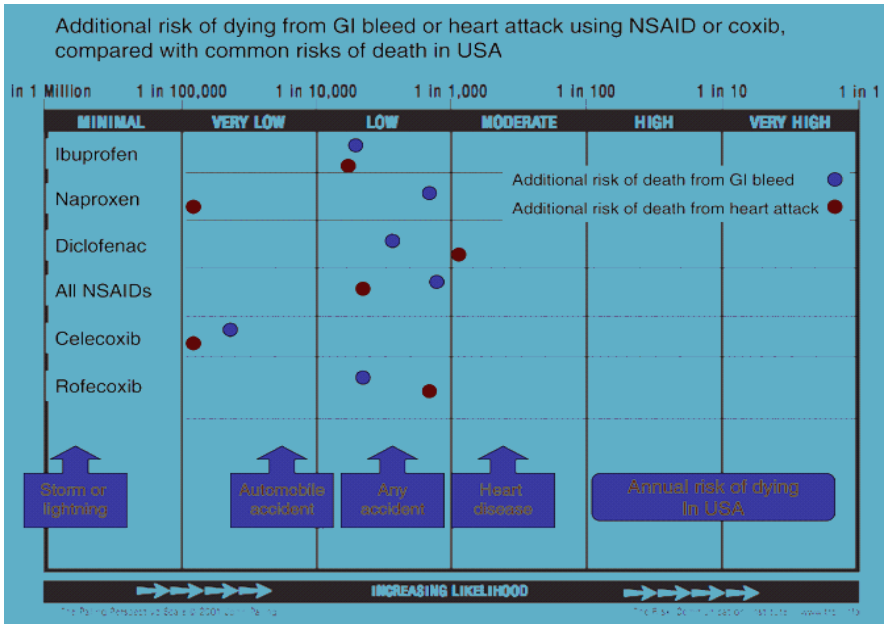
There is no evidence that celecoxib and escitalopram interact in any way. As far as we know, their biomolecular targets (COX-2 and serotonin transporter) do not have shared affinities. Escitalopram has low potential for clinically significant interactions. Studies *in vitro* have shown that the biotransformation of escitalopram to its demethylated metabolites depends on three parallel pathways (cytochrome P450 (CYP) 2C19, 3A4 and 2D6). Celecoxib, on the other hand, is metabolized by 2D6. Nevertheless, since we know it is common practice to combine celecoxib with antidepressants, mostly in non-psychiatric settings, we conducted an internal review of all prescriptions of celecoxib in combination with an antidepressant throughout Loyola Medical Center during 2009. The results are presented in summary form below. As shown, escitalopram represented 19.2 % of all prescriptions of an antidepressant co-prescribed with celecoxib. No adverse events were recorded in a total of 224 cases receiving the combination at our institution.

Situations Resulting in Combination	No. Cases	Serious Adverse Events from Combo
Primary treatment of mood disorder	21	0
Primary treatment was the antidepressant for other than mood disorder	17	0
Primary treatment for pain (2nd depression?)	28	0
Post-surgery	6	0
Cardiovascular disease present	38	0
GI disease present	10	0
Reason unspecified for combination treatment	104	0
Total	224*	0

*Of all the incidents of antidepressant co-prescriptions with celecoxib in 2009, escitalopram was the agent co-prescribed 19.2 % of the time. There were no serious adverse events reported due to any antidepressant in combination with celecoxib at Loyola Medical Center in 2009.

NSAIDs are known to carry a risk of gastrointestinal bleeding. However, this is mostly a property of COX-1 inhibitors. Escitalopram may also impose a minor risk of prolonged bleeding through its platelet serotonin depletion property. Because of this property, we will monitor platelets during treatment. However, the risk of bleeding appears very low when the odds ratios published in a recent review article is considered (Loke 2008). The odds ratios of gastrointestinal bleeding were summarized from all prior studies of SSRIs alone, NSAIDs alone, and SSRIs + NSAIDs. A meta-analysis was performed on published papers representing a total of 153,000 patients. The article also obtained 161 potentially relevant private company reports covering four SSRIs – fluoxetine, paroxetine, sertraline, and escitalopram – in regard to adverse event frequencies involving GI hemorrhage. The article also assembled all post-marketing reports of adverse events submitted to the FDA or to the Canadian Adverse Events Database. From this large database they concluded that the odds ratio of developing GI bleeding from an SSRI alone was 2.36. By comparison, the odds ratio of developing GI bleeding from an NSAID alone was 3.16. Finally, the odds ratio of developing GI bleeding from concomitant use of an

SSRI and an NSAID was 6.33. However, it is important to realize this was for *all* NSAIDs combined. COX-2 inhibitors were found to be involved in only a fraction of the total NSAID+SSRI adverse event cases producing GI bleeding. A much lower rate of bleeding risk is expected in our study because the median ages and durations of treatment in the review exceed those of the Loke review (median age 66 years, median length of drug exposure 25 weeks). Also, the studies assembled were not limited to the 400 mg/d dose of celecoxib we will prescribe. Therefore, developing a bleeding diathesis from the combination treatment should be very low: we estimate no more than 1 per 350 cases.



The above graph compiled by the Risk Communicator Institute illustrates the very low risk celecoxib poses for GI bleed or heart attack compared to other agents. It is directly comparable to that of naproxen and lower than that of ibuprofen, both of which are sold without prescription.

Another potential risk to subjects concerns confidential information. It will be explained that our study is not designed to produce confidential data of any kind that presents any known risk to the subjects. However, it will be acknowledged in plain language that we cannot guarantee that in some unpredictable way either their background information or the samples could become a future liability.

III. Accounting for Pain Perception

No previous study with an NSAID has considered analgesia as a possible confounding variable in the reported augmented antidepressant response to the anti-inflammatory add-on agent. Because NSAIDs possess analgesic properties and physical pain can be variably present in depression, the issue must be considered. To underscore the point, the Sequenced Treatment Alternatives to Relieve Depression multi-center study found that 80% of 2,876 outpatients with MDD complain of painful physical symptoms (Pilowsky et al, 1984; Husain et al, 2007; Leuchter et al 2009). None of the previous studies on celecoxib excluded patients with active physical pain or sought to quantify the pain dimension during treatment. As a result, we feel it is important to control for and evaluate pain perception. Therefore, subjects with a pre-existing pain condition will not be enrolled in the study. Furthermore, we will use the Short-Form McGill Pain Inventory to assess the visit-to-visit pain perception of subjects.

DATA ANALYSIS AND STATISTICAL METHODS

I. Statistical Analysis

Specific Aim 1 is to determine if combined pharmacotherapy of escitalopram + celecoxib will result in earlier and/or improved affective responses compared to escitalopram + PBO. Additionally, Specific Aim 1 seeks to control for pain perception in this analysis. For this aim, the patients in the two treatment arms will be compared on HAM-D, HAM-A, MADRS, BDI, and BAI scores. This will be accomplished with an analysis of covariance conducted at weeks 1, 2, 4 and 8. Baseline mood scores and McGill Pain Inventory scores will be used as covariates. In addition, the number of patients who are and are not classified as remitters at week 8 (those having a HAM-D score of 7 or less) will be tallied and cross-referenced with treatment group (escitalopram + celecoxib or escitalopram + PBO). The frequencies in the resulting 2-by-2 contingency table will be analyzed with the Pearson Chi-Square Test for Association.

For Specific Aim 2, dealing with treatment safety and tolerability, the numbers of patients who do and do not experience adverse events at weeks 1, 2, 4, and 8 will be tallied and cross-referenced with treatment group. The frequencies in the resulting 2-by-2 contingency table will be analyzed with the Pearson Chi-Square Test for Association. Additionally, to understand whether any pharmacodynamic interaction exists between escitalopram and celecoxib, blood levels of escitalopram and celecoxib drawn at weeks 4 and 8 will be compared for patients in the two treatment groups with an analysis of covariance using baseline levels as a covariate. If there is no pharmacokinetic interaction then we expect that there will be no statistically significant results for these analyses.

For Specific Aim 3, determining whether there is a correlation between blood concentration of pro-inflammatory biomarkers and the psychiatric effectiveness of the combined treatment, three types of analyses will be conducted. First, at the end of treatment we expect that general inflammation will be lower for patients taking celecoxib. Hence, the week-8 measures of TNF-alpha and hsCRP for patients in the two treatment groups will be compared with an analysis of covariance using baseline values as a covariate. Second, based upon information from our previous research, we anticipate that patients with the highest inflammation values, who receive celecoxib with escitalopram, will derive the greatest therapeutic benefit. Hence, we expect an inflammation-by-study group interaction. Therefore, the association of Week 8 HAM-D scores and baseline TNF-alpha values will be examined by way of multiple regression analysis. Week 8 HAM-D scores will be the criterion variable. The regression equation will include predictor terms for baseline HAM-D scores (to control for pre-existing differences in mood) , baseline TNF-alpha values, a dichotomous variable for study group (escitalopram+celecoxib vs. escitalopram+PBO), and the product of baseline TNF-alpha and study group. It will be the statistical significance of the last term (the product) that tests the inflammation-by-study group interaction. This analysis will be repeated with hsCRP. Third, the change in inflammation across the eight weeks of therapy will be correlated with the change in depression scores. The difference in HAM-D scores from week 8 and baseline will be correlated with the difference in TNF-alpha and then hsCRP using the Pearson Correlation Coefficient. This will be done for patients in the monotherapy group and then for those in the combination therapy group. The same analysis will be repeated using HAM-A, MADRS, BDI and BDI.

For Specific Aim 4, the effectiveness of celecoxib in inhibiting PGE2 will be analyzed by comparing PGE2 scores at week-8 for patients in the two treatment groups with an analysis of covariance using baseline PGE2 scores as a covariate. We anticipate that the adjusted mean for PGE2 at week-8 will be lower for patients receiving celecoxib than that for patients receiving PBO.

An approach for statistical analysis of Specific Aims 5, 6, and 7 is currently under development.

All statistical analyses will be conducted using the latest version of SPSS software for Windows. If a subject drops out after completing 6 weeks on active medication (after randomization), we will use the last observation carried forward (LOCF method) to analyze the data. If a subject has completed at least one week on active medication after randomization, but drops out of the study at any time between week-1 and week-8, s/he will be included in the intent-to-treat analysis. Since this project seeks to provide proof-of-concept in bipolar depression, and is therefore exploratory in nature, we propose the tests of significance use a 0.05 alpha level. However, if this proposal develops further we may adjust our desired level of statistical significance to be in line with the number of tests so that the family wise Type I error rate could be held at 5%. This issue will be considered in the future.

II. Determination of Sample Size

Although a number of tests of statistical significance are planned for the proposed study, our required sample size is being based upon the primary outcome of treatment effect (Specific Aims 1). In looking at differences in mean HAM-D scores from the studies by Akhondzadeh et al. 5 and Mueller et al. 6 we found that the effect sizes of an antidepressant + anti-inflammatory agent over an antidepressant + placebo at week 3 of treatment were 0.53 and 0.52, respectively. In the proposed research we anticipate that the effect size will be slightly higher due to the fact that variation in depression scores attributable to baseline levels and pain perception will be eliminated in an analysis of covariance. Hence, we anticipate that the effect size will be about 0.60 for escitalopram + celecoxib over escitalopram + PBO. As noted earlier, we anticipate that the drug combination will lead to a therapeutic response that is earlier than what is normally seen at week-3 of treatment. We thus expect to see a treatment effect at week-2 of therapy, and expect that the magnitude of the effect will increase over the course of the study. The increasing separation between the different treatment groups is similar to what was found in the studies mentioned above which observed rising treatment effects during the course of therapy. From this, our computations indicate that a sample size of 45 patients per group will provide 80% power for an analysis of covariance to detect an effect size of 0.60 at week-2 of therapy using a 0.05 level of significance. Thus, a total of 90 patients completing treatment will be acquired for the proposed work. 30 of the total patients will be enrolled and randomized at our sister site in Munich, Germany. Given that the therapeutic effect size is expected to increase over time, the proposed sample size will be adequate throughout the course of the study. Subjects lost to attrition will be replaced.

References

Adams, MD. et al. Complementary DNA sequencing: expressed sequence tags and human genome project. *Science* **252**, 1651-1656 (1993)

Akhondzadeh S, Jafari S, Raisi F, Nasehi AA, Ghoreishi A, Salehi B*, et al* (2009). Clinical trial of adjunctive celecoxib treatment in patients with major depression: a double blind and placebo controlled trial. *Depress Anxiety* **26**(7): 607-611.

Akhondzadeh S, Tabatabaee M, Amini H, Ahmadi Abhari SA, Abbasi SH, Behnam B (2007). Celecoxib as adjunctive therapy in schizophrenia: a double-blind, randomized and placebo-controlled trial. *Schizophr Res* **90**(1-3): 179-185.

Alvarez-Soria, M.A. et al. Long term NSAID treatment inhibits COX-2 synthesis in the knee synovial membrane of patients with osteoarthritis: differential proinflammatory cytokine profile between celecoxib and aceclofenac. *Ann Rheum Dis* **65**, 998-1005 (2006).16476713

Berk M, Conus P, Kapczinski F, Andreazza AC, Yucel M, Wood SJ*, et al* (2010). From neuroprogression to neuroprotection: implications for clinical care. *Med J Aust* **193** (4 Suppl): S36-40.

Besedovsky, HO. et al. Cytokines as modulators of the hypothalamus-pituitary-adrenal axis. *J Steroid Biochem Mol Biol* **40**, 613-618 (1991).1659887

Bresee CJ, Delrahim K, Maddux RE, Dolnak D, Ahmadpour O, Rapaport MH (2006). The effects of celecoxib augmentation on cytokine levels in schizophrenia. *Int J Neuropsychopharmacol* **9**(3): 343-348.

Calabrese, JR. et al. Depression, immunocompetence, and prostaglandins of the E series. *Psychiatry Res* **17**, 41-47 (1986).2935897

Chenevard, R. et al. Selective COX-2 inhibition improves endothelial function in coronary artery disease. *Circulation* **107**, 405-409 (2003).12551863

Chrapko, WE, P. Jurasz, M.W. Radomski, N. Lara, S.L. Archer and J.M. Le Mellédo, Decreased platelet nitric oxide synthase activity and plasma nitric oxide metabolites in major depressive disorder, Biol Psychiatry 56 (2004) 129 - 134.

Cleeland, CS. & Ryan, K.M. Pain assessment: global use of the Brief Pain Inventory. *Ann Acad Med Singapore* **23**, 129-138 (1994).8080219

Cooke, JP, The pivotal role of nitric oxide for vascular health, Can J Cardiol 20 Suppl B (2004) 7B - 15B.

M. Crilly, C. Coch, M. Bruce, H. Clark and D. Williams, Indices of cardiovascular function derived from peripheral pulse wave analysis using radial applanation tonometry: a measurement repeatability study, Vasc Med 12 (2007) 189-197.

Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* **9:** 46-56.

Dinan, T. et al. Investigating the inflammatory phenotype of major depression: focus on cytokines and polyunsaturated fatty acids. *J Psychiatr Res* **43**, 471-476 (2009).18640689

Drexhage RC, Knijff EM, Padmos RC, Heul-Nieuwenhuijzen L, Beumer W, Versnel MA*, et al* (2010). The mononuclear phagocyte system and its cytokine inflammatory networks in schizophrenia and bipolar disorder. *Expert Rev Neurother* **10**(1): 59-76.

Drexhage RC, van der Heul-Nieuwenhuijsen L, Padmos RC, van Beveren N, Cohen D, Versnel MA*, et al* (2010). Inflammatory gene expression in monocytes of patients with schizophrenia: overlap and difference with bipolar disorder. A study in naturalistically treated patients. *Int J Neuropsychopharmacol*: 1-13.

Elgün S and H. Kumbasar, Increased serum arginase activity in depressed patients, Prog Neuropsychopharmacol Biol Psychiatry 24 (2000) 227 - 232.

Funakoshi-Tago, M. et al. Celecoxib potently inhibits TNFalpha-induced nuclear translocation and activation of NF-kappaB. *Biochem Pharmacol* **76**, 662-671 (2008).18644347

Garcia-Bueno, B., Madrigal, J.L., Perez-Nievas, B.G. & Leza, J.C. Stress mediators regulate brain prostaglandin synthesis and peroxisome proliferator-activated receptor-gamma activation after stress in rats. *Endocrinology* **149**, 1969-1978 (2008).18079203

Ghaemi, SN, Rosenquist KJ, Ko JY, Baldassano CF, Kontos NJ, Baldessarini RJ (2004). Antidepressant treatment in bipolar versus unipolar depression. *Am J Psychiatry* **161**(1): 163-165.

Giles, TD. Aspects of nitric oxide in health and disease: a focus on hypertension and cardiovascular disease, J Clin Hypertens (Greenwich) 8 (2006) 2 - 16.

Gjeilo, KH., Stenseth, R., Wahba, A., Lydersen, S. & Klepstad, P. Validation of the brief pain inventory in patients six months after cardiac surgery. *J Pain Symptom Manage* **34**, 648-656 (2007).17629665

Halaris, A., H. Zhu, Y. Feng and J.E. Piletz> Plasma agmatine and platelet imidazoline receptors in depression, Ann N Y Acad Sci 881 (1999) 445-451.

Hayward, CS M. Kraidly, C.M. Webb and P. Collins, Assessment of endothelial function using peripheral waveform analysis: a clinical application, J Am Coll Cardiol 40 (2002) 521-528.

Himmerich, H, E.B. Binder, H.E. Künzel, A. Schuld, S. Lucae, M. Uhr, T. Pollmächer, F. Holsboer and M. Ising, Successful antidepressant therapy restores the disturbed interplay between TNF-alpha system and HPA axis, Biol Psychiatry 60 (2006) 882 - 888.

Hoekstra R, Fekkes D, Loonen AJ, Pepplinkhuizen L, Tuinier S, Verhoeven WM (2006). Bipolar mania and plasma amino acids: increased levels of glycine. *Eur Neuropsychopharmacol* **16**(1): 71-77.

Husain, M.M. et al. Pain in depression: STAR*D study findings. *J Psychosom Res* **63**, 113-122 (2007).17662746

Judd LL, Akiskal HS, Schettler PJ, Coryell W, Endicott J, Maser JD*, et al* (2003). A prospective investigation of the natural history of the long-term weekly symptomatic status of bipolar II disorder. *Arch Gen Psychiatry* **60**(3): 261-269.

Judd LL, Akiskal HS, Schettler PJ, Endicott J, Maser J, Solomon DA*, et al* (2002). The long-term natural history of the weekly symptomatic status of bipolar I disorder. *Arch Gen Psychiatry* **59**(6): 530-537.

Kim YK, Jung HG, Myint AM, Kim H, Park SH (2007). Imbalance between pro-inflammatory and anti-inflammatory cytokines in bipolar disorder. *J Affect Disord* **104**(1-3): 91-95.

Kim YK, Suh IB, Kim H, Han CS, Lim CS, Choi SH*, et al* (2002). The plasma levels of interleukin-12 in schizophrenia, major depression, and bipolar mania: effects of psychotropic drugs. *Mol Psychiatry* **7**(10): 1107-1114.

Koenig, W. Inflammation and coronary heart disease: An overview. Cardiol Rev. 9 (2001): 31-35

Lanquillon, S., Krieg, J.C., Bening-Abu-Shach, U. & Vedder, H. Cytokine production and treatment response in major depressive disorder. *Neuropsychopharmacology* **22**, 370 - 379 (2000)

Laufer S, Greim C, Luik S, AyoubSS, Dehner F (2008). Human whole blood assay for rapid and routine testing of non-steroidal anti-inflammatory drugs (NSAIDS) on cyclo-oxygenase-2 activity. *Inflammopharmacology* **16**: 155-161.

Leonard BE and Song, C. Stress, depression, and the role of cytokines, Adv Exp Med Biol 461 (1999) 251-265.

Leuchter, A.F. et al. Painful physical symptoms and treatment outcome in major depressive disorder: a STAR*D (Sequenced Treatment Alternatives to Relieve Depression) report. *Psychol Med*, 1-13 (2009).19493369

Liciono, J and ML Wong, The role of inflammatory mediators in the biology of major depression: central nervous system cytokines modulate biological substrate of depressive symptoms, regulate stress responsive systems, and contribute to neurotoxicity and neuroprotection. Mol Psychiatry 4 (1999) 317-327.

Lieb, J., Karmali, R. & Horrobin, D. Elevated levels of prostaglandin E2 and thromboxane B2 in depression. *Prostaglandins Leukot Med* **10**, 361-367 (1983).6574523

Maes, M, Bosmans E, Calabrese J, Smith R, Meltzer HY (1995). Interleukin-2 and interleukin-6 in schizophrenia and mania: effects of neuroleptics and mood stabilizers. *J Psychiatr Res* **29**(2): 141-152.

Maes, M, Van der Planken, A. Van Gastel, R. Desnyder, Blood Coagulation and platelet aggregation in major depression. J Affect Disord. 40 (1996) 35-40.

Manning, JS (2005). Burden of illness in bipolar depression. *Prim Care Companion J Clin Psychiatry* **7**(6): 259-267.

Marques, A., Silverman, M., & Sternberg, E. (2010). Evaluation of stress symptoms by applying noninvasive methodologies: Measurements of neuroimmune biomarkers in the sweat, heart rate variability and salivary cortisol. *Neuroimmunomodulation*, *17*(3), 205-208.

McEniery, CM, S. Wallace, I.S. Mackenzie, B. McDonnell, Yasmin, D.E. Newby, J.R. Cockcroft and I.B. Wilkinson, Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans, Hypertension 48 (2006) 602-608.

McIntyre, A, A. Gendron and A. McIntyre, Quetiapine adjunct to selective serotonin reuptake inhibitors or venlafaxine in patients with major depression, comorbid anxiety, and residual depressive symptoms: a randomized, placebo-controlled pilot study, Depress Anxiety 24 (2007) 487-494.

Meyer, JM, V.G. Davis, D.C. Goff, J.P. McEvoy, H.A. Nasrallah, S.M. Davis, R.A. Rosenheck, G.L. Daumit, J. Hsiao, M.S. Swartz, T.S. Stroup and J.A. Lieberman, Change in metabolic syndrome parameters with antipsychotic treatment in the CATIE Schizophrenia Trial: prospective data from phase 1, Schizophr Res 101 (2008) 273-286

Miller CL, Llenos IC, Dulay JR, Weis S (2006). Upregulation of the initiating step of the kynurenine pathway in postmortem anterior cingulate cortex from individuals with schizophrenia and bipolar disorder. *Brain Res* **1073-1074**: 25-37.

Miller AH, Maletic V, Raison CL (2009). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* **65:** 732-741.

Miller, G.E., Rohleder, N. & Cole, S.W. Chronic interpersonal stress predicts activation of pro- and anti-inflammatory signaling pathways 6 months later. *Psychosom Med* **71**, 57-62 (2009).19073750

Müller N, Krause D, Dehning S, Musil R, Schennach-Wolff R, Obermeier M*, et al* (2010). Celecoxib treatment in an early stage of schizophrenia: results of a randomized, double-blind, placebo-controlled trial of celecoxib augmentation of amisulpride treatment. *Schizophr Res* **121** (1-3): 118-124.

Müller N, Riedel M, Schwarz MJ, Engel RR (2005). Clinical effects of COX-2 inhibitors on cognition in schizophrenia. *Eur Arch Psychiatry Clin Neurosci* **255**(2): 149-151.

Müller N, Schwarz MJ, Dehning S, Douhe A, Cerovecki A, Goldstein-Muller B*, et al* (2006). The cyclooxygenase-2 inhibitor celecoxib has therapeutic effects in major depression: results of a double-blind, randomized, placebo controlled, add-on pilot study to reboxetine. *Mol Psychiatry* **11**(7): 680-684.

Müller N, Strassnig M, Schwarz MJ, Ulmschneider M, Riedel M (2004a). COX-2 inhibitors as adjunctive therapy in schizophrenia. *Expert Opin Investig Drugs* **13**(8): 1033-1044.

Müller N, Ulmschneider M, Scheppach C, Schwarz MJ, Ackenheil M, Moller HJ*, et al* (2004b). COX-2 inhibition as a treatment approach in schizophrenia: immunological considerations and clinical effects of celecoxib add-on therapy. *Eur Arch Psychiatry Clin Neurosci* **254**(1): 14-22.

Myint AM, Kim YK (2003). Cytokine-serotonin interaction through IDO: a neurodegeneration hypothesis of depression. *Med Hypotheses* **61**(5-6): 519-525.

Myint AM, Kim YK, Verkerk R, Park SH, Scharpe S, Steinbusch HW*, et al* (2007a). Tryptophan breakdown pathway in bipolar mania. *J Affect Disord* **102**(1-3): 65-72.

Myint AM, Kim YK, Verkerk R, Scharpe S, Steinbusch H, Leonard B (2007b). Kynurenine pathway in major depression: evidence of impaired neuroprotection. *J Affect Disord* **98**(1-2): 143-151.

Nery FG, Monkul ES, Hatch JP, Fonseca M, Zunta-Soares GB, Frey BN*, et al* (2008). Celecoxib as an adjunct in the treatment of depressive or mixed episodes of bipolar disorder: a double-blind, randomized, placebo-controlled study. *Hum Psychopharmacol* **23**(2): 87-94.

Nicholson, A, H. Kuper and H. Hemingway, Depression as an aetiologic and prognostic factor in coronary heart disease: a meta-analysis of 6362 events among 146 538 participants in 54 observational studies, Eur Heart J 27 (2006) 2763-2774.

Nutt, D, S. Argyropoulos, S. Hood and J. Potokar, Generalized anxiety disorder: a comorbid disease. , European Neuropsychopharmacology 16 (2006) s109-s118

O'Brien, SM., Scully, P., Fitzgerald, P., Scott, L.V. & Dinan, T.G. Plasma cytokine profiles in depressed patients who fail to respond to selective serotonin reuptake inhibitor therapy. *J Psychiatr Res* **41**, 326 - 331 (2007)

Papaioannou, TG, E.N. Karatzis, K.N. Karatzi, E.J. Gialafos, A.D. Protogerou, K.S. Stamatelopoulos, C.M. Papamichael, J.P. Lekakis and C.I. Stefanadis, Hour-to-hour and week-to-week variability and reproducibility of wave reflection indices derived by aortic pulse wave analysis: implications for studies with repeated measurements, J Hypertens 25 (2007) 1678-1686.

Piletz, JE H. Zhu, S. Madakasira, P. Pazzaglia, C. Lindsay DeVane, N. Goldman and A. Halaris, Elevated P-selectin on platelets in depression: response to bupropion, J Psychiatr Res 34 (2000) 397 – 404

Pilowsky, I., Spence, N., Cobb, J. & Katsikitis, M. The Illness Behavior Questionnaire as an aid to clinical assessment. *Gen Hosp Psychiatry* **6**, 123-130 (1984).6714666

Post RM, Denicoff KD, Leverich GS, Altshuler LL, Frye MA, Suppes TM*, et al* (2003a). Morbidity in 258 bipolar outpatients followed for 1 year with daily prospective ratings on the NIMH life chart method. *J Clin Psychiatry* **64**(6): 680-690.

Post RM, Leverich GS, Altshuler LL, Frye MA, Suppes TM, Keck PE, Jr.*, et al* (2003b). An overview of recent findings of the Stanley Foundation Bipolar Network (Part I). *Bipolar Disord* **5**(5): 310-319.

Rapaport MH, Delrahim KK, Bresee CJ, Maddux RE, Ahmadpour O, Dolnak D (2005). Celecoxib augmentation of continuously ill patients with schizophrenia. *Biol Psychiatry* **57**(12): 1594-1596.

Rapaport MH, Guylai L, Whybrow P (1999). Immune parameters in rapid cycling bipolar patients before and after lithium treatment. *J Psychiatr Res* **33**(4): 335-340.

Riedel M, Strassnig M, Schwarz MJ, Muller N (2005). COX-2 inhibitors as adjunctive therapy in schizophrenia: rationale for use and evidence to date. *CNS Drugs* **19**(10): 805-819.

Riondino, S. et al. Lack of biological relevance of platelet cyclooxygenase-2 dependent thromboxane A2 production. *Thromb Res* **122**, 359-365 (2008).18295304

Rybakowski, JK, A. Wykretowicz, A. Heymann-Szlachcinska and H. Wysocki, Impairment of endothelial function in unipolar and bipolar depression, Biol Psychiatry 60 (2006) 889 - 891.

Sang, N., Zhang, J. & Chen, C. COX-2 oxidative metabolite of endocannabinoid 2-AG enhances excitatory glutamatergic synaptic transmission and induces neurotoxicity. *J Neurochem* **102**, 1966-1977 (2007).17539917

Satriano, J, Arginine pathways and the inflammatory response: interregulation of nitric oxide and polyamines: review article., Amino Acids 26 (2004 Jul) 321 - 329.

Selley, ML, Increased (E)-4-hydroxy-2-nonenal and asymmetric dimethylarginine concentrations and decreased nitric oxide concentrations in the plasma of patients with major depression, J Affect Disord 80 (2004) 249 - 256.

Straub, R., Buttgereit, F., & Cutulo, M. (2011). Alteration of the hypothalamic-pituitary-adrenal acid in systemic immune diseases: A role for misguided energy regulation. *Clinical and Experimenal Rheumatology*, *5*(68), 23-31.

Su KP, Leu SJ, Yang YY, Shen WW, Chou YM, Tsai SY (2002). Reduced production of interferon-gamma but not interleukin-10 in bipolar mania and subsequent remission. *J Affect Disord* **71**(1-3): 205-209.

Tan, G., Jensen, M.P., Thornby, J.I. & Shanti, B.F. Validation of the Brief Pain Inventory for chronic nonmalignant pain. *J Pain* **5**, 133-137 (2004).15042521

Tondo L, Isacsson G, Baldessarini R (2003). Suicidal behavior in bipolar disorder: risk and prevention. *CNS Drugs* **17**(7): 491-511.

Tsai SY, Chen KP, Yang YY, Chen CC, Lee JC, Singh VK*, et al* (1999). Activation of indices of cell-mediated immunity in bipolar mania. *Biol Psychiatry* **45**(8): 989-994.

Tsai SY, Yang YY, Kuo CJ, Chen CC, Leu SJ (2001). Effects of symptomatic severity on elevation of plasma soluble interleukin-2 receptor in bipolar mania. *J Affect Disord* **64**(2-3): 185-193.

Tsao, CW, Y.S. Lin, C.C. Chen, C.H. Bai and S.R. Wu, Cytokines and serotonin transporter in patients with major depression, Prog Neuropsychopharmacol Biol Psychiatry 30 (2006) 899 - 905.

Tuglu, C, S.H. Kara, O. Caliyurt, E. Vardar and E. Abay, Increased serum tumor necrosis factor-alpha levels and treatment response in major depressive disorder, Psychopharmacology (Berl) 170 (2003) 429 - 433.

Ur, E, White, PD, Grossman A (1992). Hypothesis: cytokines may be activated to cause depressive illness and chronic fatigue syndrome. *Eur Arch Psychiatry Clin Neurosci* **241:** 317-322.

van der Werf-Eldering, M., Riemersma-van der Lek, R., Burger, H., Holthausen, E., Aleman, A., & Nolen, W. (2012). Can variation in hypothalamic-pituitary-adrenal (hpa)-acis activity explain the relationship between depression and cognition in bipolar patients?. *Plos One*, *7*(5), 37119. doi: 10.1371/journal.pone.0037119

von Känel, R, U. Hepp, B. Kraemer, R. Traber, M. Keel, L. Mica and U. Schnyder, Evidence for low-grade systemic proinflammatory activity in patients with posttraumatic stress disorder, J Psychiatr Res 41 (2006) 744-752.

Weber, T, J. Auer, G. Lamm, M.F. O'Rourke and B. Eber, Arterial stiffness, central blood pressures, and wave reflections in cardiomyopathy-implications for risk stratification, J Card Fail 13 (2007) 353-359.

Weber, T, R. Maas, J. Auer, G. Lamm, E. Lassnig, M. Rammer, M.F. O'Rourke, R.H. Boger and B. Eber, Arterial wave reflections and determinants of endothelial function a hypothesis based on peripheral mode of action, Am J Hypertens 20 (2007) 256-262.

Wilkinson, IB, I.R. Hall, H. MacCallum, I.S. Mackenzie, C.M. McEniery, B.J. van der Arend, Y.E. Shu, L.S. MacKay, D.J. Webb and J.R. Cockcroft, Pulse-wave analysis: clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function, Arterioscler Thromb Vasc Biol 22 (2002) 147-152.

Wilkinson, IB, H. MacCallum, J.R. Cockcroft and D.J. Webb, Inhibition of basal nitric oxide synthesis increases aortic augmentation index and pulse wave velocity in vivo, Br J Clin Pharmacol 53 (2002) 189-192.

Williams, B, P.S. Lacy, S.M. Thom, K. Cruickshank, A. Stanton, D. Collier, A.D. Hughes, H. Thurston and M. O'Rourke, Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study, Circulation 113 (2006) 1213-1225.

Yang, H. & Chen, C. Cyclooxygenase-2 in synaptic signaling. *Curr Pharm Des* **14**, 1443-1451 (2008).18537667

Yoshimura, R. et al. Higher plasma interleukin-6 (IL-6) level is associated with SSRI- or SNRI-refractory depression. *Prog Neuropsychopharmacol Biol Psychiatry* **33**, 722-726 (2009).19332097

Zunszain, P., Anacker, C., Cattaneo, A., Carvalho, L., & Pariante, C. (2011).
Clucocorticoids, cytokines and brain abnormalities in depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(3), 722-729.