STRESS AND INFLAMMATION IN THE PATHOPHYSIOLOGY MDD

A Phase IV, randomized, double-blind, placebo-controlled, one-phase study of the effects of escitalopram and celecoxib on depression.

Principal Investigator (1) *Yvette Sheline, MD*

Center for Neuromodulation in Depression and Stress,

Department of Psychiatry

3700 Hamilton Walk, D307

215-573-0082

Sheline@pennmedicine.upenn.edu

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Food and Drug Administration **Exempt from IND regulations, letter dated 08/18/2015 (attached in Appendix)**

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Table of Contents

Contents

Study Summary

Title	*Stress and Inflammation in the Pathophysiology of Depression*
Short Title	*Stress and Inflammation*
IRB Number	*819654*
Phase	*Phase 4*
Methodology	*Double-blinded study or open-label; Randomized, placebo-controlled, one-phase study*
Study Duration	*This study will last 6-12 weeks, dependent on whether the participant enters the open-label phase of the study.*
Study Center(s)	*Single-center.*
Objectives	*1: Characterize neuroanatomical and neuropsychological correlates of abnormal cytokines in MDD.* *2: Characterize the reversibility of brain dysfunction, including cognitive impairment, with treatment of depression and its association with inflammation.*
Number of Subjects	*100 depressed subjects, 50 control subjects*
Main Inclusion and Exclusion Criteria	*18-80, depressed, males and females, without psychosis or other concomitant psychiatric illnesses, or significant cardiac, liver, or GI disease.*
Drugs	*Escitalopram 10 mg/day for 3 days and then 20 mg/day for 39 day (taper down of 10 mg for 3 days) with placebo or Escitalopram 10 mg/day for 3 days and then 20 mg/day for 39 day (taper down of 10 mg for 3 days) with celecoxib (400 mg/day)*

Duration of administration	*6-12 weeks*
Statistical Methodology	*The design of this trial is simple repeated measures design.*
Safety Evaluations	*This is a low-risk study. Subject safety will be closely monitored. Subjects reporting of side effects will be used to evaluate individual safety of high doses of escitalopram and celecoxib. Additionally, heart rate monitoring will allow us to assess the safety of celecoxib for blood pressure risks.*
Data and Safety Monitoring Plan	*The sponsor is responsible for monitoring the data quality and the ongoing safety of subjects. The PI will be monitoring the data quality and the ongoing safety of subjects.*

Co-Investigator

Name:	Title:	Contact Information:
Carsten Skarke	MD	8037 Maloney Bldg Hospital of the University of Pennsylvania 3600 Spruce Street Philadelphia, PA 19104 Phone: 215-746-8330 Fax: 215-573-8996 e-mail: cskarke@ upenn.edu

1 BACKGROUND AND STUDY RATIONALE

This study will be conducted in full accordance with all applicable University of Pennsylvania Research Policies and Procedures and all applicable Federal and state laws and regulations including [as applicable include the following regulations as they apply 45 CFR 46, 21 CFR Parts 50, 54, 56, and 312, and Good Clinical Practice: Consolidated Guidelines approved by the International Conference on Harmonisation (ICH).] All episodes of noncompliance will be documented.

1.1 Introduction

Major depression (MDD) is a cause of disability worldwide, but the etiology of MDD remains unclear. An emerging concept is that inflammation may have relevance, especially in MDD. We have data supporting increased inflammatory cytokines in MDD compared with controls that are associated with decreased hippocampus and amygdala volumes. MDD also had impairment in neuropsychological function in episodic memory, executive function, decreased hippocampus and amygdala resting state functional connectivity. Antidepressant treatment improved IL-6 levels, neuropsychological testing and resting state connectivity.

We will recruit patients with MDD (n = 100) and controls (n = 50) to characterize immune function, brain structure and connectivity and neuropsychological function. Using the Hospital of University of Pennsylvania's Pepper Lab we will measure participants' c-reactive protein (CRP) levels, and stratify participants according to CRP level (i.e. high (CRP ≥3) and low (CRP <3)). All participants with MDD (n=100), will receive a selective serotonin reuptake inhibitor (SSRI) antidepressant and we will randomize them to SSRI plus placebo (n≤50) or SSRI + celecoxib (n≤50). We will assess participants pre- and post-treatment with peripheral and central cytokine levels, neuropsychological tests and brain resting state functional connectivity.

Hypothesis 1: a) Compared with matched controls, MDD will have abnormalities in peripheral and inflammatory cytokines, neuropsychological function, hippocampal, amygdala and prefrontal cortex (PFC) structure and functional connectivity; b) Peripheral/central inflammatory cytokine levels will be associated with volume loss in hippocampus and amygdala; and c) Cumulative duration of depression will correlate with volume loss in hippocampus, amygdala, and PFC. d) Across the lifespan there will be an age effect interaction with depression.

Hypothesis 2: a) Once subjects have completed the antidepressant treatment, they will have greater normalization of IL-6 and IL-10, greater improvements in memory, executive function, and in resting state functional connectivity compared to their baseline measures; b) Improvement in clinical depressive scores will correlate with normalization of inflammatory cytokines; c) Those randomized to the addition of celecoxib (vs placebo) will have additive improvements in cytokines, resting state functional connectivity and depression scores.

1.2 Background and Relevant Literature

Depression is a common cause of morbidity, disability and mortality with an increasing worldwide prevalence. A key distinguishing feature of MDD versus younger MDD is its association with comorbid medical illness, such as vascular diseases often resulting in decreased cognitive function. The etiological factors contributing to MDD are not entirely understood, but the vascular depression hypothesis posits that cerebrovascular disease contributes to the development and severity of depression in older adults by causing ischemic white matter lesions of subcortical limbic structure projections to the frontal cortex or in frontal cortical white matter tracts; these interruptions cause alterations in mood regulation and cognition. In addition, there is mounting evidence that patients with major depression (MDD), particularly those with MDD, have increased oxidative stress, resulting in part from increased peripheral blood inflammatory biomarkers. Compared with nondepressed individuals, those with MDD have elevations of inflammatory cytokines and their soluble receptors in peripheral blood and CSF. Further, they have elevations in blood concentrations of acute phase reactants, including acute phase proteins, adhesion molecules, chemokines, and mediators of inflammation such as nitric oxide synthase (NOS) and prostaglandins. Increased cytokines include interleukin (IL)-1, tumor necrosis factor (TNF) alpha, and IL-6, one of the most commonly and reliably elevated peripheral biomarkers in MDD. In addition to an overall correlation with the depressed state, there are correlations between specific depressive symptoms and inflammatory markers. These include associations of sleep impairment, fatigue and cognitive dysfunction with IL-6. Evidence for an association with depression also includes the ability of proinflammatory factors to induce depressive symptoms, which were correlated with blood cytokine concentrations. In the case of prolonged exposure to cytokines, there is a marked risk of becoming depressed, with approximately 25% of patients exposed to interferon (IFN)- experiencing symptoms of MDD. IL-6 has been shown to interfere with the production of serotonin from tryptophan by increasing breakdown of tryptophan, thus reducing serotonin levels (and increasing depression risk) and preferentially increasing the synthesis of kynurenine and its neurotoxic metabolites, 3-hydroxykynurenin and quinolinic acid. IL-6 drives this metabolic shunt and IL-10 partially counters it.

Further, the inflammation hypothesis of aging proposes an age-related increase in cytokine production, resulting in a chronic proinflammatory state, which has a detrimental effect on cellular function. Elevated plasma IL-6 is a common finding in older adults who are at greater risk of disease and disability. Given the strong correlation between proinflammatory markers and illness, inflammation is particularly important in MDD. Increased plasma IL-6 is a risk factor for many diseases of aging, including hypertension, atherosclerosis, cardiovascular ischemia and type 2 diabetes, all of which are more common in MDD. IL-6 was called the gerontologists cytokine by William Ershler, who proposed that this cytokine regulates a key human aging pathway, given the consistent association between elevated plasma IL-6 and poor health outcomes. In healthy older adults elevated plasma IL-6 has a linear inverse correlation with cognitive performance. A sizeable and growing number of older people are at risk for IL-6 mediated cognitive effects. Chronically elevated IL-6 is associated with mild cognitive deficits,

even in apparently healthy community dwelling elders. Elevated IL-6 augments the risk of stroke and dementia and predicts future cognitive decline. Despite this evidence of a link between plasma IL-6 and CNS impairment the specific mechanisms remain largely unknown. We believe it is likely that with each repeated episode of depression and resultant exposure to elevated inflammatory cytokines in brain, there is progressive regional loss of inhibitory neurons in brain regions that are particularly sensitive to inflammatory damage, such as the hippocampal CA2-3 region. We hypothesize that this volumetric loss is critically associated with development of brain dysfunction and risk of repeated depressive episodes.

A meta-analysis (1) that included 14 trials and 6262 participants was recently conducted to examine the use of non-steroidal anti-inflammatory drugs (NSAIDs) and cytokine inhibitors. The pooled effect estimate suggested that anti-inflammatory treatment reduced depressive symptoms compared with placebo. Subanalyses emphasized the antidepressant properties of celecoxib on remission and response, particularly in combination with antidepressant. Among the 6 studies that reported adverse events there was no evidence of an increased number of GI or cardiovascular events after 6 weeks or infections after 12 weeks.

A meta-analysis of five (2) post-approval clinical trials associated lower depression scores with celecoxib treatment, 200 mg qd over 6 weeks, in patients with active osteoarthritis, n=607, compared to patients exposed to placebo (2). These clinical trials were highly protected against bias by following a multicenter, randomized, double-blinded, placebo-controlled, active-comparator, parallel-group design (2). The authors write that a significant increase in systolic or diastolic blood pressure and heart rate was not evident after 6 weeks of celecoxib treatment (2). This supports a good safety profile for this celecoxib dosing regimen, in particular, when considering that 48% of patients (291/607) had hypertension at baseline, 20% (121/607) hyperlipidemia, and 17% (103/607) diabetes mellitus as baseline conditions. The high morbidity is also reflected in that 51% (310/607) of patients report at baseline to receive pharmacotherapy to treat cardiovascular disease (2). CRP levels have been shown to highly correlate with cytokine levels. Validation of commercially available methods of CRP has been conducted and shown that semi-quantative CRP strips can distinguish between normal and high levels of CRP within individuals very simply (3).

1.3 Name and Description of the Study Drugs

Study drug intervention consists of escitalopram and celecoxib in doses approved for clinical use. Dose ranges for escitalopram are 10-20 mg/day. The dose of celecoxib is 400 mg/day.

2 Study Objectives and Investigational Plan

Aim 1: Characterize neuroanatomical and neuropsychological correlates of abnormal cytokines in MDD.

Aim 2: Characterize the reversibility of brain dysfunction, including cognitive impairment, with treatment of depression and its association with inflammation.

*2.1 Primary outcome variable(s)**

1) Cytokine levels will be measured in blood: Primary measure will be IL-6 pre- and post-treatment.
2) Hippocampus, including subvolume analyses and Amygdala volumes: Left and right volumes will be separately measured and standardized by brain volumes before being entered in analyses. Prefrontal cortical thickness: L & R orbitofrontal, superior frontal and frontal pole.
3) Antidepressant treatment response determined using the MADRS score following antidepressant treatment covaried on baseline MADRS score.
4) Neuropsychological function as determined by factor scores from episodic memory and executive function.
5) Resting state functional connectivity of the hippocampus and amygdala to anterior cingulate (AC) and dorsolateral prefrontal cortex (DLPFC).

2.2 Study Design

100 depressed and 50 control subjects, age 18-80, male and female, will be recruited for this study. Depressed participants will be stratified based on their c-reactive protein (CRP) levels (i.e., "High" CRP≥3 and "Low" CRP<3). All participants will be started on either 10 mg of escitalopram + placebo or 10 mg of escitalopram + celecoxib (400mg/day) daily for 3 days. Following this lead-in dose of the SSRI they will then take 20 mg daily thereafter for 39 days and have no changes in their dose of placebo or celcoxib. Follow-up visits for all depressed participants will occur at 1 week, 2-weeks, and 4-weeks, following the start of treatment. The study coordinator and PI will monitor side effects and vital signs, and the PI may adjust the medication dosage depending on the patient's response to treatment and/or the presence of side effects. Final dose (10 mg versus 20 mg, or a dose between 10 mg and 20 mg, e.g., 15 mg) will be determined by clinical response and side effects. This decision will be based on an ongoing discussion between the participant and the study doctor. Participants who remit will have their end of study visit with a blood draw, and neuropsych testing at 6 weeks. Participants will then taper off medication at 10 mg for 3 days (if taking 20 mg during study). Based on past studies, less than 10% of participants will be taking less than 20 mg/day of SSRI at the end of the study.

Participants initially randomized to escitalopram + placebo, who do not remit will be treated with 6 weeks of escitalopram + celecoxib. They will not repeat the baseline visit, but will re-complete medication visits at week 1,2, and 4, After the 6 weeks of escitalopram + celecoxib they will undergo a 3rd MRI, neurocognitive session, and blood draw. Those who complete the study at this point will taper off medication at 10 mg for 3 days.

Participants will have total study participation of 6-12 weeks. Participants may have unscheduled visits occur throughout the course of the study (screening-completion), if participants are unable to complete the required assessments within the allotted time period. If this occurs participants will be compensated for the travel to these additional visits, but no changes will be made to their human subjects payment. Non-depressed, control group participants will not receive the study drug, and they will not attend the medication check visits at week 1, 2, and 4. Therefore, they will have 3-4 study visits: 1-2 screening visits, 1 baseline visit: blood draw , MRI, and neuropsych testing; Week 6 visit: blood draw, MRI scan, and neuropsych testing.

Participants will be recontacted six months post-study completion to assess efficacy and longevity of the treatment. At the six-month follow-up, participants will be asked to complete a series of questionnaires regarding their medical and psychiatric history. This follow-up appointment is optional.

2.3 Study duration

The study will be ongoing until recruitment is completed. The goal is to complete recruitment in four years. Individuals will participate in the study for approximately 6-12 weeks (from screening through to the last assessment, dependent on whether or not they enter the open-label phase of the study).

2.4 Resources necessary for human research protection

To reduce risks to participants, only physicians and scientists with extensive training and experience will supervise and perform procedures.. Additionally, participants will be provided with phone numbers of research personnel whom the participants may call if they are experiencing any discomfort so that the research personnel can coordinate appropriate response and care. Medication will be packaged by UPenn's Investigational Drug Service (IDS) and will be dispensed to the research team who will pick up the medication on the day it is to be given to the participant. If there is a gap between picking up the medication and dispensing it, the pills will be kept in locked cabinets behind locked doors until the appointment. While taking the medication, participants will be closely monitored for any side effects from taking escitalopram or celecoxib. Several safeguards will be put into place for protecting the confidentiality of research material. Staff members will also be trained to understand the importance of confidentiality. Information gathered about individual participants is maintained in secured storage areas at each site. All phone screenings during the recruitment process will be conducted in a private office area, and after asking the potential participant if they are in an environment where they feel comfortable answering questions related to their eligibility to participate. All aspects of the informed consent process will be conducted in a private consultation room. Study procedures will occur in a private room by personnel trained in following procedures whereby the privacy of the participant is maintained. All psychological assessments will be conducted by experienced and highly trained researchers or clinicians. All assessments will be conducted in

private rooms to ensure privacy and the maintenance of confidentiality. Only information necessary for the completion of this study will be collected. All computer data obtained from the laboratory and from research interviews will be identified by a code number. All data will be kept in locked file cabinets and only made available to qualified research personnel. Only code numbers will appear on any data and documents used for evaluation or statistical analysis for this study. Publications emanating from this research will not identify individual patients. HIPAA compliance will be enforced as per University of Pennsylvania policy. Reports from patients' clinical records concerning research observations will not be available to outside medical facilities without the written consent of the participant. It is not anticipated that additional medical or psychological services will be necessary as a consequence of this research; however, should that event occur, participants will be referred to the appropriate service provider and the study coordinator and PI will follow-up to ensure that services were received if necessary.

2.5 Target population

One-hundred depressed subjects, aged 18-80, right handed, male and female (50 with high-CRP levels (CRP ≥3) and 50 with low-CRP levels (CRP<3). In addition, 50 non-depressed controls, matched on demographic and vascular risk factors.

2.6 Subjects Enrolled at Penn

N=150

2.7 Accrual

One hundred depressed participants will be recruited from the community, from outpatient psychiatry clinics at the University of Pennsylvania (General Outpatient Psychiatry, Family Medicine, Geriatric Psychiatry, the Treatment Resistant Depression Clinic, the HUP consult liaison services), and from the Veterans' Administration Medical Center to complete this study with usable data. Fifty non-depressed control participants will also be recruited from the community and the aforementioned sources to complete this study with usable data.

Careful consideration was provided to the determination of sample size requirements. The following section breaks down the statistical methods and theoretical justification for our sample size of 150 participants: 100 depressed (High and Low CRP groups) and 50 control.

Aim 1: Characterize neuroanatomical and neuropsychological effects of abnormal cytokines in MDD. Hypothesis 1a: Inflammatory cytokines will be elevated centrally and peripherally relative to matched controls; neuropsychological performance will be poorer in MDD; hippocampus, amygdala and PFC volumes will be decreased in MDD; and connectivity of hippocampus and amygdala to rostral AC and DLPFC will be decreased in MDD. The power for the IL-6 mean differences was based on our preliminary IL-6 data where the mean (SD) difference is 2.1 (3.6), with 80% power, two-tailed t-test at 5% significance level. A t-test was used to estimate the power to compare the mean difference of regional correlations (functional connectivity) and neuropsychometric function between controls and depressed subjects. To achieve 80% power

with a two-tailed test and 5% significance level, an effect size (mean difference/SD) of at least .49 can be detected for a sample of 150 subjects, where 50 are controls. Based on our preliminary data, the group difference of the mean (SD) Fisher-z of the amygdala-subgenual AC is 0.23 (0.17), and of hippocampus-DLPFC is -0.24 (0.12). The preliminary neuropsychometric data supports this power analysis and requires a group difference of the mean (SD) for episiodic memory of -1.44 (2.96) and for executive function of -1.64 (3.35).

Hypothesis 1b: Cytokine levels will be correlated with hippocampal and amygdala volumes and PFC thickness; To examine correlational hypothesis 1b, we used a Fisher's r-to-Z transformation test for a correlation between IL-6 and volumetric measures. To achieve 80% power with a two-tailed Z test and 5% significance level, a correlation of |.23| can be detected for a sample of 150 subjects. Based on our preliminary data for IL-6 and for left CA2-3 we have a correlation of .33.

Aim 2: Characterize the effects of treatment on depression and the association with inflammation. Hypothesis 2a: Subjects randomized to treatment will have greater normalization of IL-6 and functional connectivity compared to those randomized initially to placebo. To achieve 80% power with a two-tailed t-test and 5% significance level, a mean difference of 2.1 can be detected with a standard deviation of 3.6 for a sample of 50 medicated and 50 nonmedicated subjects. Based on our preliminary data for IL-6, the mean (SD) difference, between the nonmedicated and medicated group for IL-6 is 2.7 (3.6). We will test Hypothesis 2a for improvement in functional connectivity with repeated measures ANOVA with the treatment group x time interaction result testing whether improvement in the outcome variables (increase in hippocampus and amygdala connectivity to DLPFC and AC) is greater in the treatment group than the placebo group, over and above non-specific changes over time (e.g., practice effects, naturalistic changes) that would be seen also in the control group. Our sample size of 100 with 50 in each group provides 80% power to detect a moderate effect size of .38 with .7 intraclass correlation on the group x time interaction, based on a two-tailed F-test with 5% significance level and assuming 10% attrition.

2.8 Key inclusion criteria

Inclusion Criteria for all Participants:

1) Age 18-80, right-handed male or female, any race;
2) Absence of clinical dementia
3) English speaking
4) Blood pressure not exceeding 150/90 mmHg, treated or untreated
5) Normal result on liver-function test
6) No history of ulcer disease or GI bleeding
7) No renal insufficiency
8) Weight greater than 110 lbs

Additional Inclusion Criteria for Depressed Participants:

1) DSM-IV criteria for MDD
2) PHQ-8 Score equal to or greater than 10

2.9 Key exclusion criteria

Stress and Inflammation exclusions:

1) Known history of relevant severe drug allergy or hypersensitivity (e.g. to Citalopram or Escitalopram, and/or to celecoxib, aspirin, or other NSAIDs; known demonstration of allergic-type reactions to sulfonamides);
2) Does not speak English;
3) Cannot give informed consent;
4) MRI contraindications (e.g., foreign metallic implants, pacemaker);
5) Known primary neurological disorders, such as Parkinson's disease, Alzheimer's disease, traumatic brain injury, cognitive impairment or dementia,
6) Known severe inflammatory disease such as systemic lupus erythematosis, known autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis;
7) Clinical Dementia Rating Scale score greater than 0;
8) Diagnosis of a chronic psychiatric illness other than MDD at the discretion of the study doctor;
9) Significant handicaps (e.g. uncorrected hearing or visual impairment, mental retardation) that would interfere with testing;
10) Bleeding diathesis;
11) Severe Medical problem, which in the opinion of the investigator would pose a safety risk to the subject;
12) Clinically significant cardiovascular disease that will be assessed on a case-by-case basis. Clinically significant cardiovascular disease usually includes one or more of the following: cardiac surgery or myocardial infarction within the last 4 weeks; unstable angina; acute decompensated congestive heart failure or class IV heart failure; current significant cardiac arrhythmia or conduction disturbance, particularly those resulting in ventricular fibrillation, or causing syncope or near syncope; uncontrolled high blood pressure; QTc greater than 450msec (by history for subjects with cardiac disease); documented prior stroke;
13) Clinically significant abnormalities on EKG. Primary AV block or Right bundle branch block are not necessarily exclusionary;
14) Current diagnosis of cancer
15) Current diagnosis of HIV, active Hepatitis B and/or Hepatitis C
16) Use of an Investigational medicine within the past 30 days;
17) Use of Coumadin, Warfarin within the past 2 months;
18) Current treatment with psychotropic drugs or drugs that affect the CNS such as beta-blockers, mood stabilizers, antipsychotics, steroids or non-steroidal anti-inflammatory medications or other antidepressants. No subjects will be included in the study unless

they have been off all psychotropics for at least 3 weeks, except in the case of fluoxetine, where 5 weeks off treatment will be required;

19) Current alcohol or substance abuse disorder, schizophrenia or other psychotic disorder, bipolar disorder, or current OCD;
20) Abnormal liver-function test
21) Current diagnosis of ulcer disease, Chron's disease, GI bleeding or anemia
22) Weight less than 110 lbs
23) Renal insufficiency
24) Any other factor that in the investigator's judgment may affect patient safety or compliance (e.g. distance greater than 100 miles from this facility);

Additional Exclusion Criteria for Depressed Subjects:

1) Active suicidality or current suicidal risk as determined by the investigator

2.10 Populations vulnerable to undue influence or coercion

During the consent process, all potential participants are made aware and reminded that their participation is completely voluntary and that they may choose to not participate or withdraw from participation at any time. All researchers are made aware of the importance of the voluntary participation and the need to convey this fact to all potential participants throughout the consent process and during participation. The voluntary nature of participation is reviewed with the potential participant as is the ability to withdraw at any time should a participant choose to do so.

3 Study Population

3.1 Subject recruitment

In addition to recruiting participants from the community, several physicians and collaborators have expressed interest and willingness to provide referrals and contacts to facilitate recruitment for this study. We will use the Penn Data Store to identify potentially eligible participants. We will be incorporating Penn Data Store (part of DAC) as a recruitment resource. We will send the DAC a list of basic inclusion criteria for a report every 2 weeks. Due to the sensitive nature of the diagnosis (depression) we are seeking, we will first be contacting potential participant's diagnosing provider requesting to contact his/her patient; we will attempt to contact the provider 2 times via Epic and we will seek expressed approval for permission to contact each patient (we will not attempt to contact any patients for which we had not received written approval from their diagnosing provider). We will attempt (up to 2 times) to contact the patient/potential participant. If these individuals have an email on file, we will first attempt to contact via email; otherwise we will use the phone number provided in EPIC. Please find scripts attached for the 1st provider contact, 2nd provider contact, participant email, and phone script (participant).

Flyers and brochures will be distributed throughout the greater Philadelphia area with a heavy concentration in medical facilities such as HUP, Perelman Center, and Penn Radnor. We also

will run advertisements in locally circulated newspapers and paper publications as well as radio stations. We also will advertise on electronic platforms such as the iConnect portal at the University of Pennsylvania, Craigslist, and social media sites.

We will utilize a social media campaign ran by Splash Clinical, a research study marketing company, which will advertise on Facebook, Craigslist, Google, and Instagram. Splash Clinical will create the social media page on each platform to advertise this study, with the exception of Facebook. Splash Clinical will utilize our current Facebook page for the Center for Neuromodulation in Depression and Stress. Splash Clinical creates the advertisements we will post for the campaign, monitors the traffic, and provides us with diagnostics of this campaign. Interested participants will click on an advertisement that will populate in their newsfeed/Google search, and be directed to a landing page that provides a brief description of our study and a link to the RedCap Self-Screener, which previously has received IRB-approval. We will also run a social media campaign internally. We will utilize the Center for Neuromodulation in Depression and Stress Facebook page to post these advertisements. We also would like to utilize the "boost" post or "boost" page feature to allow us to target a specific population when we are advertising our study.

The PennBioBank will also act as a recruitment resource. We will provide the Penn BioBank with a list of ICD-10 codes that we would like included or excluded in a participant's medical record and they will provide us with a report of eligible participants for whom they have a tissue sample for (namely plasma or serum). We will then reach out to these potential participants as they have signed an agreement when they donated the sample to be contacted for research studies in the future. We will call them at the phone number they had previously provided the BioBank, state that we attained their information from the Biobank and that they had previously consented to being contacted by Penn researchers, and then we will continue with our normal phone script.

University of Pennylvania's iConnect, a database of clinical research studies at the University of Pennsylvania added a feature that allows individuals with certain conditions, to sign up as part of a volunteer registry. We will utilize this registry to find older individuals who are suffering from depression and inflammation. They also have a feature to utilize a pre-screener form for individuals who find our study on their website, which we will be implementing to help lower the rate of false positives.

Additionally, we will be using the IRB-approved protocol #826348, A Feasibility Study: Department of Psychiatry Research Initiative (PI: Yvette Sheline, MD). Protocol #826348 is a pilot study the will continue through August 2017; the study collects basic contact and psychiatric intake information from Penn Behavioral Health Corporate Services & Departmentally. Only individuals who meet the basic criteria for this study will be contacted. Please see details of 826348 for further information, if necessary.

3.2 Subject compensation

Total compensation for completion of the entire study is $215 for the experimental group, $310 for experimental group that also completes the open label phase, and $120 for the control group (non-depressed) participants. If a participant takes part in a portion of the study, but does not complete the entire study, he/she will be paid incrementally in proportion to the degree of participation. If a potential participant passes the phone screen, he/she will be given an appointment to conduct the baseline informed consent. Once consented, the patient will undergo a more thorough diagnostic screening interview, EKG and blood draw. If the patient is found to be ineligible, it will be explained that he/she will not participate in the study and he/she will be excused. If the participant completes the blood draw at this screening visit but has been determined, ineligible they will be given $10.00 compensation for the blood draw. Following the screen, those who are eligible for participation will receive the full informed consent form and they will continue with the full baseline assessment battery. Unscheduled visits may occur if participants cannot complete the assessments required within the allotted time. If this does occur participants will receive travel compensation, but no changes will be made to their human subjects payment. Furthermore, participants in the depressed group will be paid an additional $10 for the second blood draw as well as additional funds in $5 increments for interim follow up visits occurring in week 1, 2, and 4. Control group participants do not have weekly follow up visits, thus they will not receive these interim payments. Subjects will be compensated through University of Pennsylvania supported Greenphire ClinCard. This is a reloadable prepaid card (similar to a debit/credit card) which allows funds to be available immediately. Study staff will provide participants with a ClinCard Cardholder FAQ: US document.

Subjects will receive compensation at the end of their study completion. If subjects do not fully complete the study, they will receive compensation for the parts of the study they did complete, based on the following outline:

Payment regimen for Depressed Group:

Baseline assessment & full study consent process = $20;
Baseline MRI = $20;
Baseline Blood Draw = $10
Week 1 assessment = $5;
Week 2 assessment = $5;
Week 4 assessment = $5;
Week 6 MRI = $25;
Week 6 Blood Draw = $10
Week 6 Neuropsychological Testing = $25;
Optional additional blood collection = $50
Optional saliva samples = $20
Optional 6-month follow-up = $20

Those who complete all study visits, participate in the optional additional blood collection, provide the optional saliva samples, and participate in the 6 month follow-up, will be paid a total of **$215.**

Those who complete the open-label phase of the study will receive an additional $95 (including optional saliva collection) broken down below. These participants will receive $310 total including the optional saliva sample collection
Week 1 assessment- $5
Week 2 assessment- $5
Week 4 assessment- $5
Final Neuropsychological assessment- $25
Final MRI- $25
Final Blood Draw- $$10
Optional Saliva Collection - $20
Optional 6 month follow-up (same as above)- $20

Payment regimen for Control Group:
Baseline assessment & consent process = $20;
Baseline MRI = $20;
Baseline Blood Draw = $10
Final MRI and Blood Draw = $35;
Final Neuropsychlogical Testing = $25;
Control participants will be paid a total of **$120**for full study completion (Including screening visit blood draw= $10).

Subjects who do not feel comfortable with the Greenphire ClinCard may be compensated by a check, in lieu of the ClinCard. We are not stating this option in the ICF, as we would prefer all participants to use the ClinCard for consistency; however, we acknowledge that not all individuals will feel comfortable with this method and therefore, if during the consenting process or at any time during the study, a participant expresses discomfort, we will then verbally offer them the option of being paid with a check.

Transportation
We will provide participants who drive to study visits (including screening visit), a parking voucher for free parking at the Perelman Center parking ramps. Participants will also receive compensation for travel for any unscheduled visits that occur.
Participants who take public transportation to study visits (including screening visit) will be provided up to $5.00 cash for the cost of taking public transportation with proof of transportation (e.g., SEPTA regional rail ticket, Uber or taxi receipt, etc.) on their Greenphire ClinCard.

3.3 Suicidal Ideation and Behavior

Plan for assessing suicidal ideation and behavior: The drug, escitalopram, will be administered to a portion of the participants in this study. Escitalopram does interact with the central nervous system as an antidepressant. Because it is an antidepressant, it is not expected to cause or contribute to the development of suicidal ideation or behavior. However, to monitor for a potential increase in risk of suicidal thinking and behavior, a safety protocol has been developed and plans are in place to evaluate all participants for clinical worsening and suicidality throughout participation in this study.

Safety Protocol to address potential risk of clinical worsening and suicidality:

As part of this study protocol, depressed patients will be enrolled and will be receiving anti-depressant medication, escitalopram. Current suicidality at the time of consent deems potential participants as ineligible to participate, thus suicidal patients will be screened out of the study. However, it is recognized the patients could possibly experience clinical worsening and potentially become suicidal. This possibility is unlikely for those who are receiving escitalopram since this medication is an anti-depressant and it is prescribed for the specific purpose of treating depressed mood.

To address the risk of suicidality, all patients will be closely monitored and they will receive psychiatric evaluations at baseline, weeks 1, 2, 4, and 6, as well as during all of these time points during the open label phase. The Montgomery-Asberg Depression Rating Scale will be administered at each follow-up visit to evaluate the presence of depressive symptoms. Additionally, participants will be provided with phone numbers of research staff and they will be instructed to contact the staff if clinical worsening occurs. If research staff become aware of clinical worsening or suicidality, they will immediately intervene and activate safety procedures.

Safety procedures will include informing the principle investigator who will then meet with the participant to assess the situation. If the participant is in imminent danger to her/himself, s/he will be escorted to the emergency room for immediate care. If the patient is reluctant or unwilling to be escorted to the emergency room the principle investigator will call 911. If the patient expresses no immediate desire to hurt her/himself, but is still considered to be a potential risk, the principle investigator will provide emergency numbers such as suicide hotline phone numbers and the principle investigator will also provide referral phone numbers for psychiatric care, facilitating the scheduling of an appointment. The principle investigator will engage the participant in a contract whereby the participant will agree to utilize the suicide hotline phone number if necessary and s/he will follow through with the psychiatric appointment.

If it is believed that the clinical worsening is deemed related to participation in the study, the principle investigator will evaluate whether the medication regimen should be modified or if the participant should be withdrawn from participation.

All evaluations and decisions regarding a participant's clinical worsening and or suicidality will be documented and the appropriate regulatory authorities will be notified.

4 Study Intervention (Escitalopram and Celecoxib.)

The PI will utilize the services offered by IDS to obtain escitalopram and celecoxib, and placebo for celecoxib, repackaged so that the research team is blind. The drug and placebo will be picked up from IDS as needed and then stored at the Center for the Neuromodulation of Depression and Stress (CNDS) in locked cabinets behind locked doors until the appointment. Only research personnel will have keys and access to the medications. The drugs and placebo will be dispensed by the principle investigator or designee to each participant at each appointment. The medication regimen will be thoroughly reviewed with the patient (i.e., dosage and duration) and written instructions will be provided. Additionally, all participants will have contact information for research personnel should they have any questions about the medication or concerns about reactions to the medication.

4.1 Description

Escitalopram and celecoxib are FDA approved drugs. Escitalopram will be administered in a tablet. Celecoxib will be administered in a capsule to hide whether it is active drug or placebo.

4.2 Intervention Regimen

Participants will take escitalopram (10 mg/day for 3 days and 20 mg/day for 39 days) plus celecoxib (400 mg/day) or escitalopram (10 mg/day for 3 days and 20 mg/day for 39 days) plus placebo for 6 weeks. Participants will be lowered to a smaller dose of the study drug, if they are unable to handle the higher dose (as determined by side effects or subjects expressing concerns about side-effects). All participants will receive a taper dose of escitalopram at 10 mg for 3 days at the completion of the study.

4.3 Receipt

IDS will package and deliver the drugs or study coordinators will pick them up as needed for participants. Drug accountability will be maintained by IDS and study coordinators. Before study drugs are dispensed to enrolled study participants, pill count will be conducted by the attending nurse, study coordinator or investigator. Study participants are encouraged to personally check that they received the right number of pills.

4.4 Storage

Room temperature, protect from light, protect from moisture.

4.5 Preparation and Packaging of Verum Study Drug

The Investigational Drug Service will purchase CELECOXIB 200mg capsules from Pfizer, Inc. These capsules will be over-encapsulated into opaque Size 1 pharmaceutical-grade, BSE-free

certified, gelatin capsule shells and then backfilled with lactose monohydrate NF as described below.

Capsules:

- Size 1 Green
- Source: Fagron, Inc, 2400 Pilot Knob Rd, St. Paul, MN 55120
- Item # 800710

Lactose Monohydrate, NF

- Source: Fagron, Inc, 2400 Pilot Knob Rd, St. Paul, MN 55120
- Item # 801787

The capsules will be prepared using a GMP-grade machine capable of loading and filling 300 capsules at a time (Profill-324™ Machine, Serial # 3FB.49). All surfaces in contact with the capsule or filler, are type-316 surgical stainless steel.

Capsules are filled in an enclosed pharmaceutical compounding area which is not accessible to general traffic and in which food items are not allowed. All persons working with the capsules have been previously trained in the capsule loading and filling procedure and all items used in the preparation are verified by two persons before the start of the session and again at the end of the session. Only one type of product is made at one time; the product is completed, all containers sealed and product moved away from the machine, then the machine is then disassembled and cleaned and the work area cleaned, before a second product may be prepared on the machine.

Persons working with the machine first enter through an anteroom in which hands are washed thoroughly and a hair net, gown or scrubs, and gloves are worn, before entering the compounding area. The area where the capsules are prepared is cleaned with 70% isopropyl alcohol prior to the start of the procedure. The removable top and bottom plates from the capsule filling machine are washed in a laboratory-equipment washer (LabConCo FlaskScrubber™), with a high-heat drying cycle, between uses.

The capsules are loaded into a filling tray by placing empty gelatin shells into the hopper of the capsule loader tray, which orients and drops the shells into precut holes in the capsule filler tray. The capsule halves are separated and the filled tray is then removed from the loader and placed into the filling machine, where the shells are then separated, leaving the bottom half to be filled, while the top half of each shell is held in a tray which is set aside. A stainless-steel frame is placed over the filler apparatus containing the bottom halves of the capsules; this frame prevents powder from being lost during the filling process.

Once the capsules are separated, a total of 300 CELECOXIB capsules are first counted and verified, then the operator places one whole tablet into each capsule. A second person then verifies visually and signs the batch worksheet to document that each capsule shell contained one tablet and that no shells were empty. Once this process is complete, lactose monohydrate is backfilled over the capsules until flush with the rim of the capsule shell.

At this point the stainless-steel frame is removed and the tray containing the lids for each capsule is placed back over the capsule bottoms. The capsules are rejoined, removed from the machine, then placed in a Type 2 HDPE plastic container with a tightly sealing lid.

The container is sealed and labeled clearly with the contents (drug, strength, quantity), an internal lot number and use-by date which are assigned at the time of preparation. The use-by date will be either 12 months or the remaining expiration of any component, whichever is less), the name of the study for which the capsules have been prepared, and the statement "Caution: Drug Limited by Federal Law to Investigational Use"

Finished capsules will be dispensed in blister packs pursuant to an individual prescription for each study participant, provided by the investigator/prescriber. A prescription label will be affixed, including directions, study name, medication name (blinded description) and quantity, investigator name, subject ID number and the statement 'Caution: new drug limited by Federal (USA) law to investigational use only".

Caution: New Drug Limited by Federal (USA) Law to Investigational Use Only	**University of Pennsylvania – Investigational Drug Service** 3600 Spruce St – Ground Maloney – Philadelphia PA 19104 215-349-8817 AH4429177 RX # XXXXXXXXX Dr. _____ Patient: XXXXXX (######) Date: _____ **TAKE ONE (1) CAPSULE BY MOUTH TWICE EACH DAY AS DIRECTED.** IND# ###### Study Period: ____ **CELECOXIB 200MG OR PLACEBO CAPSULES #10** Study: IRB#819654 Refills: 2	INVESTIGATIONAL DRUG **[BARCODE HERE]** Rx# XXXXXXXXX

4.6 Blinding

The PI and all but one member of the study team will be blinded. The unblinded member will perform the randomization and will enter the group into a password protected excel sheet. This team member will be able to break the blind in an emergency.

4.7 Administration and Accountability

Study drug will be delivered by the UPENN Investigational Drug Service. Escitalopram will be administered once daily, in the morning or evening, with or without food. Single doses of celecoxib can be given without regard to timing of meals.

The Investigator will maintain adequate records showing the receipt, dispensing, return, or other disposition of the investigational product, including the date, quantity, batch or code number, and identification of subjects (subject number and initials) who received the investigational product. Investigational Product may not be relabeled or reassigned for use by other subjects.

4.8 Subject Compliance Monitoring

Participants will be asked to bring back their empty pill bottles/packs for pill counts to assess compliance. If pills have not been taken, the participant will be engaged in discussing issues of compliance and how these can be addressed by the study team. If compliance remains poor he PI will discuss with study participants the possibility of discontinuation/withdrawal.

Post study, compliance can be objectively assessed by measuring drug levels in plasma samples and possibly in CSF as well.

4.9 Return or Destruction of Investigational Product

Any extra drug returned will be given to IDS.

5 Study Procedures

5.1 Informed Consent Process / HIPAA Authorization

100 depressed participants will be recruited from the community, from outpatient psychiatry clinics at the University of Pennsylvania (General Outpatient Psychiatry, Family Medicine, Geriatric Psychiatry, the Treatment Resistant Depression Clinic, the HUP consult liaison services), and from the Veterans' Administration Medical Center. Fifty non-depressed control participants will also be recruited from the community and the aforementioned sources. These participants initially will be contacted by research staff via telephone to conduct a phone screen interview and also to explain the study, including the time commitment required for participation and the basic procedures to be completed. This information is required to determine eligibility and is directly related to our inclusion/exclusion criteria. The information obtained through the phone screen is our way of ruling out specific and obvious things that will determine if a person meets preliminary inclusion/exclusion criteria without having to make a trip to this facility. We are requesting a waiver of documentation of informed consent for this procedure; verbal informed consent will be obtained from all subjects. Individuals who are patients in the Penn system will be asked for verbal consent during the pre-screening to release their PennChart electronic medical record. Data from the PennChart electronic medical record will be viewed only. The information will not be downloaded or stored. Individuals without a PennChart electronic medical record will have one created for them in order to participate in full screening study procedures (i.e. blood draw and EKG). Individuals who are not in the Penn system will be asked to complete an medical record release authorization form at the screening visit after their have signed the informed consent. Upon receipt of their medical records, personal health information and identitifiers will be redacted to mitigate the risk of lost of confidentiality and privacy. For those who pass the initial phone screening, an appointment will be scheduled for an in-person visit to complete the informed consent process and to conduct a diagnostic screen, blood draw to determine CRP levels, the presence of HIV, Hepatitis B and C, autoimmune disease, and rheumatoid factor, and EKG to further assess eligibility. There will be time between the phone screen and the in person visit so that participants can consider participation and discuss the study with family and friends. Following the diagnostic assessment and EKG, if a participant continues to be eligible for participation, s/he will receive a second consent that addresses the expectations for participation in the entire study. Additionally, participants will be provided a copy of the consent to keep for their files which will contain all research staffs' contact information. Prior to participation, the research staff will thoroughly review the consent form, answering any and all questions. The participant will be made aware that participation is entirely voluntary and he/she may stop at any time during the study, including after signing the consent form. As mentioned previously, unscheduled visits may occur if assessments required are not completed within the allotted time.

5.2 Recruitment, screening and baseline assessments

Brief Summary of Procedures (also see attached schedule of procedures and assessments):

Potential participants will be recruited from the community or they may be approached by collaborating clinicians or research team members (see recruitment plans) to explore potential participant's interest in taking part in a research study. Those who are interested will receive a telephone call from research staff to conduct a telephone screen. Those who pass the phone screen will receive an appointment to come to the research location to review and sign the informed consent form for screening purposes and to complete baseline diagnostic assessment screen, blood draw to test for CRP level, Hepatitis B and C, HIV, and rheumatoid arthritis, and will also complete an EKG to determine eligibility. Those who are eligible will return for a baseline visit and will review and sign another informed consent form for participation in the full protocol. Once fully consented, participants will undergo a series of visits to conduct multiple assessments which will include:

(1) Psychological and Neuropsychological Tests at baseline and week 6; Open Label at week 6;

(2) Medical Examination;

(4) 3-4 Blood Collections (blood draw at screening, blood draw at baseline, blood draw at week 2 for liver function and hematocrit assessment, and one at Week 6) for those who enter the open-label phase they will receive an additional 2 blood draws (one at Open Label Week 2 to assess for liver function and hematocrit levels, and one at Open Label Week 6). All participants will be asked if willing to participate in additional blood collection for immune profiling.

(5)2-3 MRIs, one at baseline, one at week 6, and one at open-label week 6; the baseline scan will occur before treatment begins;

(6) 2-3 fMRI (one at baseline which will occur during the same baseline MRI session, one at Week 6, and one at open-label week 6);

(7) Optional Nutrition Assessment. Participants will be asked to do a 24-hour dietary recall. The food recall will also be collected using the Nutrition Data System for Research (NDSR). Participants are guided through a multiphase interview where they are guided to recall all the food they ate in the preceding 24 hours.

(8) Participants will be closely monitored for problems resulting from participation. They will be provided with contact information for research personnel should any problems or questions arise, and they will be contacted by research personnel during their participation to ascertain the presence of any problem.

Treatment Design:

All subjects will have an initial telephone screening. Screened participants will have an appointment to come to the research facility to conduct informed consent for the diagnostic screen and blood test to measure CRP levels and test for HIV, Hepatitis B and C, autoimmune diseases and rheumatoid factor. This visit is expected to last approximately 90 minutes. Informed consent will be obtained, and then participants will undergo a blood draw to assess c-reactive protein level, as they will be stratified depending on the current level. Subjects will participate in a structured clinical interview, EKG, and a review of medical history with the research staff. All participants will be given the screener for Structured Clinical Interview for DSM-IV (SCID-1) to ascertain the presence or absence of Major Depressive Disorder (MDD) and to rule out other psychiatric disorders. They will also be given the Hamilton Rating Scale for (HAMD) and Montgomery-Asberg Depression Rating Scale (MADRS). Those with MDD may be enrolled in the experimental condition. Potential Controls may be enrolled in the control condition if they have no psychiatric disorders and their HAMD score is less than or equal to 7. Participants will receive an EKG to screen for any abnormalities or conditions that would suggest they should not receive medication thus be deemed ineligible at this point. Participants will be asked medical screening questions (e.g., from the Framingham Scale to determine a vascular risk factor), and they will be assessed with the Clinical Dementia Rating (CDR) scale to determine whether there is any cognitive impairment. At the screening, the participants will be asked to fill out the Perceived Stress Scale (PSS) and Life Events Stress (LES). A trained study team member will then perform a neuropsychiatric test on the participants that include questions about the participants' memory, attention, spatial abilities, general abilities, motor control, and problem solving.

At this point, all participants who remain eligible will undergo a second informed consent to address the next steps in participation. Following the acquisition of the second consent, an appointment will be scheduled for the participant to conduct the baseline assessments. At these baseline assessments participants will receive their actigraph which will monitor their physical activity throughout the course of the study. These baseline assessments include neuropsychological tests, which will take approximately 1 hour to complete. Also at baseline, participants will receive anMRI (detailed further below).Following completion of all baseline assessments, the experimental group participants will receive the drug. Participants will take 10 mg of the antidepressant + placebo or 10 mg of the antidepressant + celecoxib for 3 days, and then they will increase the antidepressant to 20 mg for the remaining 39 days of the study. Final dose (10 mg versus 20 mg) will be determined by clinical response and side effects. This decision will be based on an ongoing discussion between the participant and the study doctor. Participants will be closely monitored for side effects via phone calls from research staff and in person inquiries during the follow-up visits. They will be provided with contact information (phone numbers) for research personnel should any problems or questions arise, and they will also be instructed to contact research personnel with any problems, questions, or concerns. Participants in the experimental group will have scheduled appointments at weeks 0, 1, 2, 4, and

6. At the end of the 6 weeks participants will have their final physical and psychological assessment. Individuals remitting will be given an additional 2 weeks of escitalopram at this time; this is done so that individuals have enough time to schedule a visit with a provider of their choice, if they wish to remain on the medication. At the end of the study, all depressed participants will be transitioned into follow-up care with their own doctor or given a referral. For those who do not remit and had received escitalopram + placebo initially, they will be eligible for the open-label phase of the study. During this phase they will be given escitalopram + celecoxib. They will follow the same study procedures as the initial phase of the study (Medication visits Week 1, Week 2, Week 4, 3rd and Final MRI, neurocognitive testing, and blood draw).

5.3 Assessment measures and neuropsychiatric tests

The following assessment measures and neuropsychiatric tests will be used:

Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I - Abbreviated version). The Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) is a semi-structured interview for making Axis-I diagnoses according to the DSM-IV, and usually takes 60-90 minutes to administer. We will perform only the overview portion;

Montgomery-Asberg Depression Rating Scale (MADRS). The ten-item MADRS will be used to determine the presence of and severity of current depressive symptoms, and will be the primary outcome measure of depression severity in this study. The MADRS is included as it was used in our previous studies and will allow for comparison;

Hamilton Rating Scale for Depression (HAMD). All patients and controls will also be assessed using the HAMD, 17-item version, to determine the presence of any current depressive symptoms. The HAMD is included as it is more widely used in the US than the MADRS;

Montreal Cognitive Assessment (MOCA): This short test of visual and verbal functioning and delayed recall gives an indication of cognitive decline and dementia..

Framingham scale. For each subject we will rate a vascular risk factor (VRF) total score using the approach of the Framingham Study (Wolf et al., 1991), which gives a validated number based on the presence and severity of VRFs, weighted according to their predictive power for stroke;

Cumulative Illness Rating Scale-Geriatric (CIRS-G). A history of major illnesses and treatments will be taken, and the Cumulative Illness Rating Scale-Geriatric (CIRS-G) will be completed by the evaluating physician at the start of the trial (Miller et al., 1992). The CIRS-G covers all major systems (cardiovascular, pulmonary, renal, genitourinary, etc), and provides scores for current and past medical illnesses, including a global composite score that will be used as a measure of medical morbidity;

The following Self-Report Questionnaires will be distributed:

Perceived Stress Scale (PSS). We will use the PSS score, a validated measure of subjective life stress to characterize the relationship between inflammatory cytokine levels, subjective stress, depression severity and treatment response. It is the most widely used measure of subjective stress;

Life Events Stress (LES) Scale. Each event is assigned a weight for the severity of the stressor, e.g. death of spouse vs. friction at work. Scores for all relevant events are summed to yield a total life events stress score. This scale will be used to determine the cumulative amount of stress experienced by each participant;

Maltreatment and Abuse Chronology of Exposure (MACE) Scale There is reason to believe that childhood maltreatment can affect the trajectory of brain development and enhance the risk of medical and psychiatric disorders. We will use this scale to further test this theory. The 'Maltreatment and Abuse Chronology of Exposure' (MACE) scale (1) can be used to assess not only the type and severity of abuse exposure but also the timing of the exposure. There is increasing interest in childhood maltreatment as a potent stimulus that may alter trajectories of brain development and enhance the risk for medical and psychiatric disorders. Although a number of other scales exist for retrospective assessment they fail to provide detailed information on the timing of the exposure, critical for delineation of sensitive periods. Using the MACE (2) both dose dependent and timing effects were demonstrated for some disorders, whereas overall amount of adverse experiences was the critical. In our current study the variables obtained from the MACE will be included in predictive models to help understand which brain circuits are affected in particular symptom clusters and disorders. This will help us improve our predictive models, given the strong relationships obtained using the MACE. Answers to the MACE will be anonymized and entered into the database. As with all procedures, participation is voluntary and it will be explained to participants that the interview might be uncomfortable or upsetting. In the event that a participant becomes upset they will be asked if they would like to stop the questions. Participants will be asked if they would like counseling and referred for care if appropriate.

The following comprises the Complete Neuropsychological Battery. This will be performed at the beginning and end (weeks 0 and 6):

Controlled Oral Word Association (COWA)/Verbal Fluency: In these tests, which are widely validated tests of frontal lobe function (Cohen, 1993), subjects are given 60 seconds to generate as many words as possible that begin with a specific letter of the alphabet or to name as many examples in a particular category (such as animals), respectively. Subjects receive one point for every correct word. For the COWA, a standardized set of letters (CFL) with established norms

will be used. The animal category is the easiest of the verbal fluency categories (Monsch, 1992) and has been shown to discriminate Alzheimers Disease from depression, whereas letter fluency did not (Hart, 1988);

Trail Making Test A&B: In this test subjects are required to sequentially connect on a page of paper dots that are marked with either letters or numbers (Part A). They are then instructed to connect dots by alternating between numbers and letters sequentially (e.g. 1-A-2-B-3-C-4-) (Part B). In each part, performance is timed. This test is sensitive to deficits in visuomotor tracking, speed, and attention (Lezak, 1995), and performance is correlated with frontal activation (Segalowitz, 1992). Studies show depressed patients to be significantly slower on Part B (King, 1993). Normative studies show increased performance times with age;

Mini-Mental Status Exam: This is a short 5 minute test used to assess orientation, memory, and recall. It measures cognitive decline and dementia. Participants must score a 26 or above to participate in the study.

Word List Memory Test: In this test a 10-word list is presented to subjects three times and immediate free recall of the list is tested after each presentation. Then following a 30-minute delay, free recall is again tested. Subjects are then asked to recognize the learned words from a list of 20 words. This test measures immediate and long-term memory and allows for an assessment of both memory acquisition and retrieval;

Digits Forward: This simple test of memory and concentration subjects are presented orally with a list of digits and are required to recite them back to the examiner. The digit list becomes progressively longer. Subjects are scored based on the number of correct trials;

Digits Backward: This simple test of memory and concentration subjects are presented orally with a list of digits and are required to recite them in reverse order. The digit list becomes progressively longer. Subjects are scored based on the number of correct trials;

Digits Ascending: This simple test of memory and concentration subjects are presented orally with a list of digits and are required to recite them in ascending order. The digit list becomes progressively longer. Subjects are scored based on the number of correct trials;

Logical Memory (Immediate and Delayed Recall): This subtest of the Wechsler Memory Scale tests immediate recall. In this task a short story is read to the subject and they are required to recall the story immediately following the presentation. They are scored based on the number of correct elements recalled from the story. After a 30 minute delay the subject will be asked to recall each story again;

Digit Symbol: In this task, subjects are given a key in which symbols are assigned a digital value from 1-9. Subjects are then required to use the key to translate a list of symbols into digits;

Benton Visual Retention Test: This task has the participants look at 10 cards, one at a time, for 10 seconds, the card is removed and they are to draw what they saw on the card;

Shipley Vocabulary Test: The subject is given a word along with 4 choices and asked to circle the word that means the same thing as the given word;

Boston Naming: The subject is shown cards and asked to name the picture that appears on the card;

Stroop Color and Word Test: This task has participants read a series words that spell out different colors in a color not the same as the word itself. This is a measure of selective attention and processing speed.

MRI procedures: At baseline, all participants will be taken to University of Pennsylvania imaging facilities for neuroimaging whereby MRI and a fMRI will be conducted. At baseline, we will acquire anatomical MR images as well as functional MR images while doing the resting state connectivity task. We will also acquire functional MR images at the end of the study (end of week 6).

Image Acquisition: Structural and functional scans will be obtained in a single session on a clinically-approved 3 Tesla Siemens Trio (Erlangen, Germany) scanner, equipped with 40mT/m gradients and 200 mT/m/s slew-rates. RF transmission will use a quadrature body-coil, and reception will use a Siemens receive-only 64-channel head coil. The total time in the scanner will be approximately 1 hour. Based on our experience, this is well within patients ability to tolerate the scanning procedures without discomfort and without excessive motion. We will acquire structural images, perfusion images, and functional images as follows; all MRI imaging be reviewed and approved by CAMRIS. High-resolution Anatomical Images: A 5 min. magnetization prepared, rapid acquisition gradient echo (MPRAGE) image is acquired, which includes an inversion recovery preparation period to produce T1-weighted contrast, and has a 1x1x1mm voxel size. The MPRAGE is used to screen for gross anatomical abnormalities, to facilitate registration of lower resolution functional images into a standardized space, and to allow identification of between-group or inter-individual variation in volume of regions that could affect interpretation of functional results.

Resting Perfusion Images: A ~5 min. pulsed arterial spin labeled (ASL) perfusion MRI and ~1.5 min. reference scan are acquired to directly measure resting cerebral blood flow, using magnetically-labeled blood as an endogenous non-invasive tracer. Functional Images: Functional scans lasting ~15 minutes. fMRI will be acquired with blood oxygenation level dependent imaging (BOLD) using a whole-brain, single-shot gradient-echo (GE) echo-planar (EPI) sequence. The BOLD sequences include on-line geometric correction for spatial distortions due to magnetic field inhomogeneity using a magnetic field map acquired with a 2 min reference scan.

5.4 Blood draws

During the screening visit, all participants will have a blood draw to test for CRP levels to stratify the participants accordingly in High CRP and Low CRP groups. Participants will also have a blood sample drawn at the end of the screening visit to test immediately for Hepatitis B and C, HIV, autoimmune diseases (double-stranded DNA test), and rheumatoid factor; as well as to use in later analyses of inflammatory markers. Participants will have a blood sample drawn (approximately 16.5mL) at the screening visit, approximately 40 mL at the baseline visit, approximately 40 mL at the end of the study, 40 mL at the end of the open label phase. Blood samples will be used for doing tests for inflammatory markers and for measuring levels of Interleukin-6, Interleukin-10, TNF-alpha, and C-reactive protein. We will look at immunological markers by two methods; luminex and flow cytometry. The Luminex assay measures soluble proteins in the sera. Molecules such as IL-6 and TNF-alpha, MIP1-alpha, IFN-alpha, CRP will be assessed. We will include or alter molecules in the luminex assay based on the most recent literature as we near the end of sample collection. Flow cytometry will also be utilized. Flow cytometry will be used to measure the status of T cells which are important in suppressing such conditions as infectious disease and cancer. We will develop a series of markers that define the memory and naive phenotype of T cells along with their activation status and functionality. We will also assess additional cell subsets such as NK and macrophages. Molecules that will be assessed include but are not limited to; CD3, CD4, CD8, CD45RO, CD27, CCR7, CD69, CD33, CD68, CD56,CD16, CD38 and HLADR and CD11c -monocytes. Some blood will be retained and banked for future research. Additional blood is being collected for ex vivo isolation of human peripheral blood mononuclear cells (PMBC), which is another assessment of inflammation levels. This additional blood draw will be optional and offered to all depressed participants.

Participants will give blood for a liver-function test and for hematocrit levels to test for GI bleeding. Participants may also have 24-hour ambulatory blood-pressure monitoring. Participants may wear a cuff for 24 hours that is set to automatically take blood pressure every 15 minutes during the day and every 30 minutes during the night.

5.5 Gastrointestinal Safety

At the beginning of the study, GI/bleeding risks will be examined. Hematocrit levels will be tested in blood samples to assess this risk at baseline, and at week 2.

5.6 Protocol Non-Conform Drugs

Urine may be used to assess intake of protocol non-conform drugs by means of the Rapid Detect 10 Panel Test Dip Card, Rapid Detect, Inc., 804 South Broadway, Poteau, OK (or similar). The drug screen includes for example Amphetamines (AMP), cocaine (COC), Tetrahydrocannabinol (THC), methamphetamine (mAMP), opiates (OPI), Phencyclidine (PCP), benzodiazepines (BZO), tricyclic antidepressants (TCA), barbiturates (BAR), and methadone (MTD). This can be done both prospectively and retrospectively.

5.7 Ambulatory Blood Pressure Monitoring (ABPM)

Ambulatory blood pressure monitors (ABPM) are readily available to the investigators to assess ABP in this study protocol. The 90207 ABP monitors are compact and lightweight to optimize subject comfort and a choice of four cuff sizes further aids comfort while also maximizing accuracy.

ABPM will be guided by the AHA Scientific Statement, Council on High Blood Pressure (4). Recommendations relevant to the present study are:

- Select ABP cuff size according to Table 1,
- Select the non-dominant upper arm for ABPM measurements,
- Instruct the patient to hold the arm still by their side while the device is taking a reading.

Table 1 Cuff Sizes for ABPM

Cuff Size*	Arm circumference [cm]	Arm circumference [inches]
Small Adult	17-26	7-10
Adult	24-32	9-13
Large Adult	32-42	13-17
Extra Large Adult	38-50	15-20

* The bladder of the cuff should encircle at least 80% of the arm circumference (4).

The monitors for the 24-hr ambulatory blood pressure measurements will be preset to assess BP every 20 minutes in the daytime, defined as 0600h to 2200h, and every 30 minutes during the nighttime, defined as 2200h-0600h (**Error! Reference source not found.**). Preset ranges for acceptable BP measurements are 60–250 mmHg systolic, 30-200 mmHg diastolic, 40-230 mmHg for mean arterial values, and 200 b/min for heart rate. ABP readings should cover ≥ 80% of the expected readings with interruptions of less than 1 hour. Awake and asleep times will be determined by patient diaries. This protocol is well established (5, 6).

Blood pressure cuff is usually mounted on the non-dominant arm of the patient connected through a hose with the recorder, which initializes the blood pressure measurements following the preset paradigm.

Experience in the field, however, informed us that the 24-hr ambulatory blood pressure measurements are tolerated by the patients only in varying degrees. Therefore, we will adapt the ABP settings to achieve tolerability. The minimum frequency of 2 measurements per hour during

the day and 1 measurement per hour during the night will be observed as well as a minimum time of observation of 15 hours (e.g. 16:00 until 07:00 the following day).

5.8 Activity Diary

Patients will be asked to fill out an activity diary during each of the 24 hour ABPM session. An example of the diary form is provided in the appendix.

5.9 Actigraphy

Actigraphy will noninvasively assess and log the sleep, wake and activity patterns for each patient. In detail, data capture entails 24 hour sleep/wake measurements including total sleep time (TST), sleep latency, wake after sleep onset (WASO), sleep efficiency, energy expenditure, metabolic rates, steps taken, physical activity intensity, subject position, and ambient light levels.

This device will be applied as a wrist watch which facilitates incorporation into the clinical study setting. Actigraphy devices will be sources from e.g. ActiGraph, Pensacola, FL, http://www.actigraphcorp.com/company/ or similar suppliers.

Participants will be asked to wear this device for the course of the study 6 weeks in total.

5.10 Drug concentrations in plasma

Escitalopram and Celecoxib drug concentration will be determined in plasma and CSF (optional) using standard mass spectrometric methods.

5.11 Urinary Prostaglandin Metabolites

If urine samples are available, drug effects of celecoxib are easily quantified by measuring prostaglandin metabolites in the spot urine collections. Spot urine collections will be stored at -80°C until analysis. The urinary prostacyclin metabolite, 2,3-dinor-6-keto-$PGF_{1\alpha}$ (**PGI-M**), the thromboxane metabolite, 11-dehydro-thromboxane B_2 (**TxB-M**), possibly also including 2,3-dinor-TxB-M and the prostaglandin metabolites 7α-hydroxy-5,11-diketotetranorprostane-1,16-dioic acid (**PGE-M**) and 11β-dihydroxy-15-oxo-2,3,18,19-tetranorprost-5-ene-1,20-dioic acid (**PGD-M**) will be quantified by LC/MS/MS. These assays are routinely performed in the Institute for Translational Medicine and Therapeutics' mass spectrometry laboratories with inter- and intra-assay variabilities of 4-8%.

5.12 Microbiome Analyses

Microbiome can be analyzed through an optional stool sample or optional mouth swab (inside of both cheeks) and saliva collection. DNA from will be extracted from each sample; PCR amplified using 16S primers, and subjected to 454/Roche pyrosequencing. At least 1000 sequence reads will be used to characterize each community. The 16S sequence reads are then aligned using the NAST and GreenGenes servers and inserted into a well characterized phylogenetic trees of 16S sequences, allowing phylogenetic placement of each sequence read. As a first step in analyzing the global effects of each treament, we will compare microbial communities using UniFrac, which quantifies the similarities among microbial communities

based on phylogenetic distances. To compare two communities, sequences from both communities are placed on a common phylogenetic tree generated using ARB. The fraction of the branch length on the tree unique to each community is then measured. This provides a objective measure of community similarity based on the amount of shared evolutionary history. To compare multiple communities, distances between all pairs of communities are computed to generate a distance matrix and Principal Coordinate Analysis is used to plot communities in a scatter plot along orthogonal axes of maximal variance. Such scatter plots can be generated taking into account the abundance as well as the presence of each taxa (weighted UniFrac), or using only presence/absence information (unweighted UniFrac). The two methods thus address different questions--weighted analysis allows differences in proportional representation of community members to be assessed, while unweighted analysis discloses changes in community composition. Measures of alpha diversity such as the Chao1 and Shannon Indices will also be used to characterize communities. Saliva swab samples will be obtained for future unplanned analysis including DNA analyses specifically looking at the APOE genotype as it may affect treatment response.

5.13 Distribution and shipment of human specimens

The human specimens collected for this study will be de-identified and will not contain any Protected Health Information (PHI) prior to in-house sample distribution or shipment to external collaborators.

6 Analysis Plan

6.1 Data Analysis and Predictions

At a basic level, the design of this trial is simple repeated measures design with a) two replicates (time points) b) baseline covariates and outcomes, and c) a single between-person factor (initial case-control depression status). The data collected at two time points to be included in analysis (pre- and post-treatment) will be the depression severity, inflammatory markers, resting state functional connectivity and neuropsychometric data. There have been no published studies to date examining adjuvant celecoxib and SSRI treatment in patients who simultaneously received assessment of inflammatory biomarkers. However, based on our own small pilot sample we will have greater than 85% power to detect differences in changes in IL-6 between SSRI responders and SSRI + celecoxib responders. Volumetric data and confounders to be included in the analysis will be collected at baseline. Classic models of change scores can be used where the baseline level can be controlled giving the so-called residual change score. Use of residualized change scores has several analytic advantages: (1) control for variance (the best predictor of today's value is yesterday's value), (2) statistically each subject is started at the same point. Thus, our estimation model will be extended from the classic model of change scores to a repeated measures ANOVA (analysis of variance) or mixed model repeated measures design. A joint modeling approach with mixed models for repeated measures data can be used to address within-subject variation and between-subject variation of the repeated measurements of multiple

variables such as neuropsychological measures and inflammatory markers. Generalized linear models (GLM) will be used for the baseline data. We are aware that numerous other confounders exist (such as age, race, education, statin use and gender). When a variable is identified to be related to the outcome and covariate of interest, i.e. confounder, we will adjust for it in the model of choice. A sensitivity analysis of statin and/or ARB use will be performed via analysis by statin and ARB use to determine if it is necessary to adjust or stratify by statin and ARB use. Models consider inclusion of the list of known covariates and their interaction. The importance of these models will be assessed through inspection of the change in the parameter of interest, as well as the parameter (and p-value) for the covariate. Point estimates, p-values, and confidence intervals to test the covariate of interest will be computed. Mixed model diagnostics and GLM diagnostics will be evaluated. For the continuous outcomes, we will assess if transformations exist that can bring about near normality. In addition, the impact of skew on the stability of estimates can be assessed using bootstrap methodology. For all models involving skewed predictors, we will assess the impact of the significant outliers using bootstrap and will check the linearity assumption. Other diagnostic procedures will be used to check for the validity of the GLM assumptions such as normality of the residuals and linearity assumption. Missing data problems are ubiquitous in aging research. Missing values can arise from several sources, (1) subjects are lost to follow-up, (2) missing CRFs, or (3) particular items of a CRF are skipped. We will employ multiple imputation. In this design, we have numerous indicators of outcome and predictors. The concern is that the possibility of a Type-I error is increased if a series of univariate tests is employed. Several solutions are possible in the context of this design: 1) use of Bonferroni Corrections or the less conservative extension to Bonferroni corrections 2) use of data reduction techniques like Principal Component Analysis (PCA) , and 3) use of multivariate mixed models. Based on the estimated numbers of remitters vs non-remitters we expect to enter approximately 53 subjects in Phase II. Using the estimates from Abbasi et al. (7) for serum IL-6 comparing SSRI+celecoxib to SSRI+placebo, we will have greater than 95% power by randomizing the 53 subjects between the two groups.

6.2 Variables to be used in hypothesis testing

1) For measure of cytokine levels we will measure these in CSF and blood: Primary measure IL-6 pre- and post-treatment.
2) Hippocampus, including subvolume analyses and Amygdala volumes: Left and right volumes will be separately measured and standardized by cortical volumes before being entered in analyses. Prefrontal cortical thickness: L & R orbitofrontal, superior frontal and frontal pole.
3) Antidepressant treatment response determined using the MADRS score following antidepressant treatment covaried on baseline MADRS score.
4) Neuropsychological function as determined by factor scores from episodic memory and executive function.

5) Resting state functional connectivity of the hippocampus and amygdala to anterior cingulate (AC) and dorsolateral prefrontal cortex (DLPFC).

6.3 Analysis of Specific Aims

Aim 1: Characterize neuroanatomical and neuropsychological effects of abnormal cytokines in MDD. Hypothesis 1a: Inflammatory cytokines will be elevated centrally and peripherally relative to matched controls; neuropsychological performance will be poorer in MDD; hippocampus and amygdala volumes and PFC thickness will be decreased in MDD; and connectivity of hippocampus and amygdala will be decreased in MDD (hippocampus to DLPFC and AC; amygdala to subgenual AC). For an unadjusted analysis, a t-test can be utilized to assess depression group differences among the various outcomes. Linear regression will be used to assess whether various outcomes differ between depression status adjusting for confounders (such as age, race, education, statin use, and gender). Baseline inflammatory cytokines, neuropsychological measures, functional connectivity, and volumetric data will be treated as outcomes in the linear regression model with depression status as a covariate as well as possible other confounders as covariates.

Hypothesis 1b: Cytokine levels will be correlated with hippocampal and amygdala volumes and PFC thickness; we predict that higher IL-6 and lower IL-10 will be correlated with decreased hippocampal and amygdalar volumes and decreased PFC thickness. The Pearson moment-product correlation coefficient (or Spearmans rank correlation coefficient when normality assumptions are violated) will be used to assess relationships between cytokines and ROI measures for an unadjusted analysis. Linear regression will be used to assess whether the ROI data are related to the inflammatory markers, while adjusting for depression status and confounders. In addition, we will confirm whether an interaction term between the inflammatory markers and depression is necessary.

Hypothesis 1c: There will be a correlation between cumulative duration of depression and volume loss in hippocampus and amygdala, particularly hippocampus CA2-3. We will correlate the lifetime history of depression with hippocampal and amygdala volumes and predict a significant correlation using the Pearson product-moment correlation coefficient or Spearman rank correlation coefficient depending on normality assumptions.

Aim 2: Characterize the reversibility of brain dysfunction, including cognitive impairment with treatment of depression and its association with inflammation. Hypothesis 2a: Subjects randomized to treatment will have greater normalization of IL-6 and IL-10, greater improvements in memory, executive function and improvements in resting state functional connectivity compared to those randomized to placebo. For an unadjusted analysis, a paired t-test can be used to determine if there is a difference in the pre-treatment and post-treatment inflammatory cytokines. Mixed models will be utilized to assess if the inflammatory markers change over time adjusting for confounders. A mediator analysis will be utilized to determine whether MADRS has an effect independent of treatment on the inflammatory levels. The first

approach would be to test for an interaction effect between baseline MADRS and treatment in a mixed model with inflammatory markers as the outcome adjusting for MADRS, treatment, and an interaction between MADRS and treatment. If the interaction term is not significant we will determine if MADRS independently of treatment or additively with treatment is related to inflammatory markers. Structural equation models can be an alternative approach for the mediator analysis.

Hypothesis 2b: Improvement in clinical depressive scores post treatment will be correlated with changes in cytokine levels. Change score models or joint mixed models will be utilized to assess the relationship between depression scores (outcome) and inflammatory markers, while adjusting for confounders in the depressed subjects.

6.4 Exploratory Aims

Hypothesis 1E: Normalization of cytokine levels will be correlated with increased functional connectivity of the hippocampus and amygdala and with improvements in memory and executive function.

Hypothesis 2E: Peripheral blood cytokine levels are sufficiently sensitive to substitute for CSF levels.

Hypothesis 3E: MDD participants will have CSF abnormalities in tryptophan, kynurenine, YKL-40, and increased protein sulfenne and methionine sulfoxide conversion, specific for tissue hydrogen peroxide and superoxide, respectively.

6.5 Further Analyses

At the completion of the study, we will have a rich database with which to conduct exploratory analyses. Such analysis will include relationships between volumetric measures, CSF and serum inflammatory markers, stressful life events and perceived stress. While extreme deviations from normality provides some analytic challenges and linearity is assumed, the model is flexible in allowing covariates. Importantly, Abbasi et al. (7) provides some preliminary estimates for serum IL-6 concentrations for the conditions SSRI+celecoxib versus SSRI+placebo (7).

7 Safety and Adverse Events

In brief, both the PI and sponsor assess and evaluate all AEs. SAEs need to be reported to the sponsor within 48 hours. AEs will be reported in aggregates.

7.1 Definitions

7.1.1 Adverse Event

Expected adverse events include all adverse events listed in the escitalopram and celecoxib prescription information (see appendix). It is further expected that the incidences of these adverse events are higher in the particular age group under study.

An **adverse event** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

7.1.2 Serious Adverse Event

Serious Adverse Event

Adverse events are classified as serious or non-serious. A **serious adverse event** is any AE that, in the view of either the investigator or the sponsor, is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as **non-serious adverse events**.

7.2 Recording of Adverse Events

At each contact with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study intervention or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study intervention or study participation will be recorded and reported immediately.

The Investigator will monitor and/or ask about or evaluate AEs using non-leading questions at each visit or evaluation. The occurrence of all AEs will be documented in the CRF with the following information, where appropriate:

- AE name or term
- When the AE first occurred (start date)
- When the AE stopped (stop date), (Or an indication of "ongoing")
- How long the AE persisted
- Intensity of the AE
- Seriousness
- Actions taken
- Outcome
- Investigator opinion regarding the relationship of AE to the investigational product(s).

7.3 Relationship of AE to Study

The relationship of each adverse event to the study procedures will be characterized by the PI. The categories for classifying the Investigator's opinion regarding the relationship of an AE to investigational product(s) are listed below.

Definitely related:	An AE occurring in a plausible time relationship to study drug administration, and which cannot be explained by a concurrent disease or other drugs or events. The response to withdrawal of the drug (dechallenge) is clinically reasonable.
Probably related:	An AE with a reasonable time sequence to administration of the study drug and which is unlikely to be attributed to concurrent disease or other drugs or events. The response to withdrawal of the drug (dechallenge) is clinically reasonable.
Possibly related:	An AE with a reasonable time sequence to administration of the study drug, but which could also be explained by concurrent disease or other drugs or events. Information on drug withdrawal may be lacking or unclear.
Unlikely:	A clinical event, including laboratory test abnormality, with a temporal relationship to study drug administration which makes a causal relationship improbable, and in which other drugs, events or underlying disease provide plausible explanations.
Unrelated:	An AE with sufficient evidence to accept that there is no causal relationship to study drug administration (e.g., no temporal relationship to drug administration, because the drug was administered after onset of event; investigation shows that the drug was not administered; another cause was proven; etc.).

Unassessable (Unclassifiable):	A report suggesting an adverse reaction, which cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified.

7.4 Reporting of Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible. The UPenn IRB Reportable events policy will be accessed at http://www.upenn.edu/IRB/mission-institutional-review-board-irb/reportable-events to concur to the latest requirements.

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study intervention was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study intervention

Additionally all other events (unanticipated problems, adverse reactions, and subject complaints will be recorded and reported with respect to institutional and federal policies as described in the Penn Manual and below.

7.4.1 Follow-up report

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the IRB. The investigator is responsible for ensuring that all SAE are followed until either resolved or stable.

7.4.2 Investigator Reporting: Notifying the Penn IRB

Reportable events will be reported to the UPenn IRB observing applicable timelines as detailed at http://www.upenn.edu/IRB/mission-institutional-review-board-irb/reportable-events.

7.5 Unblinding Procedures

While the safety of the subject always comes first, it is still important to seriously consider if unblinding the study therapy is necessary to ensure a subject's safety. Authorization for unblinding must be given by the principal investigator or sponsor.
To break the code, contact the **24 hour emergency number** at the **Investigational Drug Service** at the University of Pennsylvania School of Medicine, Institute for Translational Medicine and Therapeutics:

1-800-670-3151

Unblinding will be recorded in print in the source documents and reported to the UPenn IRB.

7.6 Medical Monitoring

7.6.1 Data and Safety Monitoring Plan

7.6.1.1 Principal Investigator

The principal investigator or a designee for this clinical study, blinded to the treatment allocation, will continuously monitor efficacy and safety of the study participants.

7.6.1.2 Medical monitor

The sponsor or a designee for this clinical study, blinded to the treatment allocation, will be reviewing subjects' safety labs, SAE/AE reporting and subjects' overall study course to monitor for missed AEs/SAEs. A template similar to the one provided in the appendix will be used.

7.6.1.3 Data Safety and Monitoring Board (DSMB)

Both principal investigator and sponsor will be supported in their safety assessments by an independent Data Safety and Monitoring Board comprised of three (3) members who have expertise in psychiatry, pharmacology and biostatistics.

Meetings

The DSMB will convene as often as necessary, but at least once annually, to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed. A DSMB meeting may be requested by DSMB members, IRB, study sponsor, or study Principal Investigator at any time to discuss safety concerns. Meetings may be held by conference calls or videoconferences or as face-to-face meetings, information can be shared electronically per e-mail.

Reporting

The DSMB will issue a written report that identifies topics discussed by the DSMB and describes their individual findings, overall safety assessment and recommendations. The rationale for recommendations will be included when appropriate. This report will be circulated to the IRB and study investigators.

Conflict of Interest

No member of the DSMB should have direct involvement in the conduct of the study. Furthermore, no member should have financial, proprietary, professional, or other interests that may affect impartial, independent decision-making by the DSMB.

Responsibility

The DSMB will review cumulative data for evidence of escitalopram- and celecoxib-related adverse events. Safety data will comprise results from the laboratory blood chemistry, in-office blood pressure and ambulatory blood pressure monitoring data collected. The study Principal

Investigator, study sponsor-investigator, DSMB members and the IRB can request additional data to assess the study safety of celecoxib.

7.7 Expedited FDA Reporting Requirements

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports.

The following describes the IND safety reporting requirements by timeline for reporting and associated type of event:

- **Within 7 calendar days**
  Any study event that is all:
  - associated with the use of celecoxib, and
  - unexpected, and
  - fatal or life-threatening,

- **Within 15 calendar days**
  Any study event that is:
  - associated with the use of celecoxib, and
  - unexpected, and
  - serious, but not fatal or life-threatening

    -or-

  - a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

7.8 NIH/NIMH Reporting Requirements

Reporting to NIH/NIMH will follow the time frame as outlined above in “Expedited FDA Reporting Requirements”.

7.8.1 Additional reporting requirements

Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

7.8.2 Reporting Process

Applicable events can be reported to the FDA using Form FDA3500A or in narrative format. The report must be sent to the correct division. Specific information that must be included in the reports can be found in 21 CFR 312.32.

8 Study Administration, Data Handling and Record Keeping

8.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Several safeguards will be put into place for protecting the confidentiality of research material. Staff members will be trained to understand the importance of confidentiality. Information gathered about individual participants is maintained in secured storage areas at the site. All phone screenings done during the recruitment process will be done from a private office area, and after asking the potential participants if they are in an environment where they feel comfortable answering questions related to their eligibility to participate. All aspects of the informed consent process are done in a private consultation room. Study procedures will occur in a private room by personnel trained in following procedures in a manner in which the privacy of the participant is maintained. Only information, images, and biological samples necessary for the completion of this study will be collected. All computer data obtained from the laboratory and from research interviews will be de-identified and then only identified by a code number. All data will be kept in locked file cabinets behind locked doors and only made available to qualified research personnel. All data will be de-identified and only code numbers will appear on any data and documents used for evaluation or statistical analysis for this study. Publications emanating from this research will not identify individual patients. HIPAA compliance will be enforced as per University of Pennsylvania policy. Reports from patients clinical records concerning research observations will not be available to outside medical facilities without the written consent of the participant.

8.2 Subject Privacy

Several safeguards will be put into place for protecting the privacy of participants and the confidentiality of research material. Staff members will be trained to understand the importance of privacy and confidentiality. Information gathered about individual participants is maintained in secured storage areas at the site. All phone screenings done during the recruitment process will be done from a private office area, and after asking the potential participants if they are in an environment where they feel comfortable answering questions related to their eligibility to

participate. All aspects of the informed consent process are done in a private consultation room. Study procedures will occur in a private room by personnel trained in following procedures in a manner in which the privacy of the participant is maintained. Only information, images, and biological samples necessary for the completion of this study will be collected. All computer data obtained from the laboratory and from research interviews will be de-identified and then only identified by a code number. All data will be kept in locked file cabinets behind locked doors and only made available to qualified research personnel. All data will be de-identified and only code numbers will appear on any data and documents used for evaluation or statistical analysis for this study. Publications emanating from this research will not identify individual patients. HIPAA compliance will be enforced as per University of Pennsylvania policy. Reports from patients clinical records concerning research observations will not be available to outside medical facilities without the written consent of the participant.

An exception to confidentiality is if a subject reports child abuse or neglect or if a subject reports current suicidal or homicidal ideation of concern to the research team. Any information about child abuse or imminent intent to harm one's self or others will be reported to authorities, as required by law.

8.3 Data Disclosure

The following entities may have access to the data if requested: 1) Government representatives, (including the funding sponsor (NIH) and the Office for Human Research Protections) to complete federal or state responsibilities 2) The U.S. Food and Drug Administration 3) Hospital or University representatives, to complete Hospital or University responsibilities 4) University of Pennsylvania's Institutional Review Board, 5) future collaborators for the advancement of scientific knowledge.

8.4 Data Collection and Management

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

The UPenn-based data management software REDCap (http://project-redcap.org/) will be deployed to collect and store study data. REDCap (Research Electronic Data Capture) is a secure web application for building and managing online surveys and databases. Using REDCap's stream-lined process for rapidly developing projects, one may create and design projects using 1) the online method from your web browser using the Online Designer; and/or 2) the offline method by constructing a 'data dictionary' template file in Microsoft Excel, which can be later

uploaded into REDCap. Both surveys and databases (or a mixture of the two) can be built using these methods. REDCap provides audit trails for tracking data manipulation and user activity, as well as automated export procedures for seamless data downloads to Excel, PDF, and common statistical packages (SPSS, SAS, Stata, R). Also included are a built-in project calendar, a scheduling module, ad hoc reporting tools, and advanced features, such as branching logic, file uploading, and calculated fields.

8.5 Records Retention

Study essential documents will be retained for 7 years after completion of research.

8.6 Trial Registration

This study is registered in ClinicalTrials.gov - *NCT02389465*.

9 Study Monitoring, Auditing, and Inspecting

9.1 Study Monitoring Plan

Reporting unanticipated problems involving risks to participants or others: The PI or authorized designee will monitor the study for any unanticipated problems including adverse and serious adverse events. All unanticipated problems will be reported to the IRB and collaborators within 10 working days with the following exception: If the adverse event involved a death and indicates that participants or others are at increased risk of harm, investigators are required to submit a report to the IRBs within 3 work days.

9.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

10 Ethical Considerations

This study is to be conducted in accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

10.1 Risks

There are some potential risks associated with study participation and these have been carefully considered to substantially reduce occurrence and minimize such risks. Potential risks include:

Clinical interview and assessments: Some discomfort may be associated with the clinical assessments conducted in this study. Some participants may experience emotional discomfort when answering some questions in the questionnaires or talking about their personal situation. Participants may choose not to answer any question.

10.1.1 Risk of Breach of Confidentiality

Breaches in confidentiality could impact future insurability and/or employability.

10.1.2 EKG

Participants may experience some discomfort that is similar to removing an adhesive bandage when the EKG technician must remove the electrodes that have been placed on the participant's chest in order to conduct the test.

10.1.3 Blood draw

Participants may experience discomfort, bruising and or bleeding at the site of needle insertion when blood is drawn. Also, some people experience dizziness or feel faint. There is a slight risk of infection.

10.1.4 MRI scan

There are no known risk factors associated with MRI scans for healthy subjects except that participants may experience discomfort as they will be required to lie still in a confined area. Participants may experience claustrophobia (fear of enclosed spaces and/or anxious feelings accompanied by fast heart rate or shortness of breath) within the MRI scanner. In addition, the scanner produces a loud repetitive knocking noise during the study that some people find bothersome.

10.1.5 Escitalopram

Side effects of escitalopram include nausea, sleepiness, headache, dizziness, tremor, diarrhea, nervousness, dry mouth, increased sweating, and insomnia or ejaculation failure. Less common side effects include: rash, agitation, decreased sex drive, fatigue. Rare or uncommon side effects include: constipation, loss of appetite, vomiting, stomach upset, and vision abnormalities

10.1.6 Celecoxib

Celecoxib has been associated with an increased risk of cardiovascular events in people with cardiovascular disease (Celecoxib package insert). Side effects include nausea, diarrhea, gas, cold symptoms, sore throat, lack of energy, loss of appetite, fever, rash, back pain, and flu-like symptoms, among others. Participants would be instructed to contact study members if severe,

serious, or persisting side effects occur. There is a rare risk of stroke or heart attack; however these very serious side effects are greatest risk for those with current clinically significant cardiac disease, and we are excluding these people (clinically significant cardiovascular disease usually includes one or more of the following: cardiac surgery or myocardial infarction within the last 4 weeks; unstable angina; acute decompensated congestive heart failure or class IV heart failure; current significant cardiac arrhythmia or conduction disturbance, particularly those resulting in ventricular fibrillation, or causing syncope or near syncope; uncontrolled high blood pressure; QTc greater than 450msec (by history for subjects with cardiac disease); documented prior stroke; clinically significant abnormalities on EKG.) Celecoxib also has increased risk for gastrointestinal bleeding and hepatic impairment. We will exclude those with current ulcer disease or GI bleeding as well as those with liver function impairment (as determined by a liver function test prior to entry into the study). We are excluding clinically significant cardiovascular disease for the past 6 months.

10.2 Minimizing risks

The following considerations have been made in order to minimize risk as much as possible:

Informed Consent: All consenting procedures will be HIPAA compliant. Participants will be given a full description of all study procedures and the risks involved, and any questions the participant may have will be answered prior to consenting to the study. The consent process will be done in a private exam room.

Clinical interview and assessment: All clinical assessors have extensive experience in clinical psychiatric assessment and will make every effort to implement protocol procedures in a sensitive and supportive manner. Other measures to minimize risks include the careful assessment of each subject before the study, and close clinical scrutiny during all aspects of the study. There are no physical risks of diagnostic procedures or ratings.

Blood draw: To reduce the risks to subjects from the drawing of blood, only trained personnel will perform these procedures, and immediate medical assistance and facilities will be available at all times.

MRI scan: The potential risks related to MRI will be minimized in the following manner: Subjects are screened for exclusions such as foreign metallic implants and pacemakers to MRI participation prior to enrollment; claustrophobia from MRI will be reduced by explaining the nature of the magnetic resonance scanner in detail to all participants prior to their enrollment.

Subjects who have a history of claustrophobia will not be enrolled into the study. If the subject complains of feeling claustrophobic, the study will be terminated; to lessen the noise of the scanner, earplugs will be provided.

Treatment with Escitalopram: Participants receiving treatment with escitalopram will be monitored closely for any adverse side effects. Two-week follow-up visits will be scheduled to

evaluate response to treatment and participants will be withdrawn from the study if their depressive symptoms worsen or they have intolerable side effects to escitalopram. These patients will be referred for follow-up care.

Confidentiality: The risk of violating confidentiality will be minimized by the following: all computer data obtained from the laboratory and from research interviews will be identified by a code number; all data will be kept in locked file cabinets and only made available to qualified research personnel; participants will be asked to provide only the contact numbers or e-mail addresses which they want us to use for study related contact. HIPAA compliance will be enforced as per

University of Pennsylvania policy and approval; reports from patients clinical records concerning research observations will not be available to outside medical facilities without the written consent of the subject; all participant interaction with the PI will occur in private exam rooms or in the PIs private office; only essential, engaged study members will interact with participants during all research activities.

Others: Any unanticipated findings of potential medical significance discovered during the course of this study, such as an abnormality in the MRI scan or significant deficit during neuropsychological testing, will be reported to the PI. The participant will be informed and referred to their private physician.

10.3 Benefits

There is no promise of benefit to subjects in this study. Depressed subjects may benefit from treatment with escitalopram and may derive additional anti-depressant benefit from the combination of escitalopram and celecoxib. There is no benefit to receiving the MRI scans or to the clinical assessments. The scans and the clinical assessments are performed only for research, and the results are not routinely shared with participants. However, in the event that clinically significant MRI or EKG abnormalities are discovered, subjects will be informed of the finding and referred to their physician. An indirect benefit is contributing to scientific knowledge. This study has the potential to greatly increase our knowledge of inflammation in people with depression and may lead to new treatments.

10.4 Risk Benefit Assessment

There are risks associated with participation in this study, but they are minimal and even rare and it is believed that the benefits of conducting the study outweigh the minimal risk.

Celecoxib has been associated with an increased risk of cardiovascular events in people with cardiovascular disease. To mitigate this risk we will enforce our stringent exclusion criteria for people with cardiac risk factors, monitor blood pressure, assess gastrointestinal safety, appoint an independent data safety monitoring board (DSMB) and conduct this study under an FDA-IND for the use of celecoxib. A separate risk/benefit analysis for the use of celecoxib follows below.

Because of these precautions, the addition of Celecoxib is not a greater than minimal risk to participants. We will perform the EKG and cardiac risk assessment following the completion of the initial phase of the study before entering into the celecoxib phase in case their cardiac profile has changed. Our exclusion criteria related to cardiac risk include: lifetime history of cardiac surgery or myocardial infarction; unstable angina; acute decompensated congestive heart failure or class IV heart failure; current significant cardiac arrhythmia or conduction disturbance, particularly those resulting in ventricular fibrillation, or causing syncope or near syncope; uncontrolled high blood pressure; QTc greater than 450msec (by history for subjects with cardiac disease); documented prior stroke; Clinically significant abnormalities on EKG.

Additionally, subjects will have blood pressure monitoring at each visit, as it may be a surrogate for celecoxib thrombotic risk. There is also a risk of boredom and/or fatigue from study visits. There is also a rare risk of breach of confidentiality. We have developed procedures for Protection Against or Minimizing Risks for the participant. To reduce risks to participants, only physicians and scientists with extensive training and experience will supervise and perform procedures. Participants will be closely monitored for any side effects from taking escitalopram. There are significant benefits to the research. This study has the potential to greatly increase our knowledge in the treatment and prevention of Depression. No direct benefits are expected to the participant aside from the satisfaction of participating in a study that addresses an important health issue.

10.5 Risk / Benefit Assessment for Celecoxib

Celecoxib is a purposefully designed non-steroidal anti-inflammatory drug, or pdNSAID, which inhibits the enzyme prostaglandin-endoperoxide synthase 2, also known as cyclooxygenase-2, or COX-2. The cardiovascular hazard of NSAIDs is a direct consequence of their intended mechanism of action, inhibition of COX-2 (8, 9). Seven placebo-controlled trials of structurally distinct NSAIDs specific for inhibition of COX-2 have revealed the increase in cardiovascular complications on these drugs (8). Inhibition of the COX enzymes by NSAIDs causes suppression of prostaglandin (PG) production. PGs play important roles in pain, inflammation and pyresis, but also in platelet function, vascular integrity and the regulation of renal blood flow, volume control and vascular tone

This study exposes patients in the age group of 50-80 years diagnosed with depression to 6 weeks of oral celecoxib 200 mg bid. The factors to consider for the risk assessment are i) thrombotic risk, ii) cardiovascular risk profile at baseline, iii) blood pressure response, iv) co-administration of SSRI & COX-2 inhibitor.

10.5.1 Thrombotic risk and cardiovascular risk profile at baseline

Reports on the cardiovascular safety outcomes are available from the Adenoma Prevention With Celecoxib (APC) clinical trial which used similar dosing, 200 mg celecoxib bid, as in the present protocol as well as double the dose, 400 mg bid (10). The hazard ratio for the pre-specified composite safety end point “cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, or heart failure” was 2.6 (95% confidence interval [CI], 1.1 to 6.1) in patients taking 200

mg celecoxib bid, and 3.4 (95% CI, 1.5 to 7.9) for 400 mg celecoxib bid. Though these risk estimates are derived from studies not designed to test for cardiovascular safety but in the case of APC powered to test the efficacy and safety of celecoxib versus placebo in reducing colorectal adenoma recurrence after polypectomy, the data underscores the dose-dependent increase in cardiovascular risk observed for COX-2 selective inhibitors. Hence, **to mitigate the cardiovascular risk for the participants of this protocol, celecoxib will be administered in the lower dose of 200 mg bid.**

The presence of cardiovascular events in the past medical history elevates the hazard ratio to 8.3% in patients exposed to celecoxib compared to 1.9% for the patients on celecoxib without such a history (10). The difference in hazard ratios observed in users and non-users of low-dose aspirin may convey a similar message: patients taking cardioprotective aspirin indicating a past history of CVD show a higher hazard ratio (4.4%) than patients not on aspirin (2.3%) (10). Therefore, **to mitigate the cardiovascular risk for the participants of this protocol, patients with a past history of cardiovascular disease will be excluded.**

A difference in the cardiovascular risk (and upper gastrointestinal complications) at baseline impacts the risk for major vascular (and gastrointestinal) events during treatment with a selective COX-2 inhibitor (11). As these data estimate the per annum risk, we reached out to the senior author, Dr. Colin Baigent FRCP FFPH, (11) with the question of the adverse event profile in patients during 0-2 months of exposure to celecoxib in patients > 60 years of age. Of note is, that this overview analysis from individual patient data (11) report on the safety of high doses of celecoxib, i.e. 800 mg per day. Dr. Baigent pointed out the following:

- In this meta-analysis, there were only 5 trials involving a randomized comparison of celecoxib vs placebo in which at least 1 major vascular event occurred and only 2 trials in which at least 1 symptomatic upper GI event occurred (see (11), Webfigures 15 and 20 from the supplementary material that was published alongside the 2013 Lancet paper (11)).
- In the 5 trials in which a major vascular event (MVE) occurred, at least one MVE occurred for 30 out of 1158 patients randomized to celecoxib 800 mg (during a total of 1819 person-years) compared with 8 out of 1079 patients randomized to placebo (during a total of 1750 person-years), yielding an average event rate ratio of 2.96 (99% CI 1.21 to 7.25) ((11), Webfigure 15). Most of the information comes from the APC trial (23 versus 6 MVEs).
- In the 2 trials in which a symptomatic upper GI event, the respective counts were 16 out of 872 randomized to celecoxib 800 mg versus 11 out of 778 randomized to placebo (RR 1.32, 99% CI 0.45 to 3.86) ((11), Webfigure 20). Again, this mostly derived from the APC trial (13 versus 11).
- Given the very small numbers involved, Dr. Colin Baigent does not advise to try and subdivide these events further by age or duration of follow-up. But, it is worth noting that the effects of any coxib by duration of follow-up/baseline age were also published in the webmaterial ((11), Webfigures 2, 7, 12 and 13).

In conclusion, absolute numbers of patients in which a cardiovascular (or gastrointestinal) event occurred are too small to estimate the risk for the short-term exposure of 8 weeks in this protocol. **The strategy here to mitigate cardiovascular risk is i) to include patients below a**

Framingham 10-year risk score of 13 (15% 10-year risk), taking into consideration that the 10-year cardiovascular risk in our elderly age group (age 60-80) is estimated at 13.5% in the general population and 15% in patients with cerebrovascular risk factors, and ii) to reduce the upper age for inclusion to 80 years of age.. The feasibility of this approach is drawn from our pilot data in a sample (n=217) of late life depression and controls where we determined the Framingham score in both patients with MDD (who had higher vascular risk scores) and age matched controls: Age 60-65 mean score MDD: 9.0 (10-year stroke risk of 8% vs 7% for general population). Age 65-74 mean score: 12.2 (10-year risk of 13.5% vs average risk = 11%); 75-84 mean score: 16.2 (10-year risk of 23% vs average risk of 20%).

To explore retrospectively how adverse events relate to differences in drug effects, i.e. inhibition of prostaglandin synthesis, urine spot samples will be collected for prostaglandin metabolite analysis at select time points optionally.

10.5.2 Blood pressure

The risk of NSAIDs to induce hypertension (12, 13), sodium retention (13) and edema (13) is well recognized (14-17), but it remains unclear why some patients develop such complications and others do not. Factors associated with this risk are age, co-morbidity, preexisting hypertension and elements of drug exposure to selective COX-2 inhibitors. Latter includes dose, attained plasma concentrations, duration of action and dosing and the degree of selectivity for COX-2.

Retrospective analyses of blood pressure responses have been performed in most NSAID trials of large populations and/or those involving extended drug exposure, although these are usually not based on rigorous (or, in some cases, not even pre-specified) blood pressure measurements. In some studies, the incidence of hypertension has been recorded merely as reported adverse events or by retrospectively defined changes from baseline. Based on these assessments, all NSAIDs have increased blood pressure or induced hypertension within diverse study populations, although this appears to relate to COX-2 selectivity (confounded with drug exposure; (9)). Perhaps unsurprisingly, the rates in blood pressure increase were highly variable across studies and conditioned by the specific characteristics of the study population and by recording methods. However, the lack of information from rigorously designed and sufficiently sized trials limit any conclusions about the relative likelihood of hypertension on specific inhibitors or in subpopulations.

Only a small number of clinical trials – some of which were not powered to detect small changes – have assessed prospectively the effects of COX inhibition on blood pressure control. Of note is that young, healthy individuals are unlikely to experience an increase in blood pressure in response to traditional (t)NSAIDs, such as naproxen or ibuprofen or purposefully-designed, selective COX-2 inhibitors, like rofecoxib or celecoxib based on these studies. Older, normotensive subjects and patients with controlled, stable hypertension can be expected to have average increases of 3-5 mmHg in systolic blood pressure (18-22) with an incidence of above average responses (as defined by an increase of 20 mmHg in systolic blood pressure and a systolic blood pressure above 140 mmHg) ranging from 7-17 %. As one would expect,

hypertensive responses to COX-2 selective inhibitors were reported to be more likely in patients with elevated blood pressure as a pre-existing condition (23).

A number of retrospective studies, RCTs, and database studies, as recently reviewed (24), suggest that SSRIs including escitalopram have an overall neutral impact on the cardiovascular system including blood pressure. In a randomized clinical trial using fixed-dose escitalopram, 10 mg/d, over a period of 8 months, hypertension was the reason for discontinuation in only n=2 out of n=274 (0.7%) escitalopram-treated patient (25).

Age has an physiological effect on the glomerular filtration rate (GFR), quantified as a decline in GFR by 0.4 ml/min/year (26). COX-2 inhibitors' renal effects are a decline in the GFR (27) and an alteration of the feedback mechanism which controls renin release (28). Thus, the noted increase in aldosterone concentrations after escitalopram administration - 261±36 pmol/liter at baseline, 269±38 pmol/liter at 2 weeks, and 282±40 pmol/liter at 6 weeks (p=0.04) (29), by itself functionally not associated with changes in blood pressure in this study of normotensive males with depression (29) - might sensitize the reno-vascular system for hypertensive responses under conditions of celecoxib co-administration.

Taking the consideration outlined above into account, **our initial strategy to mitigate this risk was to identify the patients responding to celecoxib administration with an above average increase in blood pressure. The paradigm to detect such an above average signal was the use of continuous 24-hour ambulatory blood pressure monitoring (ABPM) before the start of their enrollment to establish a baseline, and under steady state conditions of celecoxib plasma drug concentrations, i.e. one to two weeks after start of the study; ABPM at the end of the study was planned to collect further safety and interventional data. Patients demonstrating an elevation of 10 mmHg in mean daytime and nighttime systolic blood pressure at 1-2 weeks after the start of the study compared to the mean daytime and nighttime systolic blood pressure at baseline and the mean daytime (that is out of bed times) systolic blood pressure is ≥150 mmHg were instructed to discontinue trial medication and excluded from the study and instructed to see their primary care physician for further evaluation and follow up.**

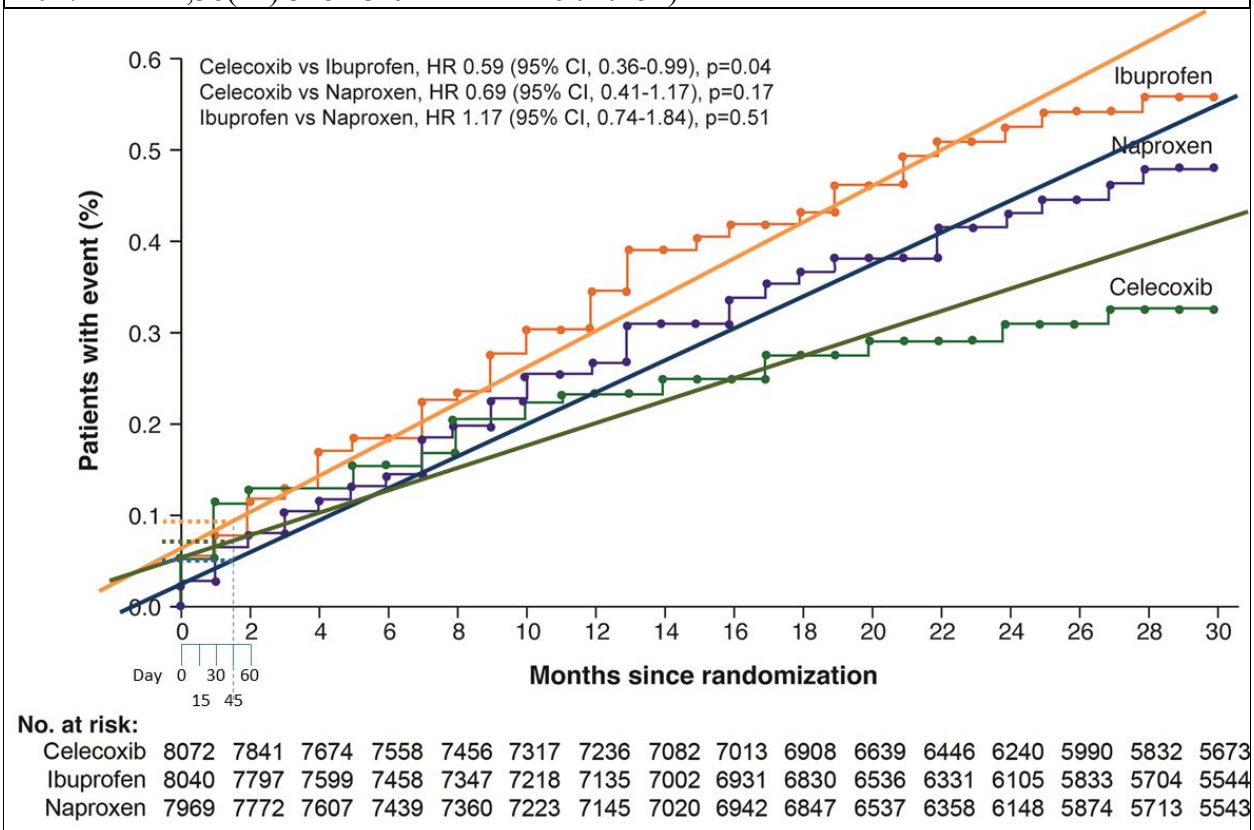
However, using this initial strategy we were only able to enroll n=2 patients during the past 2 years, concluding that the in-/exclusion criteria and protocol procedures, in particular the 24-hour ambulatory blood pressure measurements, are too demanding for the patient cohort under study. Patient screening interviews conducted by the clinical study coordinators reflected this notion among the majority of patients potentially eligible for study inclusion.

Therefore, to increase enrollment, we amend the protocol to make optional the 24-hour ambulatory blood pressure monitoring, thus focusing on the weekly in-office blood pressure measurements as described below to screen primarily for the risk of blood pressure elevation.

A similar approach to risk detection was taken in the largest prospectively randomized clinical study on NSAIDs (Nissen SE et al. N Engl J Med. 2016 Dec 29;375(26):2519-29.

Cardiovascular Safety of Celecoxib, Naproxen, or Ibuprofen for Arthritis. PMID: 27959716) where patients with osteoarthritis or rheumatoid arthritis and with elevated cardiovascular risk or established cardiovascular disease received either a high dose of ibuprofen or naproxen or a low to medium dose of celecoxib. Data on blood pressure suggests that less than 0.1% of patients exposed to NSAIDs experience hypertensive urgencies or emergencies at 45 days into dosing, which is the planned duration of celecoxib exposure for the current study. The incidence of blood pressure related events did not exceed 0.6% after 30 months of NSAID treatment.

Time to first hospitalization for hypertension in PRECISION (Ruschitzka F et al. Differential blood pressure effects of ibuprofen, naproxen, and celecoxib in patients with arthritis: the PRECISION-ABPM (Prospective Randomized Evaluation of Celecoxib Integrated Safety Versus Ibuprofen or Naproxen Ambulatory Blood Pressure Measurement) Trial. Eur Heart J. 2017 Nov 21;38(44):3282-3292. PMID: 29020251)



Regression lines were added manually per visual inspection to extrapolate the NSAID-exposure-related event risk at 45 days of administration.

In the case that patients opt in for the 24hr APBM, patients' activity patterns during the 24 hour ABPM sessions, in particular in- and out-of-bed times to differentiate the diurnal profile of blood pressure changes during day- and nighttime, will be recorded by two different methods, paper and pencil logs and body actigraphy.

In-office blood pressure measurements (in triplicates 5 minutes apart after a 5 minute resting period) will continue at weekly intervals throughout the study. In the case that all systolic blood pressures taken on ≥2 separate office visits are ≥150 mmHg systolic blood pressure or ≥90 mmHg diastolic blood pressure, a follow up 24-hour ABPM will be scheduled within one to two weeks. Here, the same criteria for study exclusion, as outlined above, will be applied.

The reference systolic and diastolic blood pressure of 150/90 mmHg in people aged 60 years and older has recently been recommended in the "JNC8 evidence-based guideline for the management of high blood pressure in adults 2014" (30).

In the case that patients opt in for the 24hr APBM, the benefit to patients is the extensive phenotyping of their blood pressure response to celecoxib. Patients exhibiting an above average blood pressure response to celecoxib will be advised to be cautionary in particular when taking over-the-counter or prescription NSAIDs for longer periods of time. In addition, the primary care physician will be advised to monitor blood pressure in such patients.

A challenge will be the high prevalence of hypertension in this age group, estimated at approximately 65% of men and 75% of women by age 70 years (31). Study enrollment can be offered to this patient cohort provided that blood pressure is adequately controlled with life style changes and/or anti-hypertensive pharmacotherapy. This option will also be offered to patients initially excluded from the study or excluded from the study due to elevated blood pressure. Here, **the benefit to patients is that their blood pressure is monitored over periods of 24 hours on multiple occasions, a diagnostic tool less common in the primary care setting. This is expected to actively engage patients in the control of their blood pressure**, **which is beneficial across all ages (32).**

High salt diet and dehydration are two conditions known to induce COX-2 expression in renal medullary interstitial cells (33-36), which play an important role in adaptive regulation of arterial pressure. Resultant vasodilator COX-2 products contribute to the regulation of medullary blood flow (MBF), which drives pressure natriuresis and diuresis. In mice, COX-2 inhibition and nonselective COX-inhibition (37), reduces acutely MBF, sodium excretion and urine volume (38). Coincident challenge of the renal autoregulatory mechanisms by vasopressors, excessive salt intake or volume depletion results in a rise in systemic arterial pressure (38, 39). This is consistent with clinical observations in young and healthy populations in which both nonselective COX inhibition and selective COX-2 inhibition have no effects on arterial pressure despite transient changes in GFR and sodium handling on initiation of dosing, while elderly volunteers show a depression of MBF and in an increase in blood pressure (40). **To mitigate the risk for a diet-drug interaction on blood pressure, patients will be screened for dietary salt intake per 24-hour dietary recall administered by the CTRC bionutrition core. If the daily salt intake exceeds 1.5 g (http://www.cdc.gov/salt/), the patient will receive dietary recommendations to follow a sodium-deplete diet for the duration of exposure to the COX-2 inhibitor in Phase 2. Furthermore, patients will be instructed how to prevent dehydration. This will be achieved by having the patients log their intake of fluids (type, volume) which will be reviewed and, where required, adjusted to meet the Institute of**

Medicine's recommended 13 cups (3 liters) of total beverages a day for men or 9 cups (2.2 liters) of total beverages a day for women (http://www.iom.edu/Activities/Nutrition/SummaryDRIs/DRI-Tables.aspx).

10.5.3 Co-administration of SSRIs and Celecoxib

Safety data from co-administration of a SSRI and celecoxib is available from a handful of studies (7, 41-43) using various dose regimens over period of times similar to the present study. All of these studies support a cardiovascular and gastrointestinal profile not different to the placebo treatments, but significant limitations arise from i) the small sample sizes, up to n=20 per group, ii) the patients selected for the study, these belong to different groups at risk, i.e. age, e.g. 24-46 years of age (41), and gender, i.e. only premenopausal women (43), and iii) the method of assessing cardiovascular and gastrointestinal adverse events, e.g. "checklist administered by a resident" as stated in (41).

Both escitalopram and celecoxib are weak inhibitors of CYP2D4. Increases in the area under plasma drug concentration over time curve (AUC) can be expected between ≥ 1.25 but < 2-fold (44). Drug clearance may decrease by 20-50% (44). **To retrospectively attribute adverse events to differences in drug exposure, plasma samples will be collected for pharmacokinetic analysis at select time points.**

10.5.1 Gastrointestinal Bleeding Risk of Celecoxib

NSAIDs, including Celecoxib, cause an increased risk of gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines. These events can be fatal. Patients with a current diagnosis of peptic ulcer disease and/or gastrointestinal bleeding will therefore be excluded. GI/bleeding risk will be screened for in medical history & physical examination. In weekly intervals, each patient will be monitored for GI pain and GI bleeds. In addition, hepatic function panel and hematocrit blood testing before entering the study, during week 1 or 2, and at the end of the study will screen for the presence of blood in stool and loss of excessive blood.

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12 Appendix

12.1 Study Flow-Chart

Procedures conducted with all participants				
Procedure	**Phone Screen**	**In-person Screen**	**Baseline**	**Week 6**
Informed Consent		X (Initial screening consent)	X (Full study consent)	X
Verbal Consent	X			
Screen, History (include GI/bleeding risks)	X			X
Screen, History, SCID, MADRS, HAMD, CGI, MOCA		X		
EKG		X		X
PE , Laboratory tests, including IL-6, IL-10, CBC with diff;		X	X	X
MRI; structural and functional			X	X
Lifetime depression, PSS, LES score			X	
Framingham VRF, CIRS-G, QOL		X		X
Neuropsychological tests (COWA, Trails A & B, WLMT, Digits Forward, Digits Backward, Digits Ascending, MMSE, Logical Memory, Digit Symbol, Benton Visual Retention, Shipley Vocabulary, Boston Naming, CogState)			X	X

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Additional procedures conducted with depressed participants initially randomized to celecoxib:					
Procedure	**Study Visits**				
	Baseline	**Wk 1**	**Wk 2**	**Wk 4**	**Wk 6**
Concomitant Medications	X	X	X	X	X
Drug Dispensed Escitalopram + celecoxib or escitalopram + placebo (weeks 0-6)	X	X	X	X	X
Psychiatrist evaluation, depression scales, Vital Signs (including optional urine samples at baseline, week 1 or 2, and 6; triplicate in-office BP weekly and monitor GI-pain/bleeding for safety),AEs, SAEs	X	X	X	X	X
PE (Lab tests including IL-6, IL-10, CBC with diff)	X	X	X	X	X
Liver function test & GI/bleeding risks	X		X		
Actigraphy (1 consecutive day minimum, optional longer),		X	X	X	
Optional Stool Sample	X		X		X
Optional Saliva samples	X	X	X	X	X
Safety (ABPM, FOBT and/or hematocrit levels)	X		X		
Safety (24-hour dietary recall, optional counseling on sodium-deplete diet)	X	X			

Additional procedures conducted with depressed participants initially randomized to placebo:									
Procedure			**Phase 1**			**Open Label Phase**			
	Baseline	**Wk 1**	**Wk 2**	**Wk 4**	**Wk 6**	**Wk 1**	**Wk 2**	**Wk 4**	**Wk 6**
Concomitant Medications	X								
Drug Dispensed Escitalopram + celecoxib or escitalopram + placebo (weeks 0-6) (Open label- Escitalopram + celecoxib)	X	X	X	X	X	X	X	X	X
Psychiatric evaluation, depression scales, Vital Signs (including optional urine samples at baseline, week 1 or 2, and 6; triplicate in-office BP weekly and monitor GI-pain/bleeding for safety),AEs, SAEs	X	X	X	X	X	X	X	X	X
Liver function test & GI/bleeding risks	X		X				X		
Actigraphy	X	X	X	X	X	X	X	X	X
Optional Saliva samples	X	X	X	X	X	X	X	X	X
Safety (ABPM, FOBT and/or hematocrit levels)	X		X				X		
Safety (24-hour dietary recall, optional counseling on sodium-deplete diet)	X	X				X	X		

12.2 Diary Recall Form (example)

To The Patient

The portable automatic blood pressure unit that you are wearing measures and records your blood pressure and heart rate at predetermined intervals. Be sure that the monitor is comfortably positioned before you leave the office, then go about your normal daily activities. The heart rate data should be used for reference only, not as clinical diagnostic data.

To obtain maximum information from the monitor, it is important that you record in this diary the initial TIME when the cuff inflates, the ACTIVITY in which you are involved when the reading is taken and the time at which you take your MEDICATIONS. It is unnecessary to list redundant activities and the time of day when the cuff inflates; just list the activities when they change (i.e., work to leisure activity). In addition, if symptoms occur between readings, push the START/STOP button on the monitor and an additional reading will be taken. Readings will then continue on a normal cycle. Be sure to describe the symptoms in the diary. Should you wish to abort a reading in progress, simply push the START/STOP button.

Important Notes

DO	once the tone sounds, avoid unnecessary movement while the blood pressure reading is being recorded. Try to keep your arm still.
DO NOT	remove the blood pressure monitor from the carrying case.
DO NOT	flip the ON/OFF switch as this will turn the blood pressure monitor off. Turn the machine off only in emergencies.
DO NOT	get the monitor wet; however, should the monitor get wet, there is no electrical shock hazard from the monitor.
DO NOT	worry if the blood pressure monitor cannot take a blood pressure reading every time it cycles. If it can't take a reading, remain still and the monitor will make one more attempt to measure your blood pressure in 60 seconds. (If a reading cannot be recorded, an event code will appear on the display.)

Spacelabs Medical
An OSI Systems Company

AMBULATORY BLOOD PRESSURE MONITOR

PATIENT DIARY

Patient Name _____

Address _____

City _____ *State* _____

Physician _____

Phone Number _____

Age _____ *Height* _____

Weight _____ *Sex* _____

Medication(s) _____

_____

_____

_____

000-0027-02 Rev. F

Log Information

TIME RECORDING STARTED _____

TIME RECORDING COMPLETED _____

TIME INTERVAL

00:00-06:00 Hrs. _____ Min.

06:00-12:00 Hrs. _____ Min.

12:00-18:00 Hrs. _____ Min.

18:00-24:00 Hrs. _____ Min.

DISPLAY ON/OFF _____

ALARM ON/OFF _____

MONITOR # _____

Afternoon NOON to 6 PM

TIME	ACTIVITY/ MEDICATION	COMMENTS/ SYMPTOMS

Morning 6 AM to NOON

TIME	ACTIVITY/ MEDICATION	COMMENTS/ SYMPTOMS

Evening 6 PM to MIDNIGHT

TIME	ACTIVITY/ MEDICATION	COMMENTS/ SYMPTOMS

000-0027-02 Rev. F

12.3 Template Medical Monitoring (example)

Subject Binder: ID _____ _____ _____ (Initials _____ _____ _____)

Medical Monitoring

Lab Work

Please specify which labs are being reviewed
Screening ☐ Close-out visit ☐ Both ☐

1. Are all out-of-range values accounted for and reviewed by the investigator?
   Yes ☐ No ☐
   If "No", Please Explain:

   _____
   _____
   _____
   _____
   _____
   _____

2. Are the ratings "Not Clinically Significant (NCS)" and "Clinically Significant (CS)", when indicated, appropriate for the out-of-range values?
   Yes ☐ No ☐
   If "No", Please Explain:

   _____
   _____
   _____
   _____
   _____
   _____

Adverse Events (AE)

1. Are all Adverse Events accounted for and reviewed by the investigator?
   Yes ☐ No ☐ N/A ☐ (No AE occurred)
   If "No", Please Explain:

   _____
   _____
   _____
   _____
   _____
   _____
   _____

Subject Binder: ID _____ _____ _____ (Initials _____ _____ _____)

2. Do all Adverse Events have a corresponding completed adverse event form?
   Yes ☐ No ☐ N/A ☐ (No AE occurred)
   If "No", Please Explain:

   _____
   _____
   _____
   _____

3. Has the overall study course for the subject for Adverse Events been reviewed?
   Yes ☐ No ☐ N/A ☐ (No AE occurred)
   If "No", Please Explain:

   _____
   _____
   _____
   _____

4. Have Adverse Events been identified, investigated, reported, and all necessary follow-ups performed?
   Yes ☐ No ☐ N/A ☐ (No AE occurred)
   If "No", Please Explain:

   _____
   _____
   _____
   _____

Serious Adverse Events (SAE)

Please describe the serious adverse event and the action taken following event:

_____
_____
_____
_____

N/A ☐ (No SAE occurred)

1. Are all Serious Adverse Events accounted for and reviewed by the investigator?
   Yes ☐ No ☐ N/A ☐ (No SAE occurred)
   If "No", Please Explain:

   _____
   _____
   _____
   _____
   _____
   _____

Subject Binder: ID _____ _____ _____ **(Initials** _____ _____ _____ **)**

2. Do all Serious Adverse Events have a corresponding completed Serious Adverse Events Form?
   Yes ☐ No ☐ N/A ☐ (No SAE occurred)
   If "No", Please Explain:
   _____
   _____

3. Has the IRB been notified within 5 calendar days of the SAE?
   Yes ☐ No ☐ N/A ☐ (No SAE occurred)
   If "No", Please Explain:
   _____
   _____
   _____

4. Has the overall study course for the subject for Serious Adverse Events been reviewed?
   Yes ☐ No ☐ N/A ☐ (No SAE occurred)
   If "No", Please Explain:
   _____
   _____
   _____

5. Have Serious Adverse Events been identified, investigated, reported, and all necessary follow-ups performed?
   Yes ☐ No ☐ N/A ☐ (No SAE occurred)
   If "No", Please Explain:
   _____
   _____
   _____

Medical Monitor's Name (print): _____

Medical Monitor's Signature: _____

Date: _____

12.4 Drug Prescribing Information

Escitalopram/Lexapro included in pdf version of protocol.

Celecoxib/Celebrex included in pdf version of protocol.

12.5 Food and Drug Administration-IND Exempt Letter

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

IND 127468

ACKNOWLEDGE/EXEMPT IND

Carsten Skarke, MD
Institute of Translational Medicine and Theraputics
University of Pennsylvania
3600 Spruce St, 8036 Maloney Bldg
Philadelphia, PA 19104

Dear Dr. Skarke:

We acknowledge receipt of your Investigational New Drug Application (IND), submitted August 6, 2015, received August 7, 2015, under section 505(i) of the Federal Food, Drug, and Cosmetic Act (FDCA) for Celebrex (celecoxib).

After reviewing the information contained in your submission, we have concluded that your study meets all of the requirements for exemption from the IND regulations and, therefore, an IND is not required to conduct your investigation. In accordance with 21 CFR 312.2(b)(4) of the regulations, FDA will not accept your application.

The IND regulations [21 CFR 312.2(b)] state that the clinical investigation of a drug product, including a biological product, that is lawfully marketed in the United States, is exempt from the requirements for an IND if all of the following apply:

1. The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication for use, nor intended to be used to support any other significant change in the labeling for the drug.
2. The investigation is not intended to support a significant change in the advertising for a prescription drug product.
3. The investigation does not involve a change in route of administration, dosage level, or patient population, or other factor that significantly increases the risks (or decreases the acceptability of risks) associated with use of the drug product.
4. The investigation is conducted in compliance with the requirements for institutional review (21 CFR Part 56) and informed consent (21 CFR Part 50).
5. The investigation is conducted in compliance with the requirements of 21 CFR 312.7, i.e., the drug may not be represented as safe or effective, nor may it be commercially distributed, for the purposes for which it is under investigation.

In addition, 21 CFR 312.2(b)(5) exempts from the IND requirements a clinical investigation that involves use of a placebo if the investigation does not otherwise require submission of an IND.

Reference ID: 3807870

We remind you that exemption from the requirements for an IND does not in any way exempt you from complying with the requirements for informed consent under 21 CFR 50.20 or from initial and continuing Institutional Review Board review under 21 CFR Part 56. You are also responsible for complying with the applicable provisions of sections 402(i) and 402(j) of the Public Health Service Act (PHS Act) (42 U.S.C. §§ 282(i) and (j)), which was amended by Title VIII of the Food and Drug Administration Amendments Act of 2007 (FDAAA) (Public Law No. 110-85, 121 Stat. 904).

Title VIII of FDAAA amended the PHS Act by adding new section 402(j) (42 USC § 282(j)), which expanded the current database known as ClinicalTrials.gov to include mandatory registration and reporting of results for applicable clinical trials of human drugs (including biological products) and devices. Please note that, if in the future you submit an application under sections 505, 515, or 520(m) of the FDCA (21 USC §§ 355, 360(e), or 360(j)(m)), or under section 351 of the PHS Act (21 U.S.C. § 262), or you submit a report under section 510(k) of the FDCA (21 USC § 360(k)), the application or submission must be accompanied by a certification that all applicable requirements of section 402(j) of the PHS Act (42 USC § 282(j)) have been met. Where available, such certification must include the appropriate National Clinical Trial (NCT) control numbers (42 USC § 282(j)(5)(B)). Additional information regarding the certification is available at: http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/FoodandDrugAdministrationAmendmentsActof2007/ucm095442.htm. Additional information regarding Title VIII of FDAAA is available at: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-014.html. Additional information on registering your clinical trial(s) is available at the Protocol Registration System website (http://prsinfo.clinicaltrials.gov/).

For additional information, a searchable version of the IND regulations is available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm.

If you have any questions, email CAPT Steven D. Hardeman, RPh, Chief, Project Management Staff, at Steven.Hardeman@FDA.GOV.

Sincerely,

{See appended electronic signature page}

Mitchell V. Mathis, MD
CAPT, USPHS
Director
Division of Psychiatry Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Reference ID: 3807870

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

STEVEN D HARDEMAN
08/18/2015
signed for Dr. Mathis

Reference ID: 3807870