DETAILED PROTOCOL

Title: Salicylate Augmentation in Depression

Principal Investigator: Jessica Harder, MD

Version Date: April 20th, 2017

I. Background and Significance:

Major depression is both common and burdensome, affecting over 15 million Americans in 2010 and costing over 210 billion dollars.[28] Moreover, existing treatments for depression often do not achieve remission. Only a third of patients will achieve remission with their first antidepressant trial, and up to a third will not achieve remission even after multiple medication trials.[1] Patients who do not respond to at least one antidepressant trial can be deemed treatment-resistant (although treatment-resistance exists along a continuum).[29] It is possible that treatment resistant depression (TRD) may require novel treatment approaches. Many of the most frequently used pharmacologic treatments for depression target the monoamine systems or are hypothesized to exert an effect by affecting monoamine function, and other drugs under evaluation are generally focused on other neurotransmitter systems.[1] A focus on other systems may yield additional treatment options for treatment-resistant depression.

Increasingly, converging lines of evidence support a role for inflammation in the pathogenesis of depression. Elevations in the inflammatory markers C-reactive protein (CRP) and interleukin-6 (IL-6) are found in patients with depression as compared to normal controls. Elevated levels of CRP can also predict the onset of depression, suggesting a role for inflammation in its pathogenesis.[2] Tumor necrosis factor α (TNF-α), too, has been demonstrated to be elevated in major depression. When healthy volunteers are given endotoxin infusions, which stimulate the release of cytokines, the cytokine levels were associated with the development of mood disorder symptoms. Administration of exogenous cytokines including TNF-α and interferon-α (IFN- α) have produced classical symptoms of depression in previously mood-stable individuals. Finally, antidepressant treatment has been demonstrated to reduce levels of IL-6 and CRP in depressed patients, and a lack of response to treatment has been associated with persistent elevations in these inflammatory markers.[2] Taken together, the above suggests a role for inflammation in the development of depression and given this, investigation of anti-inflammatory agents in the treatment of depression is indicated.

Many existing anti-inflammatory treatments are developed for the treatment of autoimmune disorders and carry risks of serious side effects including that of immunosuppression. These treatments can also be costly. There has been some interest, therefore, in anti-inflammatory agents with a more moderate side effect profile and lower cost. NSAIDs fit these criteria and have been previously evaluated for their effect in depression. Thus far studies have demonstrated significant heterogeneity across a number of domains including methodology, patient population, and medication used, and results have been inconsistent.[4] Studies examining at the effect of NSAIDs on pain in patients with medical illness have found concomitant improvements in depression symptoms. In a population of osteoarthritis patients with depressive symptoms, treatment with NSAIDs (ibuprofen, naproxen, and celecoxib) compared favorably with placebo, showing a trend toward greater reduction in depressive symptoms.[3] In patients with breast cancer and mild to moderate depression, benefits were also found

from treatment with NSAIDs, with patients receiving celecoxib showing significantly greater improvement in depression scores than those receiving diclofenac.[6]

Evaluation of the effect of anti-inflammatory treatment in patients with depression has not been limited to those with medical illness. As pain and disease effects could be major contributors to depressed mood, some studies have been done to investigate anti-inflammatory efficacy in patients with major depression without major medical comorbidities. In one randomized, double-blind, placebo controlled study celecoxib was given to patients with major depression as an adjunctive treatment to the antidepressant reboxetine (a selective norepinephrine reuptake inhibitor). A much greater improvement in depression symptoms was shown among those receiving celecoxib as the adjunct.[5] In a similar study utilizing celecoxib as an adjunctive agent to antidepressant treatment with fluoxetine, those patients given celecoxib saw significantly greater improvement.[8] In a follow-up study celecoxib used as an adjunctive agent to treatment with sertraline demonstrated both significantly improved depression scores in the celecoxib group and also significantly greater reductions in the inflammatory biomarker IL-6, with significant correlations observed between Hamilton Depression Rating Scale (HAM-D) scores and IL-6 levels.[9] Two meta-analyses of the use of celecoxib as an adjunctive treatment for patients with depression both found in favor of its use.[10,11] Unfortunately, COX-2 inhibitors such as celecoxib carry an increased risk of ischemic cardiovascular disease, to the extent that related medications rofecoxib and valdecoxib have been withdrawn from most markets.[33] Some analyses support the idea that salicylates and COX-2 inhibitors may separate from other NSAIDs in their relationship to antidepressant response.[7] Given that salicylates like aspirin are ubiquitous, with low cost and a safety profile in long-term use that is well-known, there would be an advantage to the use of salicylates in depression if indeed they prove effective.

There may be other advantages as well: aspirin, unlike the selective agent celecoxib, is technically a 'nonselective' inhibitor of cyclooxygenase, although it in fact more potently inhibits COX-1 than COX-2.[2] As there is some evidence that COX-2 has anti-inflammatory and neuroprotective activities in the brain,[13,14] inhibiting it may not be the optimal target for antidepressant treatments. Cyclooxygenase inhibition weighted towards COX-1 could be more effective. In an animal model of depression (rats subjected to chronic mild stress and showing depression-like behavior), treatment with aspirin – both alone and in combination with the antidepressant amytriptiline – was associated with a reduction in depression-like behaviors (e.g. increased sucrose preference).[12] In rats with depression-like behaviors that did not respond to fluoxetine, adjunctive aspirin treatment significantly improved the behaviors.[15] In humans, while aspirin treatment in conjunction with fluoxetine did not separate from fluoxetine-only treatment in terms of HDRS score, the combination did produce a significantly greater reduction in oxidative stress parameters,[16] which has been suggested to be associated with depression severity.[2] Furthermore, an open-label assessment of aspirin in combination with an SSRI in patients who had not responded to the SSRI alone found favorable response and remission rates, and a rapid improvement in HDRS scale in a 'responder' subgroup (that was sustained until the conclusion of the study).[18] This was interpreted as aspirin exerting an accelerating effect of antidepressant action, consistent with prior preclinical findings.[18] Some population data supports an antidepressant effect for aspirin as well: in a nested case-control study of adult men exposure to aspirin and statins were associated with a significant reduction in the likelihood of mood disorders and in a retrospective cohort study exposure to these agents reduced the risk for de novo mood disorders.[17] The potential for clinical effect of aspirin augmentation is of importance, and while a protocol for a randomized clinical trial (RCT) of aspirin treatment in bipolar depression has been described,[19] an RCT of aspirin in treatment-resistant unipolar depression is lacking. Given the large and clinically significant reduction in depression scores in the previous open-label trial of aspirin as an adjunct to antidepressant treatment, we propose to pursue an RCT to

see if the effect is borne out against placebo augmentation. Moreover, we hope to identify whether a specific pattern of biomarker elevation can predict antidepressant response to aspirin, since previous work has suggested that anti-inflammatory treatments in depression may have efficacy only in those patients with high inflammatory biomarkers prior to the treatment.[20] This could provide a biomarker signature that identifies a sub-group of patients who would benefit from aspirin augmentation treatment.

II. Specific Aims:

The primary aims of this study are:

Aim 1: To evaluate the clinical effect of aspirin augmentation on depression.

> **Aim 1a**: To examine the effect of aspirin augmentation on depression symptoms (as measured by the Hamilton Depression Rating Scale) in comparison to placebo augmentation of antidepressant treatment.
>
> **Aim 1b**: To examine the effect of aspirin augmentation on the rapidity of change in depression symptoms (as measured by the Hamilton Depression Rating Scale).

Aim 2: To assess with Luminex immunoassay the inflammatory profile of the blood of the aspirin augmentation responders compared with the non-responders.

> **Aim 2a:** To determine whether elevated serum inflammatory cytokines IL-6 and TNF-α at study initiation are associated with treatment response to aspirin.
> **Aim 2b:** To determine whether serum inflammatory cytokines IL-6 and TNF-α are lower in the augmentation responders group after treatment than in the augmentation non-responders group after treatment.
> **Aim 2c**: To determine whether the ratios of TNF-α:IL-10 and IL-6:IL-10 are associated with treatment response versus non-response.

Aim 3: To evaluate in an exploratory fashion whether other cytokines/chemokines or immune gene expression patterns at study initiation are associated with subsequent antidepressant response to aspirin.

> **Aim 3a:** To assess the panel of 60 cytokines/chemokines in the aspirin augmentation responders compared with the non-responders for potential contributions to treatment responsiveness. This analysis is exploratory.
> **Aim 3b:** To assess with Fluidigm technology the blood of the aspirin augmentation responders compared with the non-responders for RNA from three flow sorted subsets of activated memory $CD4^+$ ($CD3^+CD44^{hi}$), and $CD8^+$ ($CD3^+CD44^{hi}$) T cells, as well as $CD14^+$ monocytes. 96 T cell or monocyte-specific genes for pro-inflammatory, anti-inflammatory, chemokine, chemokine receptor, and signaling molecules will be measured. This analysis is exploratory.

Aim 4: To collect samples for eventual detailed immunologic characterization including antigen arrays and microRNAs.

Hypotheses:

Peripheral inflammatory mediators are differentially expressed in sub-populations of depressed patients, and such differences may represent indicators of responsiveness to anti-inflammatory treatment approaches.

1a: That aspirin augmentation will decrease HDRS scores versus placebo augmentation, and that a responder subgroup within the aspirin augmentation group will account for most of this signal.

1b: That aspirin augmentation will exert most of its clinical effect (steepest slope of change in HDRS) during the first 4 weeks of treatment.

2: That the serum inflammatory profile of the aspirin augmentation responders differs from the non-responders.

> **2a:** That elevated IL-6 and TNF-α at study initiation will be associated with treatment response to aspirin.
> **2b:** That IL-6 and TNF-α will be lower in the augmentation responders group versus the non-responders group after treatment.
> **2c**: That higher ratios of TNF-α:IL-10 and IL-6:IL-10 will be associated with greater treatment response to aspirin.

3: That there will be different patterns of immunologic activation between treatment responders and non-responders, and within the treatment-responders group before and after treatment with aspirin.

> **3a:** That the cytokines/chemokines assessed will show a different pattern of elevations in the aspirin-responder versus the aspirin non-responder group pre-treatment, and that the aspirin-responder group will show a differential pattern post- versus pre-treatment.
>
> **3b:** That the peripheral inflammatory trascriptome will show altered patterns of immune gene expression in the aspirin-responder versus the aspirin non-responder group pre-treatment, and that the aspirin-responder group will show a differential pattern of gene expression post- versus pre-treatment.

III. Subject Selection:

This study is a randomized, double-blind, placebo-control trial. The trial will enroll 74 adult subjects with treatment-refractory major depressive disorder, 37 per group. Assuming a non-completion rate of approximately 20%, as is customary in clinical trials of antidepressants, we will enroll a total of 74 subjects, anticipating 60 study completers. Subjects will all be recruited through outpatient psychiatric clinics affiliated with Brigham and Women's Hospital, through physician referral, print advertisements, and flyers.
Inclusion criteria:

- Aged 18-55
- Current diagnosis of major depressive disorder
- Hamilton Depression Rating Scale score of $\geq$21

- Stable treatment regimen (no medication changes or changes in psychotherapy treatment in past 8 weeks, and no participation in stepped treatments, such as completion of a course of cognitive behavioral therapy, during the trial)
- Failed to remit with up to 2 sequential antidepressant trials, or combination of 1 antidepressant and 1 augmentation agent
- Women of childbearing age must agree to use an approved method of contraception for the duration of the study

Exclusion criteria:

- Active suicidal ideation
- History of manic episodes or psychosis
- Alcohol or substance use disorder within the past 6 months
- Comorbid neurologic condition affecting the central nervous system
- Comorbid autoimmune condition
- History of thyroid disease or a current abnormal TSH
- Active or recent (within the past month) infection (such as otitis, pneumonia, urinary tract infection); temperature > 100.3 or WBC count > 11 K/microL will be considered evidence of active infection even in the absence of other symptoms
- History of GI bleed
- History of stroke
- History of a bleeding disorder
- Platelet count < 150,000/mm^3 on initial screening
- On a blood-thinning agent or taking NSAIDs daily
- Current use of oral steroids or other immunomodulating medications
- Salicylate sensitivity
- Pregnancy or breastfeeding
- GFR less than 60 mL/min/1.73m^2
- Current use of another nephrotoxic medication

The decision not to restrict participation to subjects utilizing a single medication was made to better reflect clinical practice, in which a variety of SSRI (selective serotonin reuptake inhibitors) and SNRI (serotonin-norepinephrine reuptake inhibitors) are used as first-line agents, and in order to enhance the generalizability of study findings.

IV. Subject Enrollment:

Subjects will all be recruited through outpatient psychiatric clinics affiliated with Brigham and Women's Hospital, through physician referral, print advertisements, and flyers. Demographics of the subjects are expected to largely reflect the referral patterns of these participating centers. Both males and females will be enrolled, however given the gender distribution in the prevalence of depression, it is expected that approximately 2/3 of the sample size will be female and 1/3 will be male. Contact information for the study coordinator will be provided by the physician in the case of a referral, and it will also be listed on the flyer and advertisement.

When potential subjects contact the study coordinator, they will first be asked a set of screening questions as outlined in the screening phone script. These questions will confirm that the subject

meets basic inclusion criteria. Subjects who are eligible at this time will be scheduled to attend the first baseline visit, where written informed consent will be obtained.

Details about the study, including objectives, procedures, and potential risks and discomforts will be discussed with the subject by the principal investigator (Dr. Jessica Harder) or a physician co-investigator. Subjects will be given the opportunity to ask any questions and will be given ample time to consider whether they wish to participate. They will be informed that refusal to participate will not interfere with their subsequent medical care.

During the first study visit, it will also be confirmed that the subject meets all other eligibility criteria, based on the clinical interviews and their laboratory results.

V. Study Procedures:

The study design represents an 8-week randomized, placebo-control trial. The study procedures, as divided by study visits, are described below.

Study Visit 1 (screening and study week 0):
At the first study visit, the principal investigator or co-investigator study physician will obtain informed consent. Details about the study, including objectives, procedures, and potential risks and discomforts will be discussed with the subject by a physician investigator. Subjects will be given the opportunity to ask any questions and will be given ample time to consider whether they wish to participate. They will be informed that refusal to participate will not interfere with their subsequent medical care.

The clinical study staff will then administer the Hamilton Depression Rating Scale (HDRS) and the Mini International Neuropsychiatric Interview (MINI) to confirm the subject has an active diagnosis of major depressive disorder (as defined by DSM V criteria) and rule out diagnoses of exclusion (namely mania, psychosis or hypomania). Subjects with active suicidal ideation, represented by a score of >2 on HDRS item 3, will be ineligible for the study and will be referred for urgent or emergent clinical assessment as indicated in the safety plan. Subjects will complete the Quick Inventory of Depression Symptomatology-Self Report (QIDS-SR), the MGH Antidepressant Treatment Response Questionnaire, the Systematic Assessment for Treatment Emergent Events (SAFTEE), and the Childhood Experience of Care and Abuse Questionnaire (CECA-Q3). The QIDS-SR and SAFTEE are customary for antidepressant medication clinical trials. The CECA documents early life stressors that can both increase risk for depression and impact cytokine levels through the lifespan.

All subjects will have a complete blood count drawn to measure their white blood cell and platelet counts and a thyroid screening hormone test. Subjects will also have labs drawn to measure blood urea nitrogen, creatinine, and electrolytes. Subjects will be asked about co-morbid neurologic, rheumatologic, and bleeding disorders and recent history of infection. Subjects will be asked to report on their use of oral steroid medications, non-steroidal anti-inflammatory drugs (including aspirin, ibuprofen, naproxen, meloxican, celecoxib, diclofenac, nabumetone, ketoprofen, and indomethacin), warfarin, heparin, and other anticoagulants (including clopidogrel, enoxaparin, rivaroxaban, apixaban, edoxaban, dabigatran, and fondaparinux).

Subjects will be asked to report on their age at first depression diagnosis, and number of depressive episodes, as well as existing treatments for depression, including names and dosages of medications, type(s) of psychotherapy, frequency of meetings with a psychiatrist, psychologist, social worker, or other counselor, and any other treatment modalities they engage in. Female subjects will also be asked to report whether they have regular monthly menstrual cycles, the date of their last menstrual period, and whether they are taking hormonal contraceptives or not. Women of child-bearing age will also receive a qualitative urine HCG pregnancy test. Female subjects will have blood drawn for follicle-stimulating hormone (FSH), estradiol, and progesterone levels. All subjects' temperature will be taken to assess for infection. If their temperature is greater than 100.3F, they will be excluded from the study. Subjects determined to be ineligible based on the results of the screening or screening labs will be thanked for their participation and conclude their participation.

Ten mL of subjects' blood will be separated by centrifuge and the serum analyzed for an array of 60 cytokines and chemokines including C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-alpha); assays will be performed using a Luminex bead-based immunoassay. Serum will be stored into small aliquots at -80ºC to avoid repeated freeze-thaw cycles. 25 µl of serum per subject will be used for Luminex studies as previously reported.[32] Expression data will be analyzed by Luminex software. All samples will be drawn between 8:00 AM and 10:00AM in order to minimize the effect of circadian fluctuations in cytokine levels. Subject's height and weight will be taken to allow for calculation of BMI; prior work has demonstrated a U-shaped relationship between BMI and pro-inflammatory cytokines, with higher values associated with underweight and obesity.[21]

To evaluate gene profiles of peripheral immune cells, peripheral blood mononuclear cells (PBMCs) will be prepared by Ficoll-Paque PLUS gradient centrifugation from the blood in order to isolate T cells and monocytes. Frequency of $CD4^+$, $CD8^+$, and $CD14^+$ cells will be measured. PBMCs will be stored into aliquots at -80ºC. For transcriptomic analysis, Fluidigm Biomark technology will utilize microfluidic technology to perform high throughput gene expression measurements with real time PCR. RNA will be processed from flow cytometry-sorted activated/memory $CD4^+$ ($CD3^+CD44^{hi}$), and $CD8^+$ ($CD3^+CD44^{hi}$) T cells, as well as $CD14^+$ monocytes. A panel of 96 T cell or monocyte-specific genes for pro-inflammatory, anti-inflammatory, chemokine, chemokine receptor, and signaling molecules will be measured.

To allow for the eventual, detailed characterization of the subjects' immunologic profiles, whole blood samples will also be banked for subsequent processing at the Weiner Lab at Brigham and Women's Hospital/Harvard Medical School. The samples will be assayed for antigens using the following technique: over 500 individual antigens (proteins, lipids, myelin peptides) are spotted on slides by a robot, after which the slides are incubated with serum from subjects with depression and an immune signature is developed. The samples will also be assayed for microRNAs, non-coding RNAs that modulate immune function.

Subjects who meet inclusion criteria for the study will be given their pill packets for the ensuing 8 weeks, consisting of blister packs for a daily dose of aspirin 325mg in those randomized to the treatment condition and of blister packs of a placebo pill of the same size, shape, and color as the aspirin tablet in those randomized to the control condition. Subjects will be instructed to take

their pills in the evening before bed; in cardiovascular studies aspirin has had greater efficacy and better tolerability when taken at this time of day.[35, 36] Subjects will be instructed not to take aspirin over the counter during the study, and to inform study staff of their use of other over-the-counter anti-inflammatory medications. All subjects will be instructed to also continue their existing antidepressant treatment regimen but not to engage in new treatments or therapies during the course of the study. If new treatments are deemed clinically necessary during the course of the study subjects are instructed to contact study staff immediately to inform them of the change.

Study Visit 2 and 3 (weeks 2 and 4):
At study visits 2 and 3, subjects will be asked about their compliance with the study drug and about any adverse effects; they also will again complete the SAFTEE. They will be asked about any changes to their medications or psychiatric treatment. Study staff will re-administer the Hamilton Depression Rating Scale (HDRS) and subjects will again complete the QIDS-SR. Blood will be drawn for repeat cytokine/chemokine immunoassay, BUN, creatinine, and electrolytes measurements. If preferred, the subject can complete the psychometric measures by telephone and online portal and omit the blood draws at these visits.

Study Visit 4 (week 8):
At study visit 4, study staff will re-administer the Hamilton Depression Rating Scale (HDRS) and blood will again be drawn for repeat cytokine/chemokine immunoassay, including measures of CRP, IL-6, BUN, creatinine, electrolytes and TNF-alpha. Subjects will again complete the QIDS-SR and the SAFTEE, and will be asked about any changes to their medications or psychiatric treatment and any changes in their health status. Platelet count and FSH, estradiol, and progesterone levels as appropriate, will also be repeated. Follow up samples for the detailed immunologic profiling will also be drawn. Serum salicylate concentrations will be drawn (to confirm compliance with study medication in those subjects assigned to the aspirin condition, and to confirm subjects not assigned to the aspirin condition were not using it).

Subjects will be advised to follow up with their current outpatient psychiatric providers within two weeks of ending participation in the study.

Remuneration:
Subjects who complete the study including 4 in-person visits will receive a total of $200 ($45 for the first study visit, $40 each for the second and third study visits ($20 if no blood draw and subject completes questionnaires remotely), and $75 for the last study visit) in four payments sent by mail after each visit. All participants will receive complementary parking for study visits.

Data storage:
At each study visit, the study staff will enter the data into case report forms in paper binders, as well as in our institution's research electronic data capture platform (REDCap). All laboratory values will be reviewed by a physician investigator. Paper files matching the case report forms in REDCap, and including the consent form, screening materials, and any additional correspondence will be stored in a locked filing cabinet in the office of the study staff at Brigham and Women's Hospital. All electronic study data will be stored securely on REDCap and de-identified prior to data analysis. Only group data will be reported.

VI. Biostatical Analyses:

The baseline data to be collected and analyzed include: subject age at enrollment, gender, age of depression onset, number of previous depressive episodes, HDRS. HDRS will be repeated at the subsequent study visits for analysis. Cytokine levels will also be analyzed at the initial and final visits. The planned study end-point is at eight weeks after initiation, and is not based on any pre-determined treatment response. Exploratory analysis of the immunologic profiling data will be performed as well, with a goal to detect within-subject changes over the course of the study and to discern any differences between non-responders and responders.

Baseline measurements for continuous variables (e.g., age of onset, current age) will be assessed by t-test. Categorical measurements, such as gender, will be compared with Fisher's exact test or chi-square as appropriate. For the primary study outcome, the change in HDRS in the treatment versus control groups will be evaluated with analysis of covariance (ANCOVA), with initial HDRS score as a covariate.

Power calculations were based on a prior open-label trial of salicylic acid as an augmentation agent to fluoxetine, which found an average 15-point drop in HDRS score over a 4-week trial.[18] To be conservative, we are assuming a much smaller effect size, and so this study is powered to detect a 3-point difference in HDRS score, a difference which is deemed to be clinically significant.[34] 29 participants per group are required to detect this clinically significant significant difference assuming .80 power and a two-sided alpha of 0.05. Assuming a non-completion rate of approximately 20%, customary in clinical trials of antidepressants, we will enroll 74 subjects, anticipating 60 completers.

Secondary outcomes will include the response of inflammatory biomarkers in treatment responders v. treatment non-responders at 8 weeks and the degree of biomarker association with antidepressant response. For this, the association of IL-6 and TNF-α at V1 with change in HDRS from V1 to V4 in the aspirin treated group versus the placebo treated group will be evaluated with an analysis of covariance (ANCOVA) model. This model will include three variables: treatment condition (aspirin versus placebo), baseline IL-6 or TNF-α level, and an interaction term (baseline cytokine value x treatment). A similar analysis of change in HDRS scores will be performed to assess the interaction of baseline TNF-α to IL-10 and IL-6 to IL-10 ratios by treatment condition. All applicable tests will be two-tailed with an alpha set at 0.05.

For our exploratory analysis of the full panel of 60 cytokines and chemokines, we expect to perform 60 comparisons and plan to use a false discovery rate approach.[37] The interaction of the baseline value of each cytokine or chemokine with treatment condition will be evaluated. The interaction *p* values will be converted to FDR *adjusted p* values and ordered from smallest to largest. A cut point of 10% will be used to control the false discovery rate and to identify a subset of values that will be presumed to be biologically relevant. A similar procedure will be carried out for analysis of the 96 immune genes. All analyses were designed in consultation with a biostatistician.

VII. Risks and Discomforts:

Aspirin is approved for analgesia and its anti-pyretic effect, and is commonly used off-label for a number of other indications including acute coronary syndrome, acute stroke, in coronary disease, and for its anti-inflammatory effect. Unfortunately, accurate estimation of frequencies of adverse effects is deemed not feasible, in part related to some reactions being idiosyncratic.[22] Adverse effects of aspirin are often dose-related. Likely the most important adverse effect associated with aspirin administration is bleeding, which can occur throughout the body; gastrointestinal ulceration occurs in 6 to 31%. [22] Other bleeding-related conditions make up a significant proportion of the remainder of the common adverse effects of aspirin.

There is a possibility that aspirin in combination with antidepressant medications may carry an increased risk of bleeding. For example, epidemiologic studies support an increased risk for upper gastrointestinal (GI) bleeding with selective serotonin reuptake inhibitor (SSRI) use, and this is increased with concurrent use of aspirin.[24] The relative risk of upper GI bleed with an SSRI-aspirin combination in some studies reviewed ranged from 1.9 to 7.2.[25] This is important given the frequency with which SSRI medications are used as first-line agents for depression; it can be expected that many subject participants will be taking a medication in this class or a related class. However, the overall risk remains low: the number needed to harm for a GI bleed with an SSRI-aspirin combination ranged from 81 to 170 patient-years in the studies reviewed.[25] There is also some risk of intracranial hemorrhage. Shin et al[26] examined the 30 day risk of intracranial hemorrhage in Korean patients who took both antidepressants and NSAIDs and found a hazard ratio of 1.6 over use of antidepressants alone. However, as others have pointed out, it is not clear that this risk is related to the combination of the two drugs as there was no control group examined who were not taking an NSAID, suggesting that the effect could be the risk of the NSAID alone rather than a synergistic one.[27] Too, the study was performed with an entirely Korean population, raising the question of generalizability to other populations given ethnic variability in the activity of CYP450 enzymes involved in the metabolism of many antidepressants and non-steroidals.[29] Finally, the increased risk for bleeding was only statistically significant in men. This is relevant to the study population given the preponderance of women affected by depression and the expected higher female to male ratio amongst study participants. How the data above translate into risk for our population is not entirely clear, but one assessment of the number needed to harm if extrapolating from Shin's data found that the combination of antidepressants and NSAIDs gives an additional 4.1 hemorrhages/1000 patient-years, equivalent to one avoidable intracranial hemorrhage in first 30 days per 3000 men starting on the combination.[28] Assuming an equal distribution of men and women, this would mean one avoidable intracranial hemorrhage per 6000 subjects, although as stated above this number is likely to be slightly lower given higher rates of depression in women.

Serotonin-norepinephine reuptake inhibitors (SNRIs), which are another popular first-line medication for depression, appear to have similar risks for intracranial hemorrhage in depressed patients as compared to SSRIs[38] but do not carry the same risks for gastrointestinal bleeding that SSRIs do[39]. Some studies class all the serotonin blockers together to look at adverse effects and results are inconsistent: for example, in one study of patients taking either SNRIs or SSRIs and warfarin together, the risk of death from intracerebral hemorrhage was higher than with warfarin alone[43]. On the other hand, another study that evaluated patients undergoing coronary artery bypass grafting found no association between taking the combination of either SNRI or SSRI and

anti-platelet treatment (including aspirin) and risk for bleeding[40]. Unfortunately, there are no studies that directly evaluate the effect of aspirin and SNRIs on combined morbidity and mortality risk in a depressed population, so direct extrapolations to our study are challenging.

We were unable to find any studies evaluating the risks associated with bupropion, another commonly used antidepressant and augmenting agent, in combination with aspirin. It is therefore unknown if there is an increased risk of bleeding or other concern sin combining bupropion and aspirin. Mirtazapine, often used as a second-line antidepressant and occasionally used as first-line, has been shown to induce platelet antibodies in vitro[41], but has not been shown to be associated with increased risk for hemorrhage[42]. Whether mirtazapine would be associated with increased risk for bleeding in combination with aspirin is unclear. Overall, there is a lack of published reports on the safety of combining antidepressants other than SSRIs or SNRIs with aspirin. There is no evidence to suggest increased risk of bleeding in combining bupropion or mirtazapine with aspirin.

Of note, and perhaps relevant to all of the above-named antidepressants that are in common use, aspirin is already a commonly given medication for patients with a number of cardiac conditions. Given the prevalence of both cardiovascular disease and depression, it is inevitable that a large number of patients are concurrently being treated with aspirin and a variety of antidepressants. Thus, while the safety of this medication in combination with a range of antidepressants may not have been demonstrated in a clinical trial, there have been ample opportunities for risks associated with these combinations to come to clinical and scientific attention. The lack of publications reporting increased risk is as at least somewhat reassuring as to the relative safety of this combination.

The effect of aspirin on an embryo or fetus can include adverse effects up to and including fetal death. For this reason, women cannot take part in this study if they are pregnant or may become pregnant. While aspirin found in breast milk carries less clear risk, the WHO recommends against long-term therapy[23]; for this reason women cannot take part in this study if they are nursing.

With the blood draws, there are risks of pain and bruising at the phlebotomy site, infection, and lightheadedness.

The assessment of psychiatric history, including depression and substance abuse, can make some people feel uncomfortable as the information is sensitive to some subjects. Some may also be especially concerned about the confidentiality of mental health information. The study staff are trained in mental health assessments, and make every effort to make the subjects comfortable during study visits. All subjects will be assured that their history and responses will be kept confidential within the study, except as required by law, in the case of acute safety concerns, and in any instances as described in the informed consent form.

VIII. Potential Benefits:

No benefits can be guaranteed from participation in the study. However, half of all enrolled participants will be receiving an adjunctive agent that is hypothesized to help treat their

depression, and it is possible that at least some subjects will experience a decrease in the severity of their self-reported depression symptoms. In addition, even those receiving placebo may benefit from the placebo effect and from the attention of the study staff. In addition, all subjects will be permitted to continue their existing psychiatric treatments. The results of this study also have potential to benefit larger clinical populations if it is identified that aspirin is an effective adjunctive treatment for depression. Further, the data collected during the study will expand our knowledge of the role of immune biomarkers in depression and treatment response.

IX. Monitoring and Quality Assurance:

The principal investigator, Dr. Jessica Harder, will be responsible for monitoring the safety of all subjects. In the event that Dr. Harder is unavailable, physician co-investigators will be available to monitor the safety of all subjects.

All study staff are primarily located at Brigham and Women's Hospital. Study safety meetings, including the principal investigator, study coordinators, and study physicians will occur regularly to review the progress of currently-enrolled subjects and any reported side effects.

The Principal Investigator will assess all patients with regard to stopping criteria. In general terms, a subject will be discontinued from the study for the following reasons:

1) The subject has experienced an adverse event that, in the opinion of the principal investigator requires early termination
2) The subject's thoughts or behaviors become a safety risk at any time during the trial
3) The subject experiences a 20% drop in GFR during the course of the study
4) The subject withdraws consent

Specific to this study, subjects will be monitored for suicidal thoughts at both visits and for adverse events as well. Subjects who develop active suicidal ideation, represented by a score of >2 on HDRS item 3, will be referred for urgent or emergent clinical assessment. A patient who develops active suicidal thoughts will be urgently evaluated by a study physician. In any case where there are acute safety concerns arising from suicidality (such as a plan or actual intent), the patient will be sent to the BWH Emergency Department for further evaluation under Section 12a, and in compliance with hospital policies. If there are no acute safety concerns based on the urgent evaluation, the patient will be monitored according to study protocol and instructed to contact study staff immediately with any worsening in their mood or thinking. The study physician will review the urgent or emergent safety assessment and determine if the subject can continue in the study. Any subject who requires a level of care exceeding customary outpatient services (i.e., psychiatric hospitalization or participation in a partial hospital program) will be removed from the study. If a subject must be transferred from the study into clinical care, the study physicians will continue to follow the subjects until they are accepted into appropriate, longer-term care, if clinically indicated.

Any subject who discontinues the study due to adverse effects arising during the study will be advised to follow up with their primary care physician. If an adverse event occurs, or there is an

abnormal finding during laboratory testing, that information will be released to the subject's primary physician with the subject's permission and written release.

At the end of the study, study staff will arrange for any additional (or new) psychiatric follow up, as clinically indicated.

Privacy and Confidentiality:

Potential subjects will receive information about the study in the privacy of an exam room. All visits will take place in an exam room. Phone calls will take place at a number and time of the patients choosing.

After each study visit, the study staff will enter the data into case report forms in REDCap. All laboratory values will be reviewed by the Principal Investigator or another physician co-investigator. All study data will be stored securely in REDCap with access limited to study staff and de-identified prior to data analysis. Only group data will be reported. Paper files for each subject, including the consent form, screening materials, and any additional correspondence will be stored in a locked filing cabinet in the office of the study staff at Brigham and Women's Hospital. Only persons associated with the research will have access to the research data.

During all portions of this research study, the privacy and confidentiality of all participants will be maintained. Specimens will be de-identified and assigned a code number which will not contain identifiers which could link them to individual subjects. These will then be stored in the locked research lab with access only available to study staff.

No patient data obtained from the study unless it is regarded as a standard of care will be added to the medical record of the patient.

X. References:

1. Philip NS, Carpenter LL, Tyrka AR, Price LH. Pharmacologic approaches to treatment resistant depression: a re-examination for the modern era. Expert Opin Pharmacother. 2010 Apr;11(5):709-22. doi: 10.1517/14656561003614781.
2. Berk M, Dean O, Drexhage H, McNeil JJ, Moylan S, O'Neil A, Davey CG, Sanna L, Maes M. Aspirin: a review of its neurobiological properties and therapeutic potential for mental illness. BMC Med. 2013 Mar 18;11:74. doi: 10.1186/1741-7015-11-74. Review.
3. Iyengar et al. Am J Med. 2013 Nov; 126(11): 1017.e11.8.
4. Eyre HA, Air T, Proctor S, Rositano S, Baune BT. A critical review of the efficacy of non-steroidal anti-inflammatory drugs in depression. Prog Neuropsychopharmacol Biol Psychiatry. 2015 Mar 3;57:11-6. doi: 10.1016/j.pnpbp.2014.10.003. Epub 2014 Oct 16.Review.
5. Müller, N, Schwarz, MJ, Dehning S, Douhe A, Cerovecki A, Goldstein-Müller B, Spellman I, Hetzel g, Maino K, Kleindienst N, Möller H-J, Arolt V, Riedel M. The cyclooxygenase-2 inhibitor celecoxib has therapeutic effects in major depression: results

of a double-blind, randomized, placebo controlled, add-on pilot study to reboxetine. Molecular Psychiatry. 2006. 11: 680-684.

6. Mohammadinejad P, Arya P, Esfandbod M, Kaviani A, Najafi M, Kashani L, Zeinoddini A, Emami SA, Akhondzadeh S. Celecoxib Versus Diclofenac in Mild to Moderate Depression Management Among Breast Cancer Patients: A Double-Blind, Placebo-Controlled, Randomized Trial. Ann Pharmacother. 2015 Sep;49(9):953-61. doi: 10.1177/1060028015592215. Epub 2015 Jul 2
7. Gallagher PJ, Castro V, Fava M, Weilburg JB, Murphy SN, Gainer VS, Churchill SE, Kohane IS, Iosifescu DV, Smoller JW, Perlis RH. Antidepressant response in patients with major depression exposed to NSAIDs: a pharmacovigilance study. Am J Psychiatry. 2012 Oct;169(10):1065-72. doi: 10.1176/appi.ajp.2012.11091325.
8. Akhondzadeh S, Jafari S, Raisi F, Nasehi AA, Ghoreishi A, Salehi B, Mohebbi-Rasa S, Raznahan M, Kamalipour A. Clinical trial of adjunctive celecoxib treatment in patients with major depression: a double blind and placebo controlled trial. Depress Anxiety. 2009;26(7):607-11. doi: 10.1002/da.20589.
9. Abbasi SH, Hosseini F, Modabbernia A, Ashrafi M, Akhondzadeh S. Effect of celecoxib add-on treatment on symptoms and serum IL-6 concentrations in patients with major depressive disorder: randomized double-blind placebo-controlled study. J Affect Disord. 2012 Dec 10;141(2-3):308-14. doi: 10.1016/j.jad.2012.03.033. Epub 2012 Apr 18.
10. Faridhosseini F, Sadeghi, R, Farid L, Pourgholami M. Celecoxib: a new augmentation strategy for depressive mood episodes. A systematic review and meta-analysis of randomized placebo-controlled trials. Hum Psychopharmacol. 2014; 29(3): 216-223.
11. Na KS, Lee KJ, Lee JS, Cho YS, Jung HY. Efficacy of adjunctive celecoxib treatment for patients with major depressive disorder: a meta-analysis. Prog Neuropsychopharmacol Biol Psychiatry. 2014 Jan 3;48:79-85. doi: 10.1016/j.pnpbp.2013.09.006. Epub 2013 Sep 20.
12. Bhatt S, Shukla P, Raval J, Goswami S. Role of Aspirin and Dexamethasone against Experimentally Induced Depression in Rats. Basic Clin Pharmacol Toxicol. 2015 Dec 8. doi: 10.1111/bcpt.12539.
13. Maes M. Targeting cyclooxygenase-2 in depression is not a viable therapeutic approach and may even aggravate the pathophysiology underpinning depression. Metab Brain Dis. 2012 Dec;27(4):405-13. doi: 10.1007/s11011-012-9326-6. Epub 2012 Jul 7.
14. Blais V, Turrin NP, Rivest S. J Neurochem. Cyclooxygenase 2 (COX-2) inhibition increases the inflammatory response in the brain during systemic immunestimuli. 2005 Dec;95(6):1563-74. Epub 2005 Nov 8.
15. Wang Y, Yang F, Liu, YF, Gao F, Jiang W. Acetylsalicylic acid as an augmetnation agent in fluoxetine treatment resistant depressive rats. Neurosci Lett 2011;499:74-9.
16. Gałecki P, Szemraj J, Bieńkiewicz M, Zboralski K, Gałecka E. Oxidative stress parameters after combined fluoxetine and acetylsalicylic acid therapy in depressive patients. Hum Psychopharmacol. 2009 Jun;24(4):277-86. doi: 10.1002/hup.1014.
17. Williams LJ, Pasco JA, Mohebbi M, Jacka FN, Stuart AL, Venugopal K, O'Neil A, Berk M. Statin and Aspirin Use and the Risk of Mood Disorders among Men. Int J

Neuropsychopharmacol. 2016 Feb 2. pii: pyw008. doi: 10.1093/ijnp/pyw008. [Epub ahead of print]
18. Mendlewicz J, Kriwin, P, Oswald P, Souery D, Alboni S, Brunello N. Shortened onset of action of antidepressants in major depression using acetylsalicylic acid augmentation: a pilot open-label study. Int Clin Psychopharmacol. 2006 Jul; 21(4): 227-31.
19. Savitz J, Preskorn S, Teague TK, Drevets D, Yates W, Drevets W. Minocycline and aspirin in the treatment of bipolar depression: a protocol for a proof-of-concept, randomised, double-blind, placebo-controlled, 2x2 clinical trial. BMJ Open. 2012 Feb 22;2(1):e000643.
20. Raison CL, Rutherford RE, Woolwine BJ, Shuo C, Schettler P, Drake DF, Haroon E, Miller AH. A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression. 2013. JAMA Psychiatry. 70(1): 31-41.
21. Zampieri, FG, Jacob, V, Barbeiro, HV, Pinheiro da Silva, F, da Souza, HP. Influence of Body Mass Index on Inflammatory Profile at Admission in Critically Ill Septic Patients. International Journal of Inflammation. Volume 2015 (2015), Article ID 734857, 6 pages.
22. http://www.uptodate.com/contents/aspirin-drug-information?source=search_result&search=aspirin&selectedTitle=1~150#F137075
23. World Health Organization (WHO), "Breastfeeding and Maternal Medication, Recommendations for Drugs in the Eleventh WHO Model List of Essential Drugs," 2013. Available at http://www.who.int/maternal_child_adolescent/documents/55732/en/index.html
24. Andrade C, Sandarsh S, Chethan KB, Nagesh KS. Serotonin reuptake inhibitor antidepressants and abnormal bleeding: a review for clinicians and a reconsideration of mechanisms. J Clin Psychiatry. 2010 Dec;71(12):1565-75. doi: 10.4088/JCP.09r05786blu.
25. Mort JR, Aparasu RR, Baer RK. Interaction between selective serotonin reuptake inhibitors and nonsteroidal antiinflammatory drugs: review of the literature. *Pharmacotherapy*. 2006;26(9):1307–1313.
26. Shin JY, Park MJ, Lee SH, Choi SH, Kim MH, Choi NK, Lee J, Park BJ. Risk of intracranial haemorrhage in antidepressant users with concurrent use of non-steroidal anti-inflammatory drugs: nationwide propensity score matched study. BMJ. 2015 Jul 14;351:h3517. doi: 10.1136/bmj.h3517.
27. Bray, B. Study design cannot support link between intracranial haemorrhage, antidepressants, and NSAIDs. BMJ 2015;351:h4445.
28. Lewis, LS. Combined use of antidepressants and NSAIDs: NNT for intracranial haemorrhage. *BMJ* 2015;351:h4444.
29. Mercer, SW, Payne RA, Nicholl, BI, Morrison J. Risk of intracranial haemorrhage linked to co-treatment with antidepressants and NSAIDs. *BMJ* 2015;351:h3745.
30. Izawa S, Miki K, Liu X, Ogawa N. The diurnal patterns of salivary interleukin-6 and C-reactive protein in healthy young adults. Brain Behav Immun. 2013 Jan;27(1):38-41. doi: 10.1016/j.bbi.2012.07.001. Epub 2012 Jul 13.
31. Voderholzer U, Fiebich BL, Dersch R, Feige B, Piosczyk H, Kopasz M, Riemann D, Lieb K. Effects of sleep deprivation on nocturnal cytokine concentrations in

depressed patients and healthy control subjects. J Neuropsychiatry Clin Neurosci. 2012 Summer;24(3):354-66. doi: 10.1176/appi.neuropsych.11060142.

32. Bassil, R., W. Orent, M. Olah, A. T. Kurdi, M. Frangieh, T. Buttrick, S. J. Khoury, and W. Elyaman. 2014. BCL6 controls Th9 cell development by repressing Il9 transcription. Journal of immunology 193: 198-207.
33. http://www.uptodate.com/contents/cox-2-selective-inhibitors-adverse-cardiovascular-effects?source=see_link
34. Montgomery SA. Clinically relevant effect sizes in depression. Eur Neuropsychopharmacol 1994; 4: 283-284.
35. Bonten, TN, Saris, A, van Oostrom, MJ, Snoep JD, Eikenboom, J, van der Meer, PF, van der Bom, JG. Effect of aspirin intake at bedtime versus on awakening on circadian rhythm of platelet reactivity.
36. Guo W, Lu W, Xu Y, Wang L, Wei Q, Zhao Q. Relationship between Adverse Gastric Reactions and the Timing of Enteric-Coated Aspirin Administration. Clin Drug Investig. 2017 Feb;37(2):187-193.
37. Benjamini Y & Yekutieli D. The control of the false discovery rate in multiple testing under dependency. Annals of Statistics. 2001; 29:1165-1188.
38. Lee YC, Lin CH, Lin MS, Lu Y, Chang CH, Lin JW. Comparison of the effects of serotonin-norepinephrine reuptake inhibitors versus selective serotonin reuptake inhibitors on cerebrovascular events. J Clin Psychiatry. 2016 Jan;77(1):e1-7.
39. Cheng YL, Hu HY, Lin XH, Luo JC, Peng YL, Hou MC, Lin HC, Lee FY. Use of SSRI, But Not SNRI, Increased Upper and Lower Gastrointestinal Bleeding: A Nationwide Population-Based Cohort Study in Taiwan. Medicine (Baltimore). 2015 Nov;94(46):e2022.
40. Tully PJ, Cardinal T, Bennetts JS, Baker RA. Selective serotonin reuptake inhibitors, venlafaxine and duloxetine are associated with in hospital morbidity but not bleeding or late mortality after coronary artery bypass graft surgery. Heart Lung Circ. 2012 Apr;21(4):206-14. doi: 10.1016/j.hlc.2011.12.002. Epub 2012 Jan 28.
41. Arnold DM, Kukaswadia S, Nazi I, Esmail A, Dewar L, Smith JW, Warkentin TE, Kelton JG A systematic evaluation of laboratory testing for drug-induced immune thrombocytopenia. J Thromb Haemost. 2013 Jan;11(1):169-76.
42. Gahr M, Zeiss R, Lang D, Connemann BJ, Hiemke C, Muche R, Freudenmann RW, Schönfeldt-Lecuona C. Association between haemorrhages and treatment with selective and non-selective serotonergic antidepressants: Possible implications of quantitative signal detection. Psychiatry Res. 2015 Sep 30;229(1-2):257-63.
43. Löppönen P, Tetri S, Juvela S, Huhtakangas J, Saloheimo P, Bode MK, Hillbom M. Association between warfarin combined with serotonin-modulating antidepressants and increased case fatality in primary intracerebral hemorrhage: a population-based study. J Neurosurg. 2014 Jun;120(6):1358-63.