A double-blind randomized placebo-controlled study of aspirin and N-acetyl cysteine as adjunctive treatments for bipolar disorder patients

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ABSTRACT

Bipolar disorder (BD) is a severe and debilitating psychiatric illness. While available treatments benefit about 50%-60% of patients, there is a sizeable group that does not respond adequately. Bipolar depression often presents a very significant challenge for clinical management as treatment responses are often inadequate. The pathophysiology of depression is not agreed on but there is evidence for contributions of neuroinflammation and disturbed brain arachidonic acid metabolism. Furthermore, an increasing body of evidence documents a role for increased oxidative stress in brain neurons as a putative mechanism involved in neuronal damage and possibly in the pathophysiology of mood disorders. Lithium and valproate, the most commonly used medications to treat BD, have been shown in pre-clinical studies to have important effects on neuroinflammatory mechanisms, as well as oxidative stress pathways. The non-steroidal anti-inflammatory drug (NSAID) Celecoxib, a cyclooxygenase type 2 (COX-2) inhibitor, was shown to have possible effects in bipolar depression in a small double-blind controlled preliminary study. A controlled study testing the potential of aspirin in symptomatic schizophrenic patients found promising results. A double-blind placebo-controlled study also suggested therapeutic effects of N-Acetyl Cysteine (NAC) for depressive symptoms in patients with bipolar disorder. Therefore, there is a compelling rationale for conducting a double-blind controlled trial to **examine the potential of aspirin and NAC in the treatment of BD patients**. We propose to conduct a 16-week double-blind randomized placebo controlled trial with aspirin (1000 mg qam, orally) or NAC (1000 mg bid, orally) as add-on treatments to mood stabilizing agents in BD type I and II patients on a depressive or mixed episode. In addition to testing these treatments individually, we will also randomly assign patients to a combination of aspirin and NAC, to examine the potential for synergistic effects from these two medications. We will utilize a **novel drop-the-loser (DTL) adaptive clinical trial design** that is more cost-effective to screen medication leads. . This study will investigate the role of inflammatory mechanisms and oxidative stress in antidepressant response and could lead to the development of new and critically needed treatment modalities for BD patients.

Introduction and Background

Bipolar disorder (BD) is a major health problem (lifetime prevalence about 1.5%) and causes significant morbidity and burden to society. Available treatments help a substantial proportion of patients but are not beneficial for an estimated 40%-50% [1, 2]. Lithium has been the standard treatment for BD over many years [3, 4], but may have significant side effects and not be efficacious in 50-60%. Over the past two decades, several alternatives have been developed [1, 5-9] [10, 11][12-19]. Nonetheless, there still is considerable need for better treatments and understanding of disease and drug mechanisms involved [20, 21], as disability and functional impairment are still widespread amongst individuals suffering from BD.

Although the exact pathophysiology of BD and particularly of bipolar depression is unknown, evidence suggests important contributions of neuroinflammation associated with upregulated brain arachidonic acid (AA) metabolism [22, 23]. Clinical studies support the idea that BD and depression involve a brain inflammatory response and disturbed AA metabolism [24-27]. Increased levels of PGE_2 are present in platelets, saliva, and CSF of depressed patients [28-32]. In addition, increased blood levels of proinflammatory markers, particularly C-reactive protein, soluble interleukin (IL)-2 receptor, IL-6 and tumor necrosis factor (TNF)-alpha, have been reported in BD mania [33], as have increased blood levels of markers of oxidative stress (superoxide dismutase activity and catalase activity, serum thiobarbituric acid reactive substances (TBARS)) [34] [35].

In vivo rodent [36-40] and human studies [41] suggest that the inhibition of AA metabolism and the COX-2 pathway may be a common mechanism of action of mood-stabilizing drugs [22, 23]. With previous support from the Stanley Medical Research Institute (PI: JC Soares), we completed a first double-blind controlled trial with celecoxib, a selective inhibitor of COX-2, as adjunctive treatment for bipolar depression and found a non-significant but potentially clinically meaningful effect for an early, but not sustained therapeutic response [42]. Together, these preclinical and clinical findings provide strong evidence that increased COX-2 mediated PG production is involved in the pathophysiology of mood disorders, and thus that inhibition of inflammatory mechanisms is a possible avenue for antidepressant response and mood stabilization.

Another NSAID known to target AA metabolism is aspirin, which is commonly used to treat peripheral inflammation and fever. Aspirin's effects are believed to be due to decreased production of pro-inflammatory prostaglandins (PGs) and thromboxanes (TXBs), due to inhibition of COX enzymes [43]. However, an additional antiinflammatory mechanism recently was proposed. Aspirin can acetylate COX-2, and the acetylated enzyme can convert DHA to 17-OH-DHA, an antiinflammatory agent that is a precursor of anti-inflammatory resolvins [44]. In this regard, aspirin may have a synergistic interaction with lithium, which was shown to increase the brain concentration of 17-OH-DHA and reduce brain concentrations of proinflammatory PGE2 and TXB2 in a rat neuroinflammation model [45].

Preliminary clinical studies suggest a therapeutic effect of aspirin in mood disorders. One study reported that aspirin ameliorated mood in males undergoing coronary angiography [46]. A retrospective clinical pharmacoepidemiological study reported that chronic aspirin reduced the incidence of clinical worsening in patients taking lithium [47]. Additionally, a randomized double blind placebo-controlled trial reported that adjuvant aspirin therapy (1000 mg/day, 12-weeks) reduced symptoms of schizophrenia spectrum disorders, in which neuroinflammation likely plays a role [48, 49].

Increased oxidative stress may be involved in pathophysiology of mood disorders [50]. Glutathione is a key substrate for antioxidant functions in cells. Its production is limited by its precursor, cysteine. N-Acetyl Cysteine (NAC) is the acetylated derivative of cysteine. It is more bioavailable. NAC has been shown to be neuroprotective [51]. If abnormalities in oxidative stress are key mechanisms involved in pathophysiology of mood disorders, it is compelling to hypothesize that effective antioxidant drugs will have effects in alleviating the symptoms or changing the course of the illness. Lithium and valproate have been shown to have important effects on glutathione-mediated processes [52]. In a clinical trial, lithium was shown to have effects on markers of oxidative stress in patients with bipolar disorder in the manic state [53]. Berk et al [54] reported a 24 wk double-blind placebo controlled trial with NAC

as adjunct to ongoing mood stabilizing medications in patients suffering from BD where there were significant effects on depressive symptoms. Recently, results of an open label study also revealed positive results on bipolar depression during a two month extension period [55]. Furthermore, these same authors demonstrated effects of NAC on certain symptomatic domains in patients with schizophrenia, primarily on negative symptoms [56].

In view of these compelling preclinical and clinical observations, a study to test whether aspirin and NAC will ameliorate BD symptoms is highly warranted. Our study will include measurements of cytokines, inflammatory factors and markers of oxidative stress **at baseline, at 8 wks and at 16 wks** to test the hypothesis that these are mediators or modulators of therapeutic response. Our proposed study will investigate the role of neuroinflammation and oxidative stress in antidepressant response in BD and could lead to the development of new therapeutic agents for patients that may enhance or replace failed responses to commonly used medications.

Specific Aims

We propose to conduct a double-blind placebo-controlled trial with a widely available and prototypical non-steroidal anti-inflammatory agent, aspirin, and an antioxidant agent, NAC, involving symptomatic BD type I and II patients having a depressive or mixed episode. This will be the first controlled study to test the hypothesis that aspirin and NAC, by themselves or in combination, will be beneficial in treating depression in bipolar disorder patients and in promoting mood stabilization. We propose to utilize a novel drop-the-loser (DTL) adaptive design that will be more cost-effective in screening new medication leads.

Our study has the following Aims: Aim I – Examine efficacy of aspirin in treating depression in bipolar patients in a double-blind placebo-controlled add-on design; Aim II – Examine efficacy of NAC in treating depression in bipolar patients in a double-blind placebo-controlled add-on design; Aim III – Examine efficacy of combined treatment with aspirin and NAC looking for synergistic, potentiating effects; Aim IV – Examine the role of markers of neuroinflammation, oxidative stress and cytokines as possible mediators or modulators in therapeutic response in the treatment of depression in patients with BD.

Need for efficient drug development studies

Most conventional, medication-development trials in psychiatry have employed standard, fixed, parallel-group designs, involving only a few drug conditions and very large sample sizes. Trials designed in this manner have been criticized for being exceedingly long, expensive, and often uninformative if initial drug or dose selection proved to be incorrect. Two ways in which the clinical trial enterprise may be enhanced are the implementation of adaptive clinical trial designs and the application of Bayesian statistical approaches in monitoring and analyzing clinical trials [57, 58].

Adaptive designs permit critical mid-trial design modifications, based on interim analyses, without undermining the validity and integrity of the trial [59]. Adaptations to the trial are pre-specified (by design); not made in an unplanned or ad hoc manner. These adaptations are aimed at providing (1) better treatment of patients by limiting exposure to non-effective drugs or drug combinations and increasing exposure to more effective drugs or drug combinations; (2) more efficient drug development; and (3) better use of available resources.

A drop-the-loser (DTL) adaptive design is useful in phase II clinical development especially when there are uncertainties regarding the most promising drug to which Phase III resources should ultimately be allocated [60, 61]. DTL designs permit multiple treatment arms allowing for the rapid assessment of candidate drugs or drug combinations. An interim analysis plan specifies the criteria for dropping doses that fail to show clinically meaningful efficacy over placebo. Conditions satisfying interim efficacy criteria are continued to completion. This adaptive pruning permits the randomization of remaining participants to the drug condition(s) which demonstrate the most promise. As a consequence, adaptive DTL designs permit initial evaluation of a broader range of drugs or drug combinations followed by increasing the precision of effect size estimates for the more promising treatment(s).

The recommended analysis strategy when using adaptive trials is Bayesian [57] which permits statements about the probability that treatment confers benefit. This probability is calculated by combining existing information,

represented by a prior distribution, with observed data, represented by the likelihood. This yields a posterior distribution from which statements about the probability of a given effect size can be made. As treatment outcomes accumulate over the course of the trial, posterior probabilities are used to estimate treatment effects and inform the pruning of less effective treatments based on a priori decision rules.

Designing an adaptive trial typically begins with a simulation study to address issues such as timing and frequency of interim analyses, and to understand the operating characteristics associated with the criteria for dropping conditions [57]. In keeping with the proposed DTL design, a Monte Carlo simulation study was conducted (see Statistical Analyses) to assess the relative merits of this design over a non-adaptive parallel-group approach. Simulation results, support the proposed DTL design in showing that under scenarios reflecting the hypothesized treatment effects, the adaptive design allocates more subjects to the more promising treatment conditions, i.e., appropriate "pruning" of less effective conditions. Using simulated data, the design performs well as demonstrated by adequate power and Type I error rates. Thus, employing the proposed DTL design is likely to yield the same conclusions as the classical fixed (non-adaptive) design, but with greater statistical precision for the most promising treatments.

Study Design

BD type I or II patients (n=160), on a depressive or mixed episode, who were on therapeutic doses of any of the commonly utilized mood stabilizing agents (lithium, anticonvulsants, atypical antipsychotics) for at least one month and who were still symptomatic (MADRAS >20) will be enrolled. Patients will be randomly assigned to orally receive one of 4 conditions: aspirin 1000 mg (2 capsules of 500 mg) qam, NAC 1000 mg (2 capsules of 500 mg) bid, combined aspirin and NAC at same doses as given individually or matched placebo, as an add-on medication to their ongoing treatment regimen, for an 16-week double-blind trial. After the first 8 week of the double blind treatment, the responders will keep taking the same study drug, whichever it is. And the non-responders will be re-randomized for one of the other three groups of study drugs they haven't tried yet.

Patient sample:

Inclusion criteria:

1) Age 18 to 65 years
2) A diagnosis of BD type I or II according to SCID-I interview;
3) Currently in a depressive or mixed episode, based on DSM-IV/ SCID-I criteria;
4) MADRAS >20 at entry in the study;
5) No CURRENT liver, kidney, heart disease or ulcers or bleeding dyscrasia;
6) No HYSTORY of kidney dysfunction or cardiac problems;
7) ON therapeutic doses of a mood stabilizing drug (lithium, anticonvulsants, any atypical antipsychotics)
8) Allowed psychiatric co-morbid conditions, such as anxiety disorders, PTSD and substance use (as long as do NOT meet abuse or dependence criteria according to the SCID-I in the past 2 months). Current Marijuana abuse is allowed during the study.

Exclusion criteria:

1. CANNOT be on any :

Anti-inflammatory: NSAIDs:

- Aspirin (bufferin, bayer aspirin, ecotrin),
- diflunisal (dolobid, diflunisal),

- Salsalate (amigesic, salflex),
- Ibuprofen (motrin, advil),
- Naproxen (naprosyn,aleve, midol extended relief),
- Fenoprofen (nalfon),
- Ketoprofen (actron),
- dexketoprofen(ketron D),
- Flurbiprofen (ansaid),
- Oxaprozin (daypro),
- Loxoprofen (loxfen, loxonin),
- Indomethacin (indocin, indocin SR),
- Silindac (clinoril), Etodolac (lodine),
- Ketorolac (toradol), diclofenac (voltaren, cataflan),
- Nabumetone (Relafen)
- Piroxicam (feldene),
- Meloxicam (mobic),
- Tenoxicam (mobiflex),
- Lornixicam (xefo),
- mefenamic acid (ponstel),
- meclofenamic acid (meclofenamate sodium),
- celecoxib (celebrex)

Anticoagulants:

- Coumadin (Warfarin), Heparin
- Anti-oxidant agents
- Fish oil
- NAC (N-acetyl cysteine)

2. Pregnancy
3. CANNOT change the dose of the psychotropic medications during the trial

We have set our inclusion criteria to allow maximal generalizability of the results to the aggregate group of patients with BD treated in common outpatient settings, so we will allow inclusion of patients with co-morbid conditions, substance abuse, and bipolar type II. The feasibility of the proposed study is facilitated by the **University of Texas (UT) Center of Excellence on Mood Disorders**, co-directed by Drs. Jair C. Soares and Alan Swann. Our group has

an established track record of enrolling BD patients for a multitude of clinical neuroscience and clinical psychopharmacology studies.

Study procedures:

The study will be carried out at the outpatient clinics affiliated with the UT Health Science Center at Houston. After signing informed consent, patients will be submitted initially to a SCID-I interview to confirm the psychiatric diagnosis, followed by a physical examination and routine labs (CBC, liver function tests, electrolytes, kidney functions tests, thyroid function tests, urinalyses) to rule out relevant medical problems. Physically healthy BD patients on psychotropic medications or combinations will be enrolled and randomly assigned to receive, orally, aspirin 1000 mg qam, NAC 1000 mg bid, combination of aspirin 1000 mg qam and NAC 1000 mg bid, or placebo. The aspirin dose will be started at 1000 mg q day, and will remain the same for 8 weeks. NAC will be administered in 500 mg capsules, two capsules p.o bid and dose will remain the same for the duration of the study. Patients can be on any mood stabilizing agents or combinations, as well as on other psychotropic medications at study entry, and the doses of those medications cannot be changed during the trial. They cannot be on any anti-inflammatory or anti-oxidant agents or anticoagulant at the point they are enrolled. If patients decompensate significantly, and/or become acutely suicidal, participation on the trial will be terminated.

If patients qualify for the study initially based on SCID-I interview, physical examination, and routine labs (CBC, liver functions tests, electrolytes, kidney function tests, thyroid function tests, HIV testing, and urinalyses), but are not currently taking an approved psychotropic medication or combinations they are eligible to participate in a lead-in phase. This phase would be starting at their screening visit and last approximately 6 to 8 weeks depending on the patient's mood. During this lead-in phase, either the PI or Co-PI will evaluate the patient and prescribe either Lithium or Depakote. The patient will be monitored during this treatment phase by the PI or Co-PI and will return after two weeks for an evaluation. The evaluation will include CSSR-S and adverse effects. If the patient is prescribed lithium, their lithium levels will be tested. The patient will continue to follow up every two weeks. At the 4 week visit, the patient will also come in for evaluation (CSSR-S and adverse effects). At the 6 week visit, the patient will be re-evaluated for the double-blind study trial. If the patient has been on an adequate dose of the psychotropic medication for at least 4 weeks and score greater than 20 on the Montgomery-Asberg Depression Rating Scale (MADRS), then they will be enrolled. If they do not meet inclusion criteria at the week 6 lead-in phase visit, they will return at Week 8 and be re-evaluated. PI and Co-PI will continue to prescribe the psychotropic medication throughout the lead-in phase (up to 8 weeks) and through the trial (up to 16 weeks).

During the lead-in phase participants will not be compensated. They will receive a voucher for their parking for each visit. They will be responsible for filling and paying for all prescriptions in both the lead-in phase and through the trial (up to 16 weeks).

Upon completion of the first 8-week double-blind trial, patients who responded to the active medication will continued on the same study drug for an additional 8 week double-blind treatment. The non-responders to one of the study drugs will be re-randomized for one of the other three groups of study not received and will remain in treatment for an additional 8 week double-blind treatment. Treatment response will be defined as improvement in the **MADRS scores of at least 50%.**

During the first 8-week trial, patients will be seen at weeks 0, 1, 2, 3, 4, 6 and 8 for clinical assessment and mood ratings. During the continuation trial, they will be seen at weeks 8, 10, 12, 14 and 16. The main outcome measure for the study will be the scores on the Montgomery-Asberg Depression Rating Scale, MADRAS [62]. All patients will also be assessed with the Young Mania Rating Scale, YMRS [63], the Clinical Global Impression-Bipolar version, CGI-BP [64], and the UKU side-effects scale [65]. In all visits, patients will be seen by the study psychiatrist and the research staff will complete mood ratings with the MADRAS, YMRS, CGI-BD, and the UKU side-effects scale.

At baseline (before randomization), week 8 and week 16 blood will be sampled for proinflammatory markers (C-reactive protein, soluble interleukin (IL)-2 receptor, IL-6 and tumor necrosis factor (TNF)-alpha), and oxidative stress

markers (superoxide dismutase activity and catalase activity, serum thiobarbituric acid reactive substances (TBARS)), which have been reported to be elevated in BD patients [34] [33] [35]. These blood samples will be stored for possible future research on biomarkers or future genetic studies for up to 20 years, for use in pharmacogenetic research during this 20 year timeframe. The samples will be stored at the Wet Lab, in a double locked -80 degrees freezer, at [redacted] for future use studying factors of Bipolar Disorder. When (or before) the 20 year period ends, the blood sample will be destroyed.

Patients will complete neuropsychological testing at baseline and Week 16. The testing will include CANTAB, WASI, and WRAT assessments and will take approximately 3-4 hours.

At the screening visit, week 8, and week 16 we will also be testing the Lithium levels in all patients that have been prescribed and currently taking the medication for study purposes. This extra test will be conducted for the safety of the patient and more Lithium level tests can be ordered under the PI's discretion throughout the 16 week trial.

Safety considerations:

Aspirin is a safe medication for administration to humans and is approved by the FDA as an anti-inflammatory and analgesic agent. The dose proposed for this study is within the safety range as per medication package. Its side-effect profile is overall very favorable and it has met widespread use worldwide over the past decades. The main safety issues relate to the possibility of gastrointestinal complications, which are rare (and will be closely monitored in the weekly or biweekly visits that the study requires), and the propensity to interfere with coagulation and cause consequent bleeding (which will be prevented by excluding any individuals on use of anticoagulants or with active bleeding problems). There were a few case reports and small case series with other non-steroidal anti-inflammatory agents suggesting worsening of depressive symptoms related to treatment (e.g., indomethacin, ibuprofen, naproxen), but those were uncontrolled reports with individuals who suffered from other medical illnesses (rheumatologic diseases) [66] [67]. For our proposed trial, because some patients may be on lithium at the time they start on aspirin, we will exclude individuals with pre-existing cardiac and kidney disease. For patients on lithium, we will carefully monitor their serum lithium levels every two weeks during the course of the trial. Therefore, at the doses that are being proposed we do not anticipate any significant problems related to safety of the proposed intervention.

NAC is a safe compound that is commonly available and utilized over-the-counter. In a recent study [54] the tolerability was excellent and main side effects included changes in energy level, headaches, heartburn and joint pain.

Blood Draws: When the blood is drawn, there may be some minimal discomfort and/or bruising. Infection, excess bleeding, clotting, or fainting is also possible, although unlikely. All usual precautions will be taken to prevent these possibilities and these risks will be minimized by using trained staff to perform the blood draws.

Women Able to Become Pregnant: Participation in this study may involve risks to an embryo, fetus, or unborn child. If the subject is a female and able to become pregnant, a urine pregnancy test will be performed which must be negative prior to enrolling into the study, and the subject must agree not to become pregnant during the study. Urine pregnancy tests will be performed at Screening visit and week 8. The study staff will review adequate birth control methods with the subject and will remind her that she should not become pregnant during the study. Appropriate methods of birth control include: hormonal contraceptives (such as birth control pills, patches, and implants), barrier methods (such as a condom and diaphragms and spermicidal foam or jelly, surgical (hysterectomy or tubal ligation) or intrauterine device (IUD). The subject will be instructed to notify the study doctor immediately if there is a chance that she has become pregnant.

Also, if the subject is breast-feeding an infant or plan on breast-feeding an infant, she must notify the study doctor. It is not known if this drug is excreted in human milk; therefore, breast-feeding is not permitted during the study.

Breach of Confidentiality: Every effort will be made to protect the subject identity and information during the study. All lab work and scan reports will be de-identified and the medical records will be protected. However, there is a small chance that the subject information may be viewed by someone that is not involved in the research study.

The study drug must be taken only by the person for whom it has been prescribed, and it must be kept out of the reach of children or persons of limited capacity to read or understand.

By sharing your sample with the study doctor, there is a risk of the possible loss of the subject privacy. Although no identifiable (name, address, etc.) information will be shared with others outside of this research project. The clinical information obtained from subjects will be part of their medical records and maintained at UT Center of Excellence on Mood Disorders, in the Department of Psychiatry at UTHSC-H, in facilities with adequate safeguards for the protection of confidentiality. The research data will be collected and recorded using only arbitrary code numbers for identification, in order to safeguard the confidentiality. All data will be kept in a secure area. Only the members of the UT Center of Excellence on Mood Disorders research group, who will get approval from CPHS, will have access to the data files, or to the master list for the codes. For publications purposes, the patients will be designated only by their assigned codes.

These blood samples, for use in pharmacogenetic research during this 15 year timeframe, samples will be stored at the Wet Lab, in a double locked -80 degrees freezer, at [redacted]

The possibility exists that the subject information may be taken and used for reasons outside of this project. The United States' Genetic Information Nondiscrimination, Act (GINA) of 2008, does not allow for employers or health insurance companies to discriminate against the research subject based on his/her genetic information. This means that health insurance companies or employers are NOT ALLOWED BY LAW to ask or use any of the subject genetic information (DNA, RNA, etc.) gained through testing to make decisions that affect the subject or hisher family's health coverage or income in a negative way.

Statistical analyses:

Simulation Study

In preparation of this application we conducted a Monte Carlo simulation study to assess the relative merits of the proposed DTL adaptive design over a non-adaptive, parallel-group approach. Specifically, the goal of this study was to demonstrate that the DTL design would result in the maximum number of subjects being treated in the most promising conditions, i.e., appropriate "pruning" of less effective conditions. Simulation scenarios including one that posits no effect across conditions and others, assuming specific anticipated effects, permits evaluation of Type I and Type II Error rates respectively. Sample size for each simulated trial was set at n = 160. Response to treatment was defined, dichotomously, as > 50% decrease in depression score as measured on the MADRS. Specification of effects for the simulations assumed that the rate of response in the placebo condition is 25%, while 1000 mg aspirin qd and 1000 mg NAC bid have comparable effects with response rates of 50% each. The combination of 1000 mg aspirin qd and 1000 mg NAC bid is anticipated to demonstrate an additive effect yielding a 70% response rate. At 50% information gathering, defined as n = 80 subjects having completed the study, a Bayesian interim analysis determined the posterior probability that treatment conferred benefit. Benefit was defined as the absolute difference in response rate for each treatment relative to placebo. The remaining 80 subjects were allocated to the retained (non-pruned) treatments, with the probability of assignment to any given group based on the number of groups continuing in the study.

Pruning/Retention Rules. Simulation scenarios considered a variety of pruning rules that varied in stringency for 1) detecting a clinically decisive difference (>10% from placebo), and 2) selecting the best performing treatment. All simulations assumed a ~Beta(1,1) prior distribution for the probability of treatment response in all study conditions. A Beta(1,1) prior models values within the range [0,1] in which all values have the same probability of occurring. This constitutes a vague, neutral prior distribution which reflects uncertainty preceding data accrual. At 50% of data gathering (n = 80) the prior (i.e. ~Beta(1,1)) was updated with data generated from a binomial distribution for each

condition. The posterior distributions of the differences between each treatment and placebo formed the basis of pruning decisions. A decisive difference is defined as occurring if the posterior probability of there being a <10% difference between any of the treatments and placebo was < 0.20. Another way of saying this is that if there was a >80% chance that the all treatment-placebo differences were <10% then the simulation concluded that the trial had failed to find a decisive difference. If this first rule identified the existence of a decisive difference for any of the treatments, the algorithm then selected the treatment that had the highest posterior probability of conferring benefit (i.e. the p(treatment-control > 0|data) for participants. If the algorithm failed to identify a decisive treatment difference, the 1000 mg aspirin condition was retained as the active treatment. This was done to further our established programmatic goal of evaluating the anti-inflammatory effects of aspirin on depression by providing the most precise effect size estimate possible.

Study performance in terms of maximizing patients allocated to the best-performing conditions and for Type I and Type II Error rates are reported for the design under these retention/pruning criteria.

Simulation of Pruning/Retention Rules. Simulation evaluated the pruning rules for three scenarios. Scenario1 simulated clinical trials with binomial probabilities of response of 0.25, 0.50, 0.50, and 0.70 for placebo, aspirin, NAC and aspirin + NAC respectively. Scenario 2 addressed the situation in which the effect sizes are less optimistic. Scenario 2 posited binomial probabilities of response of 0.25, 0.30, 0.30, and 0.50 for placebo, aspirin, NAC and aspirin + NAC respectively. Scenario 3 simulated clinical trials with binomial probabilities of response of 0.25, 0.25, 0.25 and 0.25 for placebo, aspirin, NAC and aspirin + NAC respectively. Since this last scenario simulates data in which there is no effect, the probability of identifying a decisive effect is an estimate of the Type I Error Rate for the pruning algorithm.

Simulation of Operating Characteristics. Although Bayesian statistical approaches offer a more efficient means by which to weigh evidence, it is still essential to estimate the probability of making specific types of errors using the current design. We first evaluated, over the course of k = 200 simulated trials with the anticipated effects, how often the design fails to find the posited effect. This yields the equivalent of the Type II Error Rate, with one minus this value corresponding to the power of the design. For Scenario 1, k=200 simulated clinical trials indicated that the probability of identifying a decisive difference was >99%. Including trials for which the algorithm failed to identify a decisive difference as Type II Errors, the selection of the best performing treatment based on the highest posterior probability of benefit identified the combination 82.5% of the time. For Scenario 2, k=200 simulations suggested that the probability of identifying a decisive difference was 98.5%. Counting trials for which the algorithm failed to identify a decisive difference as Type II Errors the selection of the best performing treatment based on the highest posterior probability of benefit identified the aspirin + NAC combination 83% of the time. This corresponds to 84.5% power. Since Scenario 3 simulates data in which there is no effect, the probability of identifying a decisive effect is an estimate if the Type I Error Rate for the pruning algorithm. This final scenario identified a decisive effect in >0.5% of simulated trials. This corresponds to a Type I Error Rate of 0.005.

If N = 160 participants are randomized across four conditions in a 1:1:1:1 ratio, contrasts for treatment response rates versus placebo will use n = 40 participants per cell. For Scenario 1, assuming n = 40 per group and alpha = 0.05, power to detect the difference between the proportions 0.25 and 0.50 is 65%. Using the same assumptions power to detect the difference between the proportions 0.25 and 0.70 is >99%. For Scenario 2, assuming n = 40 per group and alpha = 0.05, power to detect the difference between 0.25 and 0.5 is 65%. More generally, for comparison of two independent proportions, sample sizes of n = 40 per group and alpha = 0.05 provide 80% power to detect a difference between 0.25 and 0.55. Such a sample is underpowered for two of the effects in Scenario 1, and all of the effects in Scenario 2. Recall, however that the proposed Bayesian adaptive design results in altered cell sizes such that the most promising treatment and placebo each receive an average of n = 60 participants. (This is substantiated by the simulation results). Assuming n = 60 participants per group and alpha = 0.05, power to detect the difference between 0.25 and 0.50 is 81.80%, while power to detect the difference between 0.25 and 0.70 is > 99%. More generally, a sample of this size provide 80% power to detect the difference between 0.25 and 0.49 with alpha = 0.05.

In summary, a Bayesian adaptive approach demonstrates adequate operating characteristics (Type I and II Error Rates) for the current trial. The method results in allocation of more participants to the most effective condition. Finally, the approach permits frequent analyses with more desirable Type I and Type II Error rates.

General Data Analytic Strategies

All statistical analyses will use SAS v. 9.2 (SAS Institute Inc., Cary, N.C.). Preliminary data analyses inspecting group differences on demographic and baseline variables will evaluate posterior distributions for differences in proportions and means, as well as for correlations between baseline variables and specified outcomes. Baseline or demographic variables for which the posterior distribution indicates there is > 99% chance of a difference across groups, and for which there is a > 99% chance of a non-zero correlation with outcomes, will be defined as potential confounders and will result in two sets of analyses: one in which the relevant variable is included as a covariate and one in which it is not. This will permit determination of the degree to which any group differences might confound conclusions regarding treatment.

Evaluation of the primary outcome, proportion of patients demonstrating > 50% decrease in depression scores on the MADRS, as a function of treatment will use a beta-binomial model and contingency tables with chi-square testing for Bayesian and Frequentist approaches respectively. Additional analyses will use the generalized linear modeling. Count, dichotomous and continuous Bayesian analyses will be conducted using Poisson (Proc GENMOD; SAS 9.2), logistic (Proc GENMOD; SAS 9.2), linear (Proc GENMOD; SAS 9.2) regression respectively. Distributional assumptions will be evaluated via inspection of residual plots and, where possible, by formal statistical tests. Violation of assumptions will be addressed, depending upon statistical technique, through the use of transformations, robust methods, and stratification.

Unless otherwise indicated in the data analytic plan, priors will be specified as neutral and diffuse. The beta-binomial model will use a ~Beta(1,1) prior distribution. For linear, Poisson, and logistic regression, priors will take the form ~N(0, 1 x 106) in the linear, log, and log(odds) scales respectively. Convergence of Bayesian analyses on the posterior distributions via Monte-Carlo Markov chain (MCMC) will be assessed via graphical (Trace Plot, Autocorrelation Plot) and quantitative (Geweke Diagnostics, Gelman-Rubin Diagnostics, and Heidelberger-Welsh Diagnostics) evidence. Evaluation of posterior distributions will permit statements regarding the probability that effects of varying magnitudes exist, given the data.

Interim Bayesian analysis with application of pruning/retention rules will be conducted when 50% of randomized subjects have reached (or should have reached) the end of study. If, at 50% of data gathering, there are additional participants continuing in active treatments that might be pruned, sensitivity analyses will inspect the degree to which potential results from these active patients might alter the conclusions of the interim analysis. If necessary, randomization will be temporarily suspended and data gathering will continue until sensitivity analyses demonstrate that statistical conclusions will remain unchanged by any patients who have not yet completed treatment. While Frequentist approaches require adjustment for multiple testing when conducting interim analyses, no such adjustment is necessary for Bayesian analyses due to its conformity to the likelihood principle [57]. Futher, Monte Carlo simulations (conducted in SAS v. 9.2) demonstrate that Type I and Type II Error Rates are at appropriate levels for the proposed design (see Simulation Study). Intention to treat principles will apply at both the interim and final analysis points; any patients having dropped out of the study will be counted as non-responders.

Structural Equation Modeling/Path Analysis will evaluate the degree to which inflammatory cytokines and markers of oxidative stress mediate the effect of treatment on MADRAS. Estimation of SEM/Path Models will employ both Frequentist and Bayesian approaches. In the context of the direct effects of treatment, mediational modeling will permit estimates of the indirect effects of treatment on MADRAS scores via inflammatory cytokines or markers of oxidative stress using the product coefficient method [68]. Frequentist ninety-five percent confidence intervals for maximum likelihood estimates will be constructed using a bootstrap resampling approach [69]. Bayesian SEM estimates for indirect effects will employ the posterior distribution of the product coefficient. All SEM/Path analyses will be conducted in MPlus v. 6.0 [70].

Significance:

This study will be the first controlled trial to test the efficacy of aspirin and NAC, compounds with potentially new mechanisms of central action in the treatment of BD patients on a depressive or mixed episode. It will test an innovative clinical trial design that we hope will make screening of possible new medication leads and these trials more cost efficient. It will examine possible novel mechanism of actions with modulation of inflammatory mechanisms and oxidative stress and could result in the development of novel, low cost, safe and widely available treatments for BD patients who have not responded to other commonly utilized alternatives.

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