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MINOCYCLINE AND ASPIRIN IN THE TREATMENT OF BIPOLAR DEPRESSION: a randomized, double-blind, placebo- controlled, parallel-group clinical trial

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ABSTRACT

Introduction: New medication classes are needed to improve treatment effectiveness in the depressed phase of bipolar disorder (BD). Extant evidence suggests that BD is characterized by neural changes such as dendritic remodeling and glial and neuronal cell loss. These changes have been hypothesized to result from chronic inflammation. The principal aims of the proposed research is to evaluate the antidepressant efficacy in bipolar depression of minocycline, a drug with neuroprotective and immune-modulating properties, and of aspirin, at doses expected to selectively inhibit cyclooxygenase 1 (COX-1). **Methods and Analysis:** One hundred and twenty outpatients between 18 and 55 years of age, who meet DSM-IV-TR criteria for BD (type I or II) and for a current major depressive episode will be recruited to take part in a randomized, double-blind, placebo-controlled, parallel-group clinical trial following a 2 x 2 design. As adjuncts to existing treatment, subjects will be randomized to receive one of four treatment combinations: placebo-minocycline plus placebo-aspirin, active-minocycline plus placebo-aspirin, placebo-minocycline plus active-aspirin, or active-minocycline plus active-aspirin. The dose of minocycline and aspirin is 100mg bid and 81mg bid, respectively. Antidepressant response will be evaluated by assessing changes in the Montgomery-Asberg Depression Rating Scale (MADRS) scores between baseline and the end of the 6 week trial. As secondary outcome measures, the anti-inflammatory effects of minocycline and aspirin will be tested by measuring pre-and-post treatment levels of CRP and inflammatory cytokines. **Ethics and Dissemination:** Minocycline has been widely used as an antibiotic in doses up to 400 mg/day. Low dose aspirin has been safely used on a worldwide scale for its role as an anti-thrombotic and thrombolytic. The study progress will be overseen by a Data, Safety and Monitoring Board which will meet once every 6 months. Results of the study will be published in peer-reviewed publications. **Registration:** Clinical Trials.gov: NCT01429272.

INTRODUCTION

The treatment of bipolar depression remains a major challenge for psychiatry. The US FDA has not approved any of the ~25 standard antidepressants for the treatment of bipolar depression, partly because these agents have not been robustly effective in BD patients¹. Thus, currently approved treatments for bipolar depression include lithium, quetiapine, and the combination of olanzapine and fluoxetine². Other treatments used include lamotrigine, conventional antidepressant agents, other atypical antipsychotics, pramipexole or riluzole (reviewed in ³). Unfortunately, the effectiveness of these options also is limited. For example, in a placebo-controlled study in which subjects receiving lithium were randomized to receive either standard antidepressant pharmacotherapy (paroxetine or imipramine) or placebo, those receiving lithium plus an antidepressant did not show a significant improvement over those receiving lithium plus placebo⁴. Similarly, in the STEP-BD trial, 42 of 179 subjects (23.5%) receiving a mood stabilizer plus adjunctive antidepressant drug treatment had a durable recovery, which did not differ significantly from 51 of 187 subjects (27.3%) receiving mood stabilizer plus placebo. Mallinger et al. reported a similar durable recovery rate in BD depressives treated with mood stabilizer plus paroxetine (27%), but found a higher rate for adjunctive monoamine oxidase inhibitors (MAOIs; 53%)⁵, consistent with the findings of previous studies comparing MAOIs vs imipramine^{6,7}. Unfortunately MAOIs are commonly unacceptable to patients.

New classes of antidepressant drugs are needed for bipolar depression. Existing agents exert their primary actions on monoaminergic systems. The efficacy of these agents contributed to the monoamine-deficiency hypothesis of depression, which continues to receive empirical support. Nevertheless, the field is in the early stages of a paradigm shift driven by evidence of dendritic remodeling and neuronal atrophy in animal models of depression, and of reductions in gray matter (GM) volume, and glial cell loss at *postmortem* in BD⁸. The neurotrophic effects of lithium, coupled with longitudinal studies demonstrating volumetric changes over time, raise the possibility that mood disorders are underpinned by a neurotoxic process^{8,9}. The final common pathway through

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2
3 which neurotoxic agents exert their effect is hypothesized to involve excess glutamatergic
4 signaling¹⁰.
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9 The glutamatergic model of mood disorders is based on the premise that excessive
10 stimulation of NMDA-glutamatergic receptors, results in neuronal atrophy and apoptosis
11 of glial and/or neuronal cells, and *ipso facto*, depression. Evidence for this hypothesis
12 derives from multiple sources. In preclinical models, riluzole, which inhibits neuronal
13 release of glutamate, ceftriaxone, which increases glutamate reuptake, and NMDA
14 receptor antagonists such as ketamine, ameliorate behavioral analogs of depression¹¹. In
15 addition, rats bred to be genetically sensitive to stress show differential expression of
16 NMDA receptors¹², and behavioral analogs of depression are abrogated in NMDA
17 receptor subunit knockout mice¹³. In humans, increased serum levels of glutamate that
18 resolve with antidepressant treatment were reported in MDD, and extended to the CSF
19 post mortem¹¹. Polymorphisms of the metabotropic glutamate receptor genes, GRM2 and
20 GRM3, and a haplotype of the glutamic acid decarboxylase (GAD2) gene were
21 associated with MDD¹⁴. Finally, ketamine induced a rapid, sustained antidepressant
22 effect in BD^{15 16} and riluzole showed promising results in treatment-resistant depression¹⁵
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One potential cause of the disruption in glutamatergic signaling in BD is dysregulation of
the immune system. Increased levels of proinflammatory cytokines such as interleukin 6
(IL-6), IL-1 β , interferon alpha (IFN α), tumor necrosis factor alpha (TNF- α)
prostaglandinE2 (PGE2), and chemokine ligand 2 (CCL2) are consistently observed in
the blood and CSF of patients with mood disorders, both at baseline and after exposure to
stressors^{17 18}. Elevated serum levels of (pro-inflammatory) positive acute-phase proteins
(e.g., haptoglobin, α 1-antitrypsin, ceruloplasmin, C-reactive protein), but reduced levels
of negative acute-phase proteins (e.g., albumin and retinal-binding protein) also are
reported in mood disorders¹⁹⁻²¹. Further, treatment of hepatitis C with IFN α is known to
induce the major depressive syndrome and/or manic symptoms in a significant minority
of patients, and the efficacy of conventional antidepressant drugs is associated with a
reduction in inflammation¹⁸. Moreover, anti-tumor necrosis factor (TNF) therapy (for

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3 psoriasis) can improve mood²². Since proinflammatory cytokines can alter brain function,
4 these data are compatible with evidence that an activated inflammatory response system
5 exists in mood disorders which plays a role in their pathophysiology²³⁻²⁶.
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10 The over-activity of the hypothalamic-pituitary-adrenal axis in mood disorders may play
11 a role in inflammation, since hypersecretion of corticotrophin-releasing hormone (CRH)
12 activates the transcription factor, nuclear factor kappa B (NF-κB). NF-κB regulates the
13 expression of proinflammatory cytokines in immune cells in the CNS and periphery, and
14 the expression of genes involved in apoptosis²⁷. In addition, NF-κB may result in the
15 expression of the class 1 major histocompatibility complex (MHC I), labeling cells for
16 removal by cytotoxic T-cells²⁷. Usually, cortisol suppresses this inflammatory response,
17 but chronic stress appears to desensitize the glucocorticoid receptor (GR) and by
18 extension, the anti-inflammatory effects of cortisol²⁷. Cytokines play a role in
19 desensitizing the system to cortisol. For example, IL1 and TNF-α retard dexamethasone-
20 induced translocation of the GR receptor from the cytoplasm to the nucleus²⁸.
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31 The immunologic and glutamatergic models of BD are complementary because a
32 proinflammatory state is one potential cause of excitotoxicity²⁷. Peripheral inflammatory
33 signals activate microglia in the brain, inducing an inflammatory cascade of cytokines
34 and free radicals. Cytokines and reactive oxygen and nitrogen species exert a direct toxic,
35 apoptotic effect on oligodendrocytes. Potentially through the loss of oligodendrocytes,
36 oxidative stress can lead to demyelination. Such a process conceivably may account for
37 the reduction in oligodendroglia found *postmortem* in the prefrontal cortex²⁹ in mood
38 disorders. The inflammatory milieu also compromises astrocyte function, leading to
39 down-regulation of glutamate transporters and impaired glutamate reuptake into
40 astrocytes, further amplifying inflammatory signaling²⁷.
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51 In addition, cytokines such as interleukin 1 (IL-1), IL-6, and TNF-α activate indoleamine
52 2, 3-dioxygenase (IDO). IDO catalyzes the breakdown of tryptophan, the amino-acid
53 precursor of serotonin, and an important regulator of T-cell function, into kynurenine
54 (Kyn)³⁰. Activation of the Kyn pathway shunts tryptophan away from 5-HT synthesis,
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3 putatively reducing serotonergic transmission. Kyn is in turn metabolized into quinolinic
4 acid (Quin), a potent NMDA receptor agonist, and neuromodulator involved in lipid
5 peroxidation, which can induce neuronal damage via oxidative stress and overstimulation
6 of NMDA receptors³⁰. Consistent with inflammation-related shunt towards Kyn
7 metabolism, the plasma tryptophan-Kyn ratio was found to correlate inversely with
8 striatal total choline (a putative cell membrane turnover biomarker) in adolescents with
9 melancholic depression³¹.
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18 The mRNA transcripts for proinflammatory genes appear particularly sensitive for
19 discriminating BD patients. Microarray gene expression profiles in purified CD14+
20 monocytes from whole blood of BD subjects, offspring of BD parents, and healthy
21 controls (HC) displayed a distinct mRNA signature representing genes from
22 inflammatory and inflammation-related pathways³². The signature showed >80%
23 sensitivity and specificity in BD subjects who were not receiving lithium or antipsychotic
24 drugs (n=11), and in affected offspring of a BD parent (n=13, of whom 10 had only
25 manifested depression). A positive signature also was present in 17 of 38 unaffected
26 offspring (45%) versus 13 of 70 healthy children (19%). Cross-sectional comparisons
27 suggested lithium and antipsychotic drugs—but not conventional antidepressant drugs--
28 down-regulated expression of most inflammatory genes. Thus, when medicated and
29 unmedicated subjects were considered together only 23 of 42 BD patients (55%) had a
30 positive signature versus 7 of 38 HCs (18%). Notably, the IL6 mRNA level remained
31 elevated in medicated BD subjects and did not differ significantly from unmedicated
32 subjects (table 1), suggesting that this assay identifies a proinflammatory diathesis even
33 in treated cases.
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Table 1: Magnitude of difference in mRNA expression between mood disordered and healthy control (HC) samples from Padmos et al.³², showing selected transcripts in unmedicated subjects vs HCs, relative to that of medicated BD subjects.

Gene Symbol	Unmedicated BD vs HC		Medicated BD vs HC		Affected offspring# vs HC	
	fold change	p-value	fold change	p-value	fold change	p-value
PDE4B	13.73*	<.001	3.42	<.001	5.79	<.001
IL6	37.92	.005	9.56	.006	935.7	<.001
CCL20	55.49	.006	6.02	.10	400.1	<.001

Legend: * - difference significant between unmedicated vs medicated BD samples; # - affected with respect to having manifested either a depressive or a manic episode
 Sample sizes: unmedicated BD n=11, medicated BD n=31, affected offspring n=13, HCs n=25 for comparisons against BD adults, n=70 for comparisons of offspring. Abbrev: BD – bipolar disorder; HC – healthy control; PDE4B - phosphodiesterase type 4B; IL6 - interleukin 6; CCL20-chemokine ligand 20

Minocycline is a second-generation tetracycline that may prevent both glutamate-induced excitotoxicity and cytokine-induced inflammation in the CNS and periphery.

Minocycline has high lipophilicity enabling efficient transfer across the blood brain barrier (BBB)³³ - its concentration in CSF reaches 11–56% of plasma concentrations³⁴. Minocycline inhibits the microglial-mediated release of proinflammatory cytokines IL-1 β , TNF- α , IL-6, and p38³⁵, while promoting release of the anti-inflammatory cytokine, IL-10³⁴. Moreover, minocycline inhibits matrix metalloproteinases which process

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3 cytokines such as TNF- α and IL-1 β into their biologically active forms³⁵. Minocycline is
4 also an effective scavenger of proapoptotic reactive oxygen species and protects against
5 excitotoxicity by preventing glutamate-induced activation of nitric oxide synthase³⁶.
6 Nitric oxide facilitates glutamate release from presynaptic neurons and inhibits glial
7 glutamate transporters, amplifying glutamatergic signaling, and contributing to
8 excitotoxic cell death¹⁰. Minocycline also upregulates a key molecular factor in the
9 apoptosis pathway, B-cell CLL/lymphoma 2 (BCL-2)³⁷, an effect shared by lithium,
10 valproate³⁸ and certain antidepressant drugs³⁹. BCL-2 represses apoptosis induced by
11 cytotoxic insults⁴⁰.
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21 **Minocycline has neuroprotective and anti-inflammatory properties.**

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24 Minocycline prevents glutamate-induced apoptosis of neurons *in vitro*⁴¹, prevents
25 ischemia-induced activation of microglia in gerbils⁴², increases hippocampal neuron
26 survival⁴³, reduces lesion-volume and improves neurological function in mice with
27 traumatic brain injury⁴⁴ and in fragile X syndrome⁴⁵, reduces pro-inflammatory cytokine
28 expression and improves neurological function and locomotor activity in rats with spinal
29 cord injury⁴⁶, attenuates MDMA-induced neurotoxicity of serotonin and dopamine
30 systems in the cerebral cortex and hippocampus of mice⁴⁷, reduces inflammation in a rat-
31 model of rheumatoid arthritis (RA)⁴⁸, and delays disease progression and demyelination
32 in rodent models of encephalitis⁴⁹, amyotrophic lateral sclerosis (ALS)⁵⁰ and
33 Huntington's Disease (HD)⁵¹. Based on these data, minocycline was employed, and has
34 shown promise as, a therapeutic agent in human diseases including HD⁵², rheumatoid
35 arthritis (RA)⁵³, and stroke⁵⁴.
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48 **Minocycline has been used to treat psychiatric disorders.**

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51 Miyaoka et al.⁵⁵ discussed 2 patients with catatonic schizophrenia who benefited from
52 minocycline. This group then conducted a 4-week trial with minocycline (150 mg/day) in
53 22 patients with schizophrenia to evaluate its efficacy as an adjunct to antipsychotic
54 drugs⁵⁶. Patients showed a significant improvement in positive and negative symptoms.
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3 Levkovitz et al.⁵⁷ recently studied 54 patients with early-stage schizophrenia treated for 6
4 months with antipsychotic medication and either minocycline (200 mg/day) or placebo in
5 a double-blind trial. Minocycline was associated with a reduction in negative symptoms
6 and improved attention/ memory.
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12 The efficacy of minocycline has not been formally tested in mood disorders. In rodents,
13 minocycline reduced immobility during the forced-swim test⁵⁸, and co-administration of
14 minocycline synergized the antidepressant-like actions of desipramine (but not
15 fluoxetine)⁵⁹. Minocycline also abrogated the depression-like behavior of rodents
16 exposed to lipopolysaccharide (LPS)⁶⁰. Levine et al.⁶¹ presented the case of a 66-year old
17 woman with severe BD, who observed that the tetracycline she took for an infection
18 alleviated her depression. When her depression returned post-treatment, minocycline was
19 reinitiated (150 mg/day). After one week her HAM-D score fell from 25 to 8.
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28 **Aspirin (Acetyl-salicylic acid, ASA) also holds potential efficacy in bipolar disorder.**

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32 The second aim of this study is to assess the antidepressant efficacy of ASA in bipolar
33 depression. Using a 2 x 2 design we will obtain data providing estimates of the effect size
34 of ASA relative to placebo, ASA relative to minocycline, and ASA in combination with
35 minocycline relative to placebo. These data also will explore the specificity of any effect
36 found for minocycline. The clinical use of low dose ASA primarily has been driven by its
37 role as an anti-thrombotic and thrombolytic. Given the exaggerated death rate from
38 cardiovascular events in BD, this action potentially is advantageous in the management
39 of BD. Nevertheless, the recent literature also supports a role for low dose ASA in the
40 management of the mood disorder itself, specifically in the amelioration of depressive
41 symptoms.
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51 The mechanism of ASA relates to its capacity to inactivate irreversibly the
52 cyclooxygenase (COX) activity of prostaglandin (PG) H-synthase-1 and PGH-synthase 2
53 (referred to as COX-1 and COX-2, respectively). Although ASA has a short half-life (15
54 to 20 min) ASA's permanent inhibition of COX-1 allows once daily dosing for anucleate
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platelets. In contrast, because nucleated cells rapidly regenerate this enzyme a shorter dosing interval is required to persistently impact COX activity in cells that mediate inflammatory processes. Moreover, ASA is 50- to 100-fold more potent in inhibiting platelet COX-1 than monocyte COX-2 activity⁶², so there is nearly a 100-fold variation in the daily dose of aspirin, as higher doses are used to target COX-2 in the management of treating peripheral inflammation (e.g., arthritis) or pain. As reviewed below, preliminary evidence obtained in BD suggests beneficial effects are achieved using ASA in low doses, where aspirin would inhibit COX-1, but not COX-2.

Aspirin has neuroprotective and anti-inflammatory properties.

In the brain, recent data indicate that genetic manipulation of COX-1 and COX-2 differentially modulate leukocyte recruitment during neuroinflammation, and suggest that reduction of COX-1 activity is neuroprotective, whereas reduction in COX-2 activity is detrimental, during a primary neuroinflammatory response (reviewed in ⁶³). Choi et al.⁶³ propose that these distinct roles reflect the predominant localization of COX-1 in microglia, which play a major role in mediating neuroinflammation, in contrast to the predominant localization of COX-2 in pyramidal neurons. For example, Choi et al.⁶⁴ examined the effects of COX-1 or COX-2 deficiency on intracerebroventricular lipopolysaccharide (LPS)-induced neuroinflammation by comparing COX-1 (-/-) and COX-2 (-/-) knockout mice to wild-type (WT) (+/+) control animals. After LPS, leukocyte infiltration and inflammatory response were attenuated in the COX-1 (-/-) mice but increased in the COX-2 (-/-) mice, compared with WT controls. In another study, Choi et al.⁶⁵ examined the effect of COX-1 genetic deletion on the inflammatory response and neurodegeneration induced by β -amyloid, and found that in COX-1 (-/-) mice, the A β ₁₋₄₂-induced inflammatory response and associated neuronal damage were attenuated compared to WT mice. Compatible with these results, in pharmacoepidemiological studies investigating whether chronic NSAID use reduced the risk of developing Alzheimer's disease (AD), indomethacin, a preferential COX-1 inhibitor, showed beneficial effects, while COX-2 selective inhibitors, failed to show any beneficial effect in AD patients with mild to severe cognitive impairment. These data

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3 suggest the hypothesis that inhibition of COX-1 activity may be a valid therapeutic
4 strategy to reduce the cerebral inflammatory response and neurodegeneration in
5 neuropsychiatric diseases in which neuroinflammatory components play a role in
6 pathophysiology.
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12 Other researchers hypothesized that NSAIDs would be beneficial in BD more specifically
13 because of their ability to down-regulate activity in the brain arachidonic acid (AA)
14 cascade by via interfering with phospholipase A2 (PLA2) and/or COX function. In
15 rodents Rapoport and colleagues⁶⁶⁻⁶⁸ demonstrated that conventional mood stabilizers
16 decrease the AA turnover in phospholipids and the expression of PLA2 and/or COX
17 enzymes. The PLA2 and COX enzymes catalyze, respectively, release of AA from
18 membrane phospholipid and AA conversion to eicosanoids such as prostaglandin E2 and
19 thromboxane B2. The AA cascade is involved in neuroreceptor-initiated signaling and
20 can be pathologically upregulated by neuroinflammation and excitotoxicity.
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30 Nevertheless, aspirin has additional mechanisms that may underlie benefits in
31 neuropsychiatric illness. While low-dose aspirin down-regulates AA cascade activity via
32 inhibition of COX-1 activity, in higher doses it also down-regulates COX-2 gene
33 transcription, increases levels of lipoxigenase-derived eicosanoids such as the anti-
34 inflammatory lipoxin A4, and acetylates COX-2 protein to a modified enzyme that can
35 convert unesterified AA to anti-inflammatory mediators such as 15-epi-lipoxin A4
36 (reviewed in ⁶⁹). The acylated enzyme also can convert docosahexaenoic acid (DHA) to
37 17-(R)-OH-DHA, which, like its metabolites di(R)-OH-DHA (neuroprotectin (R) D1)
38 and tri(R)-OH-DHA (resolvin (R) D1), is highly anti-inflammatory (reviewed in ⁶⁹).
39 Lithium given chronically to rats with lipopolysaccharide-induced neuroinflammation
40 also increases the brain concentration of 17-OH-DHA. Thus, there may be a synergy
41 between aspirin and lithium in forming anti-inflammatory brain DHA metabolites.
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53 **Aspirin appears effective in preliminary studies of mood disorders.**
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Pharmaco-epidemiological data in BD supportive of these hypotheses were published by Stolk et al.⁶⁹. Using the Netherlands based PHARMO Record Linkage System (which connects pharmacy dispensing records to hospital discharge records of > two million individuals since 1985), these researchers tested whether non-steroidal anti-inflammatory drugs (NSAIDs) or glucocorticoids would ameliorate bipolar symptoms. The target sample consisted of 5,145 patients receiving lithium (mean age = 48.6 ± 15 yrs; mean duration of lithium use=847 days), based upon the assumption that lithium treatment is relatively specific to individuals with BD. The main outcome measure was a calculated incidence density (ID) of medication events (change in the type or numbers of psychotropic medications prescribed, or increase [$>30\%$] in the psychotropic drug dose). Subjects receiving low-dose (≤ 80 mg/day) aspirin were 17% less likely to have a medication event, a finding that remained significant after adjusting for age, sex, chronic disease score and health care utilization. This effect was selective for low-dose ASA. In contrast, high-dose aspirin or non-selective NSAIDs (i.e., regimens expected to inhibit both COX-1 and -2), selective COX-2 inhibitors and glucocorticoids did not produce a statistically significant protection. Instead, the co-administration of non-selective NSAIDs and glucocorticoids was associated with statistically significant increases in medication events, suggesting destabilization of bipolar illness. The finding that low-dose aspirin decreased the number of medication events was particularly noteworthy since aspirin does not significantly augment serum lithium levels in contrast to selective COX-2 inhibitors which can raise lithium levels⁷⁰. These preliminary observations thus appeared consistent with the hypothesis that COX-1 inhibitors can reduce neuroinflammatory processes and thus benefit BD patients.

Notably, the observation that beneficial effects in BD were conferred by low-dose ASA, but not by nonselective COX inhibitors, COX-2 inhibitors or glucocorticoids, appeared inconsistent with the hypothesis that drugs that down-regulate AA cascade activity in general hold therapeutic potential in BD. Thus the putative neuroprotective effects associated with COX-1 inhibition may contribute specifically to the benefits of low-dose aspirin in BD observed by Stolk et al. For example, as reviewed above, aspirin and

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3 lithium may exert synergistic effects in forming anti-inflammatory brain DHA
4 metabolites (reviewed in ⁶⁹).

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9 Other data suggest that aspirin exerts antidepressant effects within the context of MDD or
10 cardiovascular illness. Mendlewicz et al.⁷¹ examined the effect of aspirin augmentation of
11 conventional antidepressant pharmacotherapy in 24 patients with MDD who had proven
12 non-responsive after 4 weeks of SSRI treatment. Participants were treated openly during
13 the subsequent 4 weeks with aspirin 160 mg/day in addition to their SSRI regimen. The
14 combined administration of SSRI plus aspirin was associated with a response rate of
15 52.4%. Remission was achieved in 43% of the total sample and 82% of the responder
16 sample. In the responder group, a significant improvement was observed within week 1
17 and this benefit persisted through day 28. In another study Ketterer et al.⁷² reported that
18 in 174 males undergoing coronary angiography (of whom 99 were taking low-dose
19 aspirin), aspirin use was associated with less depression and anxiety symptoms.
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30 In contrast, a preliminary study of the selective COX-2 inhibitor, celecoxib, was negative
31 in bipolar depression⁷³, potentially compatible with the negative results of COX-2
32 inhibitors reported by Stolk et al.⁶⁹. In a double-blind, randomized, add-on clinical trial of
33 celecoxib in patients (n = 28) studied during a depressed or mixed episode of BD, no
34 significant difference was observed between the celecoxib and placebo add-on groups at
35 study endpoint⁷³. These results contrasted with those obtained using celecoxib in unipolar
36 depression, however. In MDD, celecoxib augmentation of either reboxetine⁷⁴ or
37 fluoxetine⁷⁵ was associated with a significant therapeutic effect on depressive symptoms
38 in randomized, double-blind, add-on clinical trials.
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48 **METHODS AND ANALYSIS**

49 **Participants**

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55 One hundred and twenty male or female outpatients between 18 and 55 years of age, who
56 meet DSM-IV-TR criteria for BD (type I or II) and for a current major depressive episode
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3 will be recruited. The depressive syndrome must have been present for at least 4 weeks
4 and the minimum threshold for depression severity will be set at a 17-item HAM-D score
5 ≥ 18 . Subjects will provide written informed consent as approved by the Western
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7 Institutional Review Board.
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10 11 12 **Concurrent Medications** 13

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16 At study entry type I BD subjects must have been taking a stable dose of a mood-
17 stabilizing medication (lithium, valproate, carbamazepine, lamotrigine, antipsychotic
18 agents), for at least 4 weeks, dosed clinically to target the therapeutic range. Type II BD
19 subjects will be included irrespective of whether they present on a mood stabilizer. To
20 investigate the utility of this augmentation strategy in the population for whom
21 minocycline is most likely to prove therapeutically relevant, volunteers receiving stable
22 doses of mood stabilizing, antipsychotic, antidepressant, and/or anxiolytic drugs for at
23 least 4 weeks will be included. However, volunteers who currently are receiving more
24 than 4 psychotropic medications in a daily regimen will be excluded, since this condition
25 may signify a more brittle or complex clinical state. Subjects may remain in
26 psychotherapy or have no psychosocial intervention. Volunteers will be excluded if they
27 currently are receiving medications likely to have adverse interactions with minocycline
28 or aspirin, including warfarin, digoxin, penicillins, and isotretinoin products.
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41 For participants who enter the study, the preferred strategy will be for subjects to
42 maintain the same regimen of concurrent medications throughout the six week study so
43 that only the study drug regimen will be altered per protocol. Nevertheless, changes to
44 concurrent medications will not affect study status, so long as the medication change does
45 not target a depressive or manic symptom. If changes to concurrent medication regimens
46 are clinically required to address worsening depressive symptoms or the development of
47 manic symptoms, then the subject will be dropped from the study.
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53 54 55 **Study Design** 56 57 58 59 60

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3 Patients will participate in a randomized, double-blind, placebo-controlled, trial with a 2
4 x 2 design. As adjuncts to existing treatment, subjects will receive placebo-minocycline
5 plus placebo-aspirin, active-minocycline plus placebo-aspirin, placebo-minocycline plus
6 active-aspirin, or active-minocycline plus active-aspirin. The randomization sequences
7 will be determined by a research staff-member who is not obtaining clinical information
8 from the research subject and will be assigned by subject number at consenting. The trial
9 will be conducted over 6 weeks and will comprise 7 assessment sessions (figure 1). The
10 subject will be seen at the prescribed time intervals within a window of two business days
11 on either side of visit target date to complete the specified visits.
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21 At each session, a clinical assessment will be conducted using the rating scales listed
22 below, and treatment side-effects will be assessed and rated for severity. To preserve the
23 rater blind, the research staff member who conducts the clinical ratings will not be the
24 research staff member who assesses the presence of side effects, and will remain blind to
25 the information pertaining to side effects. Subjects who experience severe adverse effects
26 or who develop treatment-associated hypomania or mania will be dropped from the
27 study, instructed to discontinue the study medication, and referred for appropriate clinical
28 management of these adverse events.
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37 The primary outcome measure will be the change in the Montgomery-Asberg Depression
38 Rating Scale (MADRS) scores.
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42 **Medication**

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46 This pilot proof-of-concept study will adhere to the dosing limits and route of
47 administration for the FDA indications for minocycline's and aspirin's use in other
48 conditions (thus an IND is not required). A fixed dose design will be followed, and all
49 medications will be administered via the p.o. route. The pilot data extant for both study
50 drugs supports an onset of improvement within two weeks, so the six week study
51 duration is expected to provide sufficient time to detect an antidepressant effect, to
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3 provide information about the persistence of the antidepressant effect over about one
4 month from the anticipated onset of effect, and to minimize dropouts.
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9 For minocycline the starting dose will be 100 mg b.i.d. (total daily dose=200 mg). This
10 dose of 100 mg b.i.d. has been shown by a substantial literature to produce consistent
11 anti-inflammatory effects in rheumatoid arthritis and other inflammatory disorders. This
12 also is the dose used in a recent schizophrenia treatment trial⁵⁷. The associated placebo
13 capsules match the appearance of the 100 mg minocycline capsule.
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19 The starting dose of aspirin will be 81 mg p.o. b.i.d. This dose is sufficient to inhibit
20 COX-1, and appeared beneficial in stabilizing the course of BD in the pharmaco-
21 epidemiological study of Stolk et al.⁶⁹. When aspirin is used as an anti-platelet drug once
22 daily dosing is sufficient since anucleate platelets do not produce enough COX-1 to
23 overcome the irreversible inhibition of COX-1 within a 24-hour period. In contrast, in
24 nucleated cells COX-1 is replenished, so more frequent dosing is required to persistently
25 inhibit COX-1. Thus we will administer the dose in a b.i.d. regimen, according to the
26 guidelines described above. A total daily dose of 160 mg was administered in the
27 preliminary study which reported that aspirin significantly augmented the antidepressant
28 effects of fluoxetine in MDD⁷¹. The relevant placebo matches the appearance of the
29 aspirin tablet.
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40 Participants will be advised that one of the study drugs may reduce the efficacy of oral
41 contraceptives, and to avoid taking the study drugs within 3 hours of iron products or of
42 antacids containing calcium, magnesium or aluminum. They also will be advised that one
43 study drug can increase their risk for bleeding during surgical procedure or if combined
44 with other drugs or herbal preparations that reduce hemostasis.
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50 51 **Compensation**

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55 Participants will be compensated for participation in the amount of \$300.00.
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Treatment Compliance

To enhance compliance, study participants will be given an information sheet to take home detailing the procedure to be followed in the case of a missed dose, and requesting that this information be recorded for the investigators. The number of capsules and tablets remaining in each supply given to the patients will also be counted to evaluate treatment compliance. In cases where treatment compliance is poor, subjects will be excluded from the data analysis, using conventional criteria for defining adequate compliance in a clinical trial.

[Fig. 1, here]

Psychiatric Assessment and Clinical Ratings

Patients will be evaluated and followed in the outpatient clinics at LIBR or Oklahoma University School of Community Medicine in Tulsa, OK, or at the University of Kansas Medical Center Research Institute (KUMCRI) in Wichita, KS. The diagnosis of BD will be established using DSM-IV-TR criteria on the basis of an unstructured interview conducted by a psychiatrist and the MINI-Plus administered by trained psychiatric interviewers. The following rating scales will be administered: MADRS, Quick Inventory of Depressive Symptomatology (QUIDS; 16 item), Hamilton Anxiety Rating Scale (HAM-A), Young Mania Rating Scale (YMRS), Universal Fagerstrom (to assess nicotine use), Hollingshead socioeconomic scale, Sheehan Disability Scale (SDS) and the Family Interview for Genetic Studies (FIGS). Medical assessment will include a physical examination, electrocardiogram, complete blood count (CBC), electrolytes and liver-function assays (SMA 20), thyroid panel, and urinalysis, serum drug and pregnancy tests at study entry and study completion. At each follow-up session, the MADRS, HAM-A, YMRS, and Clinical Global Impressions (CGI) scale will be repeated. Physical and psychiatric symptoms will be evaluated and recorded in order to measure the side-effect profiles of minocycline and aspirin. Participants will be questioned about adverse reactions, including dizziness, photosensitivity, hyperpigmentation, gastrointestinal

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3 distress or bleeding at each assessment and will be withdrawn from the study if medically
4 necessary. Vital signs will be measured at entry and at each session.
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8 9 **Immune System Measures**

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12 The activity of peripheral cytokines correlates with inflammatory processes in the CNS.
13 Peripheral cytokines cross the BBB, and can propagate signals across the BBB in the
14 form of small, freely diffusible lipophilic molecules such as prostaglandins, which induce
15 the production of cytokines from glia⁷⁶. The measurement of peripheral markers of
16 inflammation thus serves as a valid, if indirect assessment of CNS inflammation.
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23 To explore predictors and correlates of treatment outcome, blood will be sampled for
24 testing plasma and whole blood peripheral blood monocyte (PBM) based markers of
25 inflammation at baseline and study end. These markers will include 10 cytokine proteins
26 (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IFN γ , and TNF, high sensitivity
27 (hs) CRP, and RNA expression of candidate genes from PBMCs. Candidate genes
28 include IL-6, TNF, and IRF5 (a factor that mediates monocyte polarization). The 10
29 inflammation-related cytokines and the PBMC mRNA will be assayed from plasma at
30 baseline and study end. We selected the markers IL-6, TNF and CRP because they are the
31 most widely implicated in mood disorders. The other cytokines included in the cytokine
32 bead array assays are measured simultaneously with IL-6 and have all been implicated in
33 the general regulation of inflammation. A meta-analysis of >100 studies found that IL-6
34 and CRP each were significantly elevated in depressed patients with standardized mean
35 difference scores (d) of 0.71 and 0.26, respectively⁷⁷. The associations remained
36 significant after adjustment for body-mass index (BMI) and smoking. Moreover, IL-6 has
37 been shown to modulate HPA axis function by inducing CRH release,
38 adrenocorticotrophic hormone synthesis, and corticosteroid production⁷⁸. CRP production
39 is induced by the proinflammatory cytokines, IL-1, IL-6, and IL-17, and is thus a non-
40 specific marker of systemic inflammation.
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Three blood samples will be transported to the immunology lab in the Department of Surgery at the University of Oklahoma College of Medicine for each participant at each of the sampling time-points (sessions 1 and 7). One sample will be centrifuged to obtain plasma which will be stored at -80°C until analyzed. Serum CRP, IL-6, TNF, and the other cytokines listed above will be assayed in duplicate with ELISA (CRP high-sensitivity kit, R & D Systems, Oxford, UK) or enhanced cytokine bead array flex kits (Becton Dickinson) using the manufacturer's reagents and standards. The other two samples will be used to isolate PBMCs and plasma and will be frozen until processed. Monocytes will be isolated from the PBMCs in order to assess mRNA levels similar to the method used by Padmos et al.³². This procedure utilizes monoclonal antibodies directed against human CD14 to isolate monocytes in peripheral blood monocyte cell suspensions. A magnetic cell sorting system will be used for the separation of the monocytes and flow cytometry will be used to gauge the purity of the population. Once purity is established, total RNA will be isolated from the monocytes using an RNeasy kits (Qiagen) according to the manufacturer's directions. RNA will then be reverse transcribed to cDNA using standard commercial kits. rtPCR reactions will be performed using the Dynamo Sybr Green HS Master Mix (New England Biolabs) and custom primers will be synthesized by a commercial laboratory. Real-time rtPCR reactions will be run using a Cepheid Smart Cycler II or similar instrument. Additional aliquots of serum and plasma will be stored so that other inflammatory markers can be tested in the future using Luminex bead arrays and/or additional available technologies.

Source of Compounds Tested

Minocycline and aspirin are available on a generic basis, and are manufactured within the USA by several companies. The identity of the active medicines and placebos will be blinded using placebos that match the appearance of the active drugs. The medications and placebos have been formulated by Wedgewood Pharmacy, Swedesboro, NJ. The study minocycline capsule and chewable aspirin tablet are identical in appearance to their corresponding placebos.

Outcome Measures and Data Analysis

Antidepressant response will be evaluated by assessing changes in MADRS scores. The *a priori* hypothesis that minocycline and/or aspirin plus existing medication will exert greater antidepressant effects than placebo plus existing medication will be tested in an intent-to-treat analysis using last observation carried forward for study dropouts. A secondary analysis will be performed to assess clinical improvement only in the study completers. These analyses will be statistically assessed using a group (for the four treatment cells)-by-session (1 vs. 7) repeated measures analysis of variance (ANOVA). If the ANOVA statistic is significant, between- and within-group t tests will be used in planned comparisons to identify the nature of the effect leading to the significant overall ANOVA statistic. We expect to find a significant group-by-session interaction, attributable to a greater reduction in MADRS scores in the minocycline and aspirin groups compared to the placebo group between session 1 and session 7.

In order to test whether the putative antidepressant effects of minocycline or aspirin have a rapid onset, as a *post hoc* analysis the ANOVA will be repeated using MADRS ratings from the assessment that follows the first week of exposure to active drug versus the corresponding change under placebo; i.e. session 2. *Post hoc* tests will be performed to assess the significance of changes in the secondary clinical outcome measures (QUIDS 16, HAM-A, YMRS, CGI-I). The rate of completion in the two cells also will be considered an outcome measure. The completion rate in the minocycline arm may be influenced more by dropouts due to side effects while the completion rate in the placebo group may be influenced more by dropouts due to non-response.

We will test the hypothesis that minocycline and aspirin reduce inflammation (e.g. CRP, IL-6, IL-6 mRNA) more than placebo using statistical analyses similar to those described above. If the assay results are normally distributed then a group-by-session repeated-measures ANOVA with CRP, IL-6 and nine other cytokine levels as dependent variables, and BMI, smoking status, and time of blood draw as covariates, will be used to assess anti-inflammatory effects of minocycline and aspirin. If the CRP or inflammatory

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3 cytokine data are not normally distributed (Kolmogorov-Smirnov test) or if the equality
4 of statistical variance assumption across assessments is violated (Levene's test), then
5 Friedman's ANOVA will be used to test for CRP or inflammatory cytokine differences
6 between groups. If the Friedman's ANOVA statistic is significant, Wilcoxon sign-ranked
7 tests will be used for post-hoc analysis of group differences. Nonspecific factors that
8 influence CRP and inflammatory cytokine levels include time of day, presence of
9 infection, treatment with anti-inflammatory medications, smoking, obesity, and alcohol
10 abuse. We will attempt to control for these potential confounds by measuring BMI and
11 recording NSAID and nicotine use (Universal Fagerstrom scale), and by excluding
12 individuals who have recently abused substances or who have intercurrent infections. The
13 serum CRP concentration shows minimal diurnal variability in adults⁷⁹ but IL-6 and other
14 cytokine levels vary across time of day⁸⁰. To minimize cytokine measurement variability
15 due to circadian fluctuations, we will schedule patient assessment sessions at the same
16 time each day. Since this may not always be possible, we will record the time of day that
17 each blood-draw is made, divide the day into quartiles: 7am-10am, 10am-12pm; 12pm-
18 3pm, and 3pm-6pm, and use these data as a covariate in the statistical analyses.
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33 To test whether baseline levels of CRP and inflammatory cytokines predict response to
34 minocycline or aspirin, we will subclassify the participants using conventional criteria⁸¹
35 as achieving full response ($\geq 50\%$ reduction in MADRS score from baseline), partial
36 response ($< 50\%$ but $\geq 25\%$ reduction), or nonresponse ($< 25\%$ reduction). Patients
37 achieving remission (post-treatment MADRS score ≤ 10) will also be identified. A non-
38 parametric alternative to the ANOVA statistic, the Mann-Whitney test, will be used to
39 compare remitted and non-remitted groups in baseline levels of inflammatory cytokines
40 and CRP if the data are not normally distributed.
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49 **Statistical Power**

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53 A recent meta-analysis of 96 antidepressant treatment studies found that the average
54 effect size of a placebo treatment is 1.69 compared with 2.50 for an antidepressant
55 treatment⁸². We calculated that in order to detect a difference in-group means of 0.81
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3 (2.50-1.69) with an 80% probability (2-sided test, $\alpha=0.05$), we will require a sample size
4 of 26 subjects per group (http://hedwig.mgh.harvard.edu/sample_size/size.html). Thus
5 given our sample size of 30 per group we should have sufficient power to test Specific
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7 Aim 1, allowing for a 13% drop-out rate.
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12 As discussed above, a recent meta-analysis⁷⁷ of cross-sectional studies of serum-derived
13 IL-6 and CRP in depression calculated effect sizes of 0.71 for IL-6 and 0.26 for CRP.
14 Based on these effect sizes a sample size of 26 would yield >80% probability of detecting
15 significant depression-related changes in IL-6, but only a 60% probability of detecting a
16 depression-related change in CRP. There are 3 reasons why we believe that these CRP
17 power estimations are not applicable to this study. Firstly, the effect sizes derived from
18 the meta-analysis are based on cross-sectional studies. Given the effect of variables such
19 as smoking, diet, exercise, and BMI on proinflammatory cytokines, a within-subjects
20 design is likely to reduce non-depression-related sources of variance, and substantially
21 increase statistical power. Secondly, we are not only examining the effect of mood on IL-
22 6 and CRP levels, but are treating patients with minocycline and aspirin, drugs known to
23 possess anti-inflammatory properties. We therefore suggest that our proposed study is
24 likely adequately powered to detect any true changes in plasma IL-6, CRP, and the other
25 inflammatory cytokines across treatment blocks.
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39 Regarding IL-6 mRNA gene expression in peripheral blood monocytes, Padmos et al.³²
40 reported a 38-fold increase in IL-6 mRNA levels in unmedicated patients with BD
41 compared with HC. Since minocycline reduces IL-6 levels (see above) we expect our
42 study to have very high power to detect differences between groups, as well as changes in
43 response to minocycline. The simultaneous detection of nine other inflammation-related
44 cytokines, in addition to IL-6 (using newer more sensitive technology) will provide much
45 finer resolution of the effects on inflammatory cascades than that measured in previous
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ETHICS AND DISSEMINATION

Gender/Minority/Pediatric Inclusion for Research

Women and Minorities will be included in the study without prejudice according to their representation in the study population. Participants will be recruited from the greater metropolitan areas of Tulsa, OK and Wichita, KS and efforts will be made to ensure that our subject population resembles the gender, ethnic and racial composition of these areas.

Exclusion Criteria

The following exclusion criteria apply: 1) inability to provide informed consent; 2) age of onset of BD > 40 years; 3) serious risk of suicide; 4) current delusions or hallucinations sufficient to interfere with the capacity to provide informed consent; 5) current manic symptoms [depressed BD patients with concurrent manic symptoms have been found to be more likely to experience adverse reactions in antidepressant treatment trials⁸³]; 6) medical illness including as hepatic impairment, renal dysfunction, bleeding diatheses (e.g., hemophilia), cerebrovascular disease or heart disease, hypertension that is inadequately controlled by medication, diabetes mellitus, or known peptic ulcer disease; 7) abuse of drugs or alcohol within the preceding 6 months, or substance dependence within the last 5 years; 8) daily alcoholic beverage consumption equivalent to ≥ 3 oz. of alcohol; 9) asthma or known allergies or hypersensitivities to tetracycline antibiotics, aspirin or other NSAIDs; 10) current use of drugs that could increase the risks associated with aspirin or minocycline administration, namely other antibiotic medications, other NSAIDs or anticoagulants (e.g., warfarin), acetazolamide, or methotrexate; 11) known HIV or other chronic infection including, but not limited to viral hepatitis. 12) Pregnant or nursing women, and women who are attempting to conceive during the 6 week study period, will also be excluded.

Specimens, Records, and Data Collection

A physician, registered nurse, or trained phlebotomist will utilize a sterile technique to draw 60 ml of blood by venipuncture. Participants will also be asked to submit a urine

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3 sample. A physician, registered nurse, or trained technician will collect EKG data from
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5 the subject in a private exam room.
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8 9 **Recruitment and Consent Procedure**

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12 Volunteers will be recruited from the community as well as from the clinical services at
13 the Laureate Psychiatric Clinic and Hospital and the Oklahoma University School of
14 Community Medicine in Tulsa, OK, and from the clinical services affiliated with the
15 KUMCRI. Volunteers may be referred from sources that include physicians, newspaper
16 advertising, self-help organizations, self-referral, and WIRB approved flyers posted at
17 local universities, schools, churches and grocery stores. Participants may be pre-screened
18 through screening protocols based at LIBR or KUCRI. We plan to recruit a total of 120
19 participants.
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28 All participant interactions including consenting will be conducted in private interview /
29 exam rooms. These rooms are secured from public areas via combination locked doors
30 that are only accessible to authorized personnel. Prospective participants will receive an
31 explanation of the objectives, procedures, and hazards of this protocol that is appropriate
32 to their level of understanding. The right of the subject to decline to participate or to
33 withdraw from the study at any time will be made clear.
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40 Non-English speaking participants will not be recruited.
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44 After the consent form is verbally explained to the participant, and any questions have
45 been answered, the researcher will leave the room to allow the participant to read the
46 consent form thoroughly. Family members will be allowed to be present and to discuss
47 the consenting process with the participant. After the consent is read, the researcher will
48 return and answer any additional questions the participant may have. The researcher will
49 remind the subject that participation is strictly voluntary and that they have the right to
50 withdraw at any time. Participants will be asked to arrive 30 minutes early in order to
51 have sufficient time for the consenting process.
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Subject Risks

The risks of behavioral testing are minimal. The risks of blood drawing are also minimal. Possible mild side effects of the blood draw include mild pain or bruising at the site of the venipuncture.

Minocycline has been used a broad-spectrum antibiotic for many years in doses up to 400 mg/day³⁴. It has been used on a chronic basis to treat acne and rheumatoid arthritis, often for many years, in hundreds of thousands of patients. The most commonly encountered side effects are upset stomach, diarrhea, dizziness, drowsiness, ataxia, vertigo, headache and vomiting. Prolonged use can be associated with pigmentation of the skin, gums or teeth. Between 1975 and 2006, the World Health Organization Collaborating Center for International Drug Monitoring listed 122 cases of adverse drug reactions to intravenous minocycline; most commonly, abnormal hepatic function and thrombocytopenia³⁴. These included cases of serious liver injury, including irreversible drug-induced hepatitis and fulminant hepatic failure that was fatal in two cases, thought to be due to triggering or unmasking autoimmune hepatitis. One case of autoimmune-related glomerulonephritis has been reported. The role of oral minocycline in precipitating these conditions has not been clearly established. Minocycline also has been associated with idiopathic intracranial hypertension (pseudotumor cerebri). Long-term trials have shown that minocycline is well tolerated. In a 2-year trial of minocycline (200 mg/day) for RA, 3 of 30 patients withdrew due to finger-nail discoloration, dizziness, or erythematous rash⁵³. Of 11 patients with HD treated with minocycline (100 mg/day) for 2 years, one complained of nausea in the first 3 weeks, and two of sedation⁵², while in a 6-month trial of minocycline for ALS, the mean tolerated dose was 387 mg/day and the most common adverse effects were gastrointestinal⁸⁴. Five of 36 patients with schizophrenia withdrew from a 6-month trial of minocycline (200 mg/day) due to indigestion (n=2), pigmentation (n=2), or a suicide attempt (n=1)⁵⁷.

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Low dose aspirin has been safely used in many millions of patients on a worldwide scale for its role as an anti-thrombotic and thrombolytic. A meta-analysis of >100 randomized trials in high-risk patients indicated that low-dose ASA reduced cardiovascular death by 15% and prevented nonfatal vascular events by about 30%⁸⁵. These data stand in striking contrast to the data obtained in COX-2 inhibitors, which can increase cardiovascular risk. In clinical trials of several COX-2 selective and nonselective NSAIDs of up to three years duration have shown an increased risk of serious cardiovascular (CV) thrombotic events, myocardial infarction, and stroke, which have in many cases been fatal⁸⁶. Patients with known CV disease or risk factors for CV disease are at greater risk for such events during chronic treatment with COX-2 inhibitors. Evidence from human pharmacology and genetics, genetically manipulated rodents, and other animal models and randomized trials indicates that this is consequent to suppression of COX-2-dependent cardioprotective prostaglandins, particularly prostacyclin⁸⁷.

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Aspirin does not cause a generalized bleeding abnormality unless given to patients with an underlying hemostatic defect (e.g., hemophilia, uremia, or that induced by anticoagulant therapy). Aspirin-induced impairment of primary hemostasis cannot be separated from its antithrombotic effect and is similar at all doses ≥ 75 mg/d⁸⁸. The risk of intracranial bleeding is exceedingly rare (<0.1% in high risk populations), but is higher in individuals with cerebrovascular disease⁸⁵. Hypertension that is inadequately controlled by medication often is considered a contraindication to aspirin because of the concern that possible benefits in the prevention of cardiovascular events may be counterbalanced by an increased risk of cerebral bleeding. However, hypertensive patients whose blood pressure is well-controlled appear protected from myocardial infarction by aspirin therapy without an increase in the number of cerebral hemorrhages or strokes⁸⁹. Moreover, aspirin therapy does not affect blood pressure or the response of hypertension to antihypertensive agents^{88 90}.

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NSAIDs as a class can cause serious gastrointestinal (GI) adverse events including inflammation, bleeding, ulceration, and perforation of the stomach, small intestine, or large intestine, which rarely have proven fatal. In controlled clinical trials the percentage

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3 of patients reporting one or more gastrointestinal complaints has ranged from 4% to
4 16%⁸⁸. The mechanism underlying this adverse effect appears attributable to the
5 inhibition of COX-1. Thus, the incidence of GI side effects has been higher for NSAIDs
6 with more potent effects at COX-1, such as aspirin and indomethacin. For example, in
7 controlled trials the incidence of GI side effects for aspirin and indomethacin have been
8 about twice as high as that for ibuprofen, a nonselective COX inhibitor, in equally
9 effective doses for arthritis. Nevertheless, the incidence of GI side effects associated with
10 aspirin is dose-dependent, and thus is markedly lower when using aspirin in the low dose
11 range planned for the current study. Notably, the risk of GI bleeding is not reduced by
12 using the enterically coated aspirin formulations, but is thought to be lower during
13 concomitant use of omeprazole⁸⁸. The effects of warfarin and NSAIDs on GI bleeding are
14 synergistic, such that the users of both drugs together have a risk of serious GI bleeding
15 higher than users of either drug alone. Fortunately, the risk of GI bleeding, which reflects
16 the inhibition of prostaglandins in the stomach (from systemic rather than local exposure)
17 is much smaller when using low-dose as opposed to high-dose aspirin.
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32 Low-dose aspirin has not been reported to alter renal function, and does not reduce
33 effectiveness of ACE inhibitors for HTN (in contrast to other NSAIDs)^{90 91}. However,
34 aspirin can inhibit the renal clearance of acetazolamide and methotrexate potentially
35 leading to increased blood concentrations of and toxicity from these agents. Salicylate
36 can displace other drugs which are protein-bound, especially phenytoin and valproic acid,
37 increasing their free drug concentrations in plasma. This may increase side effects,
38 toxicity and/or efficacy for displaced drugs. If the BD subjects are currently receiving
39 valproic acid preparations (e.g., divalproex) then the plasma levels of these agents will be
40 monitored for potential changes.
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49 Aspirin may cause a severe allergic reaction that may include: hives, asthma (wheezing),
50 facial swelling, shock. Aspirin overdose can be fatal at 30 g or higher.
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55 **PHI Protection**

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Paper copies of consents, screening forms, the Research Privacy Form, and any other forms, testing results or papers containing Protected Health Information (PHI) will be stored in a secured medical records room with access granted only to authorized personnel.

Electronic data that contain PHI will be managed in accordance with ISO 27000 series information security standards with policies developed from current NIST guidelines (SP 800-66) for HIPAA and HITECH compliance. Specific controls implemented to protect PHI are derived from NIST 800-122, and include (but not limited to):

- 1) Access Enforcement (AC-3) – Individual user accounts, role based access control, access control lists;
- 2) Separation of Duties (AC-5) – de-identification of data as appropriate, acquire/analyze/manage firewall;
- 3) Least Privilege (AC-6) – to ensure PHI data is only available to persons with established need for access;
- 4) Remote Access (AC-17) – Secure VPN, encrypted end devices;
- 5) Access Control for Mobile Devices (AC-19) – Password login, remote destruction capabilities;
- 6) Auditable Events (AU-2) + Monitoring: Log detailed server and network information, alert for problems;
- 7) Analysis, and Reporting (AU-6) – Procedures to audit system records for inappropriate activity.
- 8) User Identification and Authentication (IA-2) – username/secure password and two factor authentication will be required when appropriate.
- 9) Media Access, Marking, Storage, and Transport (MP-2,3,4,5) – Records will be asset tagged and marked to their PHI status, PHI data will be secured and managed by professional system administrators, and will be transported via encryption (VPN, USB, File);
- 10) Media Sanitization (MP-6) – Data will be destroyed by SFHS in accordance with their policies and procedures;

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3 11) Transmission Confidentiality (SC-9) – Encryption will be used when needed for
4 all avenues of data transmission (wireless, network, etc.).
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7 To protect subject confidentiality, blood samples will be anonymized as follows:

- 8
9 1. Last name: All participants will be assigned the last name “LIBR.”
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11 2. First name: The first name will be a secure alpha cryptographic hash based on
12 LIBR user ID. This technique is the gold standard in computer security for one-
13 way correlation of data.
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16 17 18 **Benefits versus Risks**

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21 The participant may benefit from participation if either study drug produces an
22 antidepressant effect. Participants will also receive a free clinical evaluation; more
23 frequent treatment visits than are typical in practice, diligent follow-up in terms of
24 symptoms and side effects, and physical and psychiatric monitoring during the study. The
25 risks of delaying alternative treatments are minimal in relation to the potential long-term
26 benefits to the subjects and the importance of knowledge that may reasonably result. The
27 importance of the knowledge that will likely be gained from this study clearly exceeds
28 the associated potential risks.
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38 **Alternative Treatment**

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41 It is possible that some patients may feel better with talk therapy. Participating in any
42 type of talk therapy with their psychiatrist or psychologist does not require dropping out
43 of this study. Subjects will be encouraged to contact the study investigators, particularly
44 the physician in the study, with any questions they may have regarding alternatives to
45 treatment through this research study. The study investigators will assist in referring the
46 subject to another physician for treatment after their participation in the study has ended.
47 Physical and psychological testing, blood draws, urine samples, and EKG data provide no
48 known risks to persons other than those listed in the exclusion criteria whereas the
49 combinatory power of these measures may provide information relevant to understanding
50 the pathophysiology of bipolar disorder.
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Data and Safety Monitoring Plan

This study involves more than minimal risk. The study progress will be overseen by a Data, Safety and Monitoring Board (DSMB). The DSMB is composed of three members who will meet in person or per telephone at least once every 6 months to review relevant study data including adverse events and dropout rates.

Any unanticipated adverse events will be reported immediately to the IRB of record and to the LIBR Human Protection Administrator. Any adverse events will be included in the annual IRB report.

Dissemination of Results

The study results will be presented at national and/or international biomedical scientific meetings and published in peer-reviewed journals.

REGISTRATION

In accordance with the recommendations of the International Committee of Medical Journal Editors⁹², the proposed trial is registered in a public registry (www.clinicaltrials.gov Identifier: NCT01429272).

Figure Legend

Figure 1: Schematic of Study Design

Legend: Each session number (total of 7) is encircled, with the timing between sessions indicated in weeks with a 2 business day window on either side of visit target date to complete the visit. Session 1 is the baseline (green star) and session 7 is the study end

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(purple star). Peripheral blood will be sampled at baseline and study end to assay markers of inflammation. The study duration is 6 weeks.

Author Contributions

The protocol was written by Drs. Savitz and W. Drevets and was critically reviewed by Drs. Preskorn, Teague, D. Drevets, and Yates.

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Competing Interests

Wayne Drevets, M.D. has consulted for Johnson & Johnson, Pfizer, Rules-Based Medicine, and Eisai. None of the other authors have conflicts of interest to declare.

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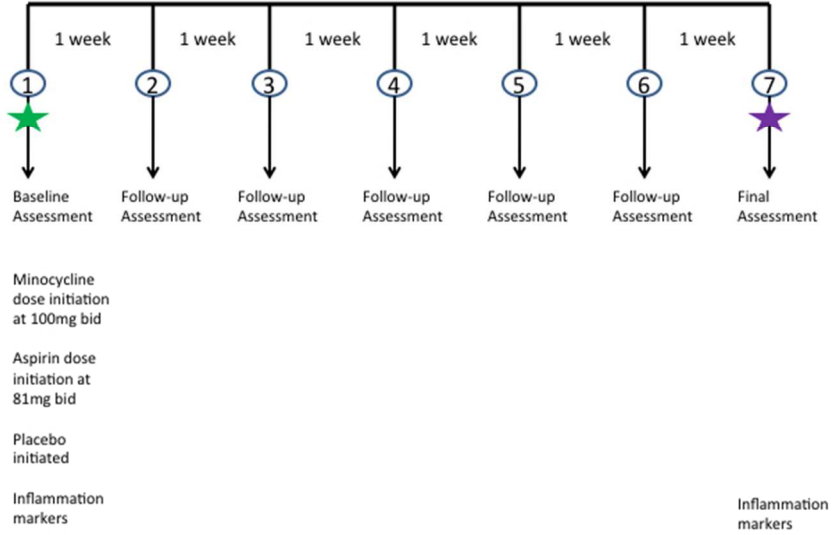
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THE FOLLOWING WERE APPROVED

INVESTIGATOR: Wayne Drevets M.D.
 6655 South Yale Avenue
 Tulsa, Oklahoma 74136

BOARD ACTION DATE: 08/22/2011
PANEL: 6
STUDY APPROVAL EXPIRES: 08/05/2012
STUDY NUM: 1126576
WIRB PRO NUM: 20111159
INVEST NUM: 160921
WO NUM: 1-683377-1
CONTINUING REVIEW: Annually
SITE STATUS REPORTING: Semi-Annual

SPONSOR: Laureate Institute for Brain Research (LIBR)

PROTOCOL NUM: 2011-002-00

AMD. PRO. NUM:

TITLE:

MINOCYCLINE AND ASPIRIN IN THE TREATMENT OF BIPOLAR DEPRESSION

APPROVAL INCLUDES:

Subject Information Sheet - Visit Five #9212383.0 - As Submitted
 Subject Information Sheet - Visit Four #9212382.0 - As Submitted
 Subject Information Sheet - Visit One #9212379.0 - As Submitted
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Robert A Taylor for

Theodore D. Schultz, J.D., Chairman

8/23/2011

(Date)

This document electronically reviewed and approved by Taylor, Robert on 8/23/2011 1:46:33 PM PST. For more information call Client Services at 1-360-252-2500.

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25 If this research is federally funded or conducted under an FWA, obtain pre-approval from WIRB for all planned
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33 subjects. FDA has not adopted the policy that all planned protocol deviations are changes in research that need prior IRB
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- 47 7. Provide reports to WIRB concerning the progress of the research, when requested.
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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-13
	2b	Specific objectives or hypotheses	20
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	14-15
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	13-14
	4b	Settings and locations where the data were collected	17
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	15-16
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	20-21
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	21
	7b	When applicable, explanation of any interim analyses and stopping guidelines	30
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	15
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	15
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	15
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	15

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2		assessing outcomes) and how	
3			
4		11b If relevant, description of the similarity of interventions	
5	Statistical methods	12a Statistical methods used to compare groups for primary and secondary outcomes	20-21
6		12b Methods for additional analyses, such as subgroup analyses and adjusted analyses	20-21
7			
8	Results		
9	Participant flow (a	13a For each group, the numbers of participants who were randomly assigned, received intended treatment, and	
10	diagram is strongly	were analysed for the primary outcome	
11	recommended)	13b For each group, losses and exclusions after randomisation, together with reasons	
12	Recruitment	14a Dates defining the periods of recruitment and follow-up	
13		14b Why the trial ended or was stopped	
14			
15	Baseline data	15 A table showing baseline demographic and clinical characteristics for each group	
16	Numbers analysed	16 For each group, number of participants (denominator) included in each analysis and whether the analysis was	
17		by original assigned groups	
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19	Outcomes and	17a For each primary and secondary outcome, results for each group, and the estimated effect size and its	
20	estimation	precision (such as 95% confidence interval)	
21		17b For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
22	Ancillary analyses	18 Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing	
23		pre-specified from exploratory	
24			
25	Harms	19 All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
26			
27	Discussion		
28	Limitations	20 Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
29	Generalisability	21 Generalisability (external validity, applicability) of the trial findings	
30	Interpretation	22 Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
31			
32	Other information		
33	Registration	23 Registration number and name of trial registry	1, 30
34	Protocol	24 Where the full trial protocol can be accessed, if available	19
35	Funding	25 Sources of funding and other support (such as supply of drugs), role of funders	
36			

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.



**MINOCYCLINE AND ASPIRIN IN THE TREATMENT OF
BIPOLAR DEPRESSION: a protocol for a proof-of-concept
randomized, double-blind, placebo-controlled, 2x2, clinical
trial**

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**MINOCYCLINE AND ASPIRIN IN THE TREATMENT OF
BIPOLAR DEPRESSION: a [protocol for a proof-of-concept
randomized, double-blind, placebo-controlled, 2x2, clinical trial](#)**

Jonathan Savitz^{1,2}, Ph.D., Sheldon H. Preskorn³, M.D., T. Kent Teague⁴,
Ph.D., Douglas A. Drevets⁵, M.D., William Yates¹, M.D., Wayne C.
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Key Words

Bipolar Depression, Clinical Trial, Minocycline, Aspirin, Inflammation

ABSTRACT

Introduction: New medication classes are needed to improve treatment effectiveness in the depressed phase of bipolar disorder (BD). Extant evidence suggests that BD is characterized by neural changes such as dendritic remodeling and glial and neuronal cell loss. These changes have been hypothesized to result from chronic inflammation. The principal aims of the proposed research is to evaluate the antidepressant efficacy in bipolar depression of minocycline, a drug with neuroprotective and immune-modulating properties, and of aspirin, at doses expected to selectively inhibit cyclooxygenase 1 (COX-1). **Methods and Analysis:** One hundred and twenty outpatients between 18 and 55 years of age, who meet DSM-IV-TR criteria for BD (type I or II) and for a current major depressive episode will be recruited to take part in a randomized, double-blind, placebo-controlled, parallel-group, [proof-of-concept](#), clinical trial following a 2 x 2 design. As adjuncts to existing treatment, subjects will be randomized to receive one of four treatment combinations: placebo-minocycline plus placebo-aspirin, active-minocycline plus placebo-aspirin, placebo-minocycline plus active-aspirin, or active-minocycline plus active-aspirin. The dose of minocycline and aspirin is 100mg bid and 81mg bid, respectively. Antidepressant response will be evaluated by assessing changes in the Montgomery-Asberg Depression Rating Scale (MADRS) scores between baseline and the end of the 6 week trial. As secondary outcome measures, the anti-inflammatory effects of minocycline and aspirin will be tested by measuring pre-and-post treatment levels of CRP and inflammatory cytokines. **Ethics and Dissemination:** Minocycline has been widely used as an antibiotic in doses up to 400 mg/day. Low dose aspirin has been safely used on a worldwide scale for its role as an anti-thrombotic and thrombolytic. The study progress will be overseen by a Data, Safety and Monitoring Board which will meet once every 6 months. Results of the study will be published in peer-reviewed publications. **Registration:** Clinical Trials.gov: NCT01429272.

INTRODUCTION

The treatment of bipolar depression remains a major challenge for psychiatry. The US FDA has not approved any of the ~25 standard antidepressants for the treatment of bipolar depression, partly because these agents have not been robustly effective in BD patients¹. Thus, currently approved treatments for bipolar depression include lithium, quetiapine, and the combination of olanzapine and fluoxetine². Other treatments used include lamotrigine, conventional antidepressant agents, other atypical antipsychotics, pramipexole or riluzole (reviewed in ³). Unfortunately, the effectiveness of these options also is limited. For example, in a placebo-controlled study in which subjects receiving lithium were randomized to receive either standard antidepressant pharmacotherapy (paroxetine or imipramine) or placebo, those receiving lithium plus an antidepressant did not show a significant improvement over those receiving lithium plus placebo⁴. Similarly, in the STEP-BD trial, 42 of 179 subjects (23.5%) receiving a mood stabilizer plus adjunctive antidepressant drug treatment had a durable recovery, which did not differ significantly from 51 of 187 subjects (27.3%) receiving mood stabilizer plus placebo. Mallinger et al. reported a similar durable recovery rate in BD depressives treated with mood stabilizer plus paroxetine (27%), but found a higher rate for adjunctive monoamine oxidase inhibitors (MAOIs; 53%)⁵, consistent with the findings of previous studies comparing MAOIs vs imipramine^{6,7}. Unfortunately MAOIs are commonly unacceptable to patients.

New classes of antidepressant drugs are needed for bipolar depression. Existing agents exert their primary actions on monoaminergic systems. The efficacy of these agents contributed to the monoamine-deficiency hypothesis of depression, which continues to receive empirical support. Nevertheless, the field is in the early stages of a paradigm shift driven by evidence of dendritic remodeling and neuronal atrophy in animal models of depression, and of reductions in gray matter (GM) volume, and glial cell loss at *postmortem* in BD⁸. The neurotrophic effects of lithium, coupled with longitudinal studies demonstrating volumetric changes over time, raise the possibility that mood disorders are underpinned by a neurotoxic process^{8,9}. The final common pathway through

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3 which neurotoxic agents exert their effect is hypothesized to involve excess glutamatergic
4 signaling¹⁰.
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9 The glutamatergic model of mood disorders is based on the premise that excessive
10 stimulation of NMDA-glutamatergic receptors, results in neuronal atrophy and apoptosis
11 of glial and/or neuronal cells, and *ipso facto*, depression. Evidence for this hypothesis
12 derives from multiple sources. In preclinical models, riluzole, which inhibits neuronal
13 release of glutamate, ceftriaxone, which increases glutamate reuptake, and NMDA
14 receptor antagonists such as ketamine, ameliorate behavioral analogs of depression¹¹. In
15 addition, rats bred to be genetically sensitive to stress show differential expression of
16 NMDA receptors¹², and behavioral analogs of depression are abrogated in NMDA
17 receptor subunit knockout mice¹³. In humans, increased serum levels of glutamate that
18 resolve with antidepressant treatment were reported in MDD, and extended to the CSF
19 post mortem¹¹. Polymorphisms of the metabotropic glutamate receptor genes, GRM2 and
20 GRM3, and a haplotype of the glutamic acid decarboxylase (GAD2) gene were
21 associated with MDD¹⁴. Finally, ketamine induced a rapid, sustained antidepressant
22 effect in BD^{15 16} and riluzole showed promising results in treatment-resistant depression¹⁵
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One potential cause of the disruption in glutamatergic signaling in BD is dysregulation of
the immune system. Increased levels of proinflammatory cytokines such as interleukin 6
(IL-6), IL-1 β , interferon alpha (IFN α), tumor necrosis factor alpha (TNF- α)
prostaglandinE2 (PGE2), and chemokine ligand 2 (CCL2) are consistently observed in
the blood and CSF of patients with mood disorders, both at baseline and after exposure to
stressors^{17 18}. Elevated serum levels of (pro-inflammatory) positive acute-phase proteins
(e.g., haptoglobin, α 1-antitrypsin, ceruloplasmin, C-reactive protein), but reduced levels
of negative acute-phase proteins (e.g., albumin and retinal-binding protein) also are
reported in mood disorders¹⁹⁻²¹. Further, treatment of hepatitis C with IFN α is known to
induce the major depressive syndrome and/or manic symptoms in [approximately 40%](#) of
patients, and the efficacy of conventional antidepressant drugs is associated with a
reduction in inflammation¹⁸. Moreover, anti-tumor necrosis factor (TNF) therapy (for

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3 psoriasis) can improve mood²². Since proinflammatory cytokines can alter brain function,
4 these data are compatible with evidence that an activated inflammatory response system
5 exists in mood disorders which plays a role in their pathophysiology²³⁻²⁶.
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10 The over-activity of the hypothalamic-pituitary-adrenal axis in mood disorders may play
11 a role in inflammation, since hypersecretion of corticotrophin-releasing hormone (CRH)
12 activates the transcription factor, nuclear factor kappa B (NF-κB). NF-κB regulates the
13 expression of proinflammatory cytokines in immune cells in the CNS and periphery, and
14 the expression of genes involved in apoptosis²⁷. In addition, NF-κB may result in the
15 expression of the class 1 major histocompatibility complex (MHC I), labeling cells for
16 removal by cytotoxic T-cells²⁷. Usually, cortisol suppresses this inflammatory response,
17 but chronic stress appears to desensitize the glucocorticoid receptor (GR) and by
18 extension, the anti-inflammatory effects of cortisol²⁷. Cytokines play a role in
19 desensitizing the system to cortisol. For example, IL1 and TNF-α retard dexamethasone-
20 induced translocation of the GR receptor from the cytoplasm to the nucleus²⁸.
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31 The immunologic and glutamatergic models of BD are complementary because a
32 proinflammatory state is one potential cause of excitotoxicity²⁷. Peripheral inflammatory
33 signals activate microglia in the brain, inducing an inflammatory cascade of cytokines
34 and free radicals. Cytokines and reactive oxygen and nitrogen species exert a direct toxic,
35 apoptotic effect on oligodendrocytes. Potentially through the loss of oligodendrocytes,
36 oxidative stress can lead to demyelination. Such a process conceivably may account for
37 the reduction in oligodendroglia found *postmortem* in the prefrontal cortex²⁹ in mood
38 disorders. The inflammatory milieu also compromises astrocyte function, leading to
39 down-regulation of glutamate transporters and impaired glutamate reuptake into
40 astrocytes, further amplifying inflammatory signaling²⁷.
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51 In addition, cytokines such as interleukin 1 (IL-1), IL-6, and TNF-α activate indoleamine
52 2, 3-dioxygenase (IDO). IDO catalyzes the breakdown of tryptophan, the amino-acid
53 precursor of serotonin, and an important regulator of T-cell function, into kynurenine
54 (Kyn)³⁰. Activation of the Kyn pathway shunts tryptophan away from 5-HT synthesis,
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3 putatively reducing serotonergic transmission. Kyn is in turn metabolized into quinolinic
4 acid (Quin), a potent NMDA receptor agonist, and neuromodulator involved in lipid
5 peroxidation, which can induce neuronal damage via oxidative stress and overstimulation
6 of NMDA receptors³⁰. Consistent with inflammation-related shunt towards Kyn
7 metabolism, the plasma tryptophan-Kyn ratio was found to correlate inversely with
8 striatal total choline (a putative cell membrane turnover biomarker) in adolescents with
9 melancholic depression³¹.
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18 The mRNA transcripts for proinflammatory genes appear particularly sensitive for
19 discriminating BD patients. Microarray gene expression profiles in purified CD14+
20 monocytes from whole blood of BD subjects, offspring of BD parents, and healthy
21 controls (HC) displayed a distinct mRNA signature representing genes from
22 inflammatory and inflammation-related pathways³². The signature showed >80%
23 sensitivity and specificity in BD subjects who were not receiving lithium or antipsychotic
24 drugs (n=11), and in affected offspring of a BD parent (n=13, of whom 10 had only
25 manifested depression). A positive signature also was present in 17 of 38 unaffected
26 offspring (45%) versus 13 of 70 healthy children (19%). Cross-sectional comparisons
27 suggested lithium and antipsychotic drugs—but not conventional antidepressant drugs--
28 down-regulated expression of most inflammatory genes. Thus, when medicated and
29 unmedicated subjects were considered together only 23 of 42 BD patients (55%) had a
30 positive signature versus 7 of 38 HCs (18%). Notably, the IL6 mRNA level remained
31 elevated in medicated BD subjects and did not differ significantly from unmedicated
32 subjects (table 1), suggesting that this assay identifies a proinflammatory diathesis even
33 in treated cases.
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Table 1: Magnitude of difference in mRNA expression between mood disordered and healthy control (HC) samples from Padmos et al.³², showing selected transcripts in unmedicated subjects vs HCs, relative to that of medicated BD subjects.

Gene Symbol	Unmedicated BD vs HC		Medicated BD vs HC		Affected offspring# vs HC	
	fold change	p-value	fold change	p-value	fold change	p-value
PDE4B	13.73*	<.001	3.42	<.001	5.79	<.001
IL6	37.92	.005	9.56	.006	935.7	<.001
CCL20	55.49	.006	6.02	.10	400.1	<.001

Legend: * - difference significant between unmedicated vs medicated BD samples; # - affected with respect to having manifested either a depressive or a manic episode
 Sample sizes: unmedicated BD n=11, medicated BD n=31, affected offspring n=13, HCs n=25 for comparisons against BD adults, n=70 for comparisons of offspring. Abbrev: BD – bipolar disorder; HC – healthy control; PDE4B - phosphodiesterase type 4B; IL6 - interleukin 6; CCL20-chemokine ligand 20

Minocycline is a second-generation tetracycline that may prevent both glutamate-induced excitotoxicity and cytokine-induced inflammation in the CNS and periphery.

Minocycline has high lipophilicity enabling efficient transfer across the blood brain barrier (BBB)³³ - its concentration in CSF reaches 11–56% of plasma concentrations³⁴. Minocycline inhibits the microglial-mediated release of proinflammatory cytokines IL-1 β , TNF- α , IL-6, and p38³⁵, while promoting release of the anti-inflammatory cytokine, IL-10³⁴. Moreover, minocycline inhibits matrix metalloproteinases which process

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3 cytokines such as TNF- α and IL-1 β into their biologically active forms³⁵. Minocycline is
4 also an effective scavenger of proapoptotic reactive oxygen species and protects against
5 excitotoxicity by preventing glutamate-induced activation of nitric oxide synthase
6 (NOS)³⁶. Nitric oxide facilitates glutamate release from presynaptic neurons and inhibits
7 glial glutamate transporters, amplifying glutamatergic signaling, and contributing to
8 excitotoxic cell death¹⁰. Minocycline also upregulates a key molecular factor in the
9 apoptosis pathway, B-cell CLL/lymphoma 2 (BCL-2)³⁷, an effect shared by lithium,
10 valproate³⁸ and certain antidepressant drugs³⁹. BCL-2 represses apoptosis induced by
11 cytotoxic insults⁴⁰. Conceivably, minocycline may additionally reduce inflammation
12 indirectly by blocking the translocation of bacteria across the intestinal barrier. In mice
13 exposed to a social stressor, bacteria translocated across the intestinal barrier stimulating
14 the release of circulating cytokines such as IL6, and increasing microbicidal activity via
15 inducible NOS⁴¹. Additionally, stress induced a change in the community structure of the
16 microflora in the cecum with a decrease the relative abundance of bacteria in the genus
17 Bacteroides and an increase the relative abundance of bacteria in the genus Clostridium.
18 Notably, these effects were blocked by pretreatment with a broad spectrum antibiotic⁴¹.
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32 33 **Minocycline has neuroprotective and anti-inflammatory properties.**

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37 Minocycline prevents glutamate-induced apoptosis of neurons *in vitro*⁴², prevents
38 ischemia-induced activation of microglia in gerbils⁴³, increases hippocampal neuron
39 survival⁴⁴, reduces lesion-volume and improves neurological function in mice with
40 traumatic brain injury⁴⁵ and in fragile X syndrome⁴⁶, reduces pro-inflammatory cytokine
41 expression and improves neurological function and locomotor activity in rats with spinal
42 cord injury⁴⁷, attenuates MDMA-induced neurotoxicity of serotonin and dopamine
43 systems in the cerebral cortex and hippocampus of mice⁴⁸, reduces inflammation in a rat-
44 model of rheumatoid arthritis (RA)⁴⁹, and delays disease progression and demyelination
45 in rodent models of encephalitis⁵⁰, amyotrophic lateral sclerosis (ALS)⁵¹ and
46 Huntington's Disease (HD)⁵². Based on these data, minocycline was employed, and has
47 shown promise as, a therapeutic agent in human diseases including HD⁵³, rheumatoid
48 arthritis (RA)⁵⁴, and stroke⁵⁵.
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Minocycline has been used to treat psychiatric disorders.

Miyaoka et al.⁵⁶ discussed 2 patients with catatonic schizophrenia who benefited from minocycline. This group then conducted a 4-week trial with minocycline (150 mg/day) in 22 patients with schizophrenia to evaluate its efficacy as an adjunct to antipsychotic drugs⁵⁷. Patients showed a significant improvement in positive and negative symptoms. Levkovitz et al.⁵⁸ recently studied 54 patients with early-stage schizophrenia treated for 6 months with antipsychotic medication and either minocycline (200 mg/day) or placebo in a double-blind trial. Minocycline was associated with a reduction in negative symptoms and improved attention/ memory.

The efficacy of minocycline has not been formally tested in mood disorders. In rodents, minocycline reduced immobility during the forced-swim test⁵⁹, and co-administration of minocycline synergized the antidepressant-like actions of desipramine (but not fluoxetine)⁶⁰. Minocycline also abrogated the depression-like behavior of rodents exposed to lipopolysaccharide (LPS)⁶¹. Levine et al.⁶² presented the case of a 66-year old woman with severe BD, who observed that the tetracycline she took for an infection alleviated her depression. When her depression returned post-treatment, minocycline was reinitiated (150 mg/day). After one week her HAM-D score fell from 25 to 8.

Aspirin (Acetyl-salicylic acid, ASA) also holds potential efficacy in bipolar disorder.

The second aim of this study is to assess the antidepressant efficacy of ASA in bipolar depression. Using a 2 x 2 design we will obtain data providing estimates of the effect size of ASA relative to placebo, ASA relative to minocycline, and ASA in combination with minocycline relative to placebo. These data also will explore the specificity of any effect found for minocycline. The clinical use of low dose ASA primarily has been driven by its role as an anti-thrombotic and thrombolytic. Given the exaggerated death rate from cardiovascular events in BD, this action potentially is advantageous in the management of BD. Nevertheless, the recent literature also supports a role for low dose ASA in the

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3 management of the mood disorder itself, specifically in the amelioration of depressive
4 symptoms.
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8 The mechanism of ASA relates to its capacity to inactivate irreversibly the
9 cyclooxygenase (COX) activity of prostaglandin (PG) H-synthase-1 and PGH-synthase 2
10 (referred to as COX-1 and COX-2, respectively). Although ASA has a short half-life (15
11 to 20 min) ASA's permanent inhibition of COX-1 allows once daily dosing for anucleate
12 platelets. In contrast, because nucleated cells rapidly regenerate this enzyme a shorter
13 dosing interval is required to persistently impact COX activity in cells that mediate
14 inflammatory processes. Moreover, ASA is 50- to 100-fold more potent in inhibiting
15 platelet COX-1 than monocyte COX-2 activity⁶³, so there is nearly a 100-fold variation in
16 the daily dose of aspirin, as higher doses are used to target COX-2 in the management of
17 treating peripheral inflammation (e.g., arthritis) or pain. As reviewed below, preliminary
18 evidence obtained in BD suggests beneficial effects are achieved using ASA in low
19 doses, where aspirin would inhibit COX-1, but not COX-2.
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31 **Aspirin has neuroprotective and anti-inflammatory properties.**

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35 In the brain, recent data indicate that genetic manipulation of COX-1 and COX-2
36 differentially modulate leukocyte recruitment during neuroinflammation, and suggest that
37 reduction of COX-1 activity is neuroprotective, whereas reduction in COX-2 activity is
38 detrimental, during a primary neuroinflammatory response (reviewed in ⁶⁴). Choi et al.⁶⁴
39 propose that these distinct roles reflect the predominant localization of COX-1 in
40 microglia, which play a major role in mediating neuroinflammation, in contrast to the
41 predominant localization of COX-2 in pyramidal neurons. For example, Choi et al.⁶⁵
42 examined the effects of COX-1 or COX-2 deficiency on intracerebroventricular
43 lipopolysaccharide (LPS)-induced neuroinflammation by comparing COX-1 (-/-) and
44 COX-2 (-/-) knockout mice to wild-type (WT) (+/+) control animals. After LPS,
45 leukocyte infiltration and inflammatory response were attenuated in the COX-1 (-/-) mice
46 but increased in the COX-2 (-/-) mice, compared with WT controls. In another study,
47 Choi et al.⁶⁶ examined the effect of COX-1 genetic deletion on the inflammatory
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3 response and neurodegeneration induced by β -amyloid, and found that in COX-1 (-/-)
4 mice, the $A\beta_{1-42}$ -induced inflammatory response and associated neuronal damage
5 were attenuated compared to WT mice. Compatible with these results, in
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7 pharmacoepidemiological studies investigating whether chronic NSAID use reduced the
8 risk of developing Alzheimer's disease (AD), indomethacin, a preferential COX-1
9 inhibitor, showed beneficial effects, while COX-2 selective inhibitors, failed to show
10 any beneficial effect in AD patients with mild to severe cognitive impairment. These data
11 suggest the hypothesis that inhibition of COX-1 activity may be a valid therapeutic
12 strategy to reduce the cerebral inflammatory response and neurodegeneration in
13 neuropsychiatric diseases in which neuroinflammatory components play a role in
14 pathophysiology.
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24 Other researchers hypothesized that NSAIDs would be beneficial in BD more specifically
25 because of their ability to down-regulate activity in the brain arachidonic acid (AA)
26 cascade by via interfering with phospholipase A2 (PLA2) and/or COX function. In
27 rodents Rapoport and colleagues⁶⁷⁻⁶⁹ demonstrated that conventional mood stabilizers
28 decrease the AA turnover in phospholipids and the expression of PLA2 and/or COX
29 enzymes. The PLA2 and COX enzymes catalyze, respectively, release of AA from
30 membrane phospholipid and AA conversion to eicosanoids such as prostaglandin E2 and
31 thromboxane B2. The AA cascade is involved in neuroreceptor-initiated signaling and
32 can be pathologically upregulated by neuroinflammation and excitotoxicity.
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42 Nevertheless, aspirin has additional mechanisms that may underlie benefits in
43 neuropsychiatric illness. While low-dose aspirin down-regulates AA cascade activity via
44 inhibition of COX-1 activity, in higher doses it also down-regulates COX-2 gene
45 transcription, increases levels of lipoxygenase-derived eicosanoids such as the anti-
46 inflammatory lipoxin A4, and acetylates COX-2 protein to a modified enzyme that can
47 convert unesterified AA to anti-inflammatory mediators such as 15-epi-lipoxin A4
48 (reviewed in ⁷⁰). The acylated enzyme also can convert docosahexaenoic acid (DHA) to
49 17-(R)-OH-DHA, which, like its metabolites di(R)-OH-DHA (neuroprotectin (R) D1)
50 and tri(R)-OH-DHA (resolvin (R) D1), is highly anti-inflammatory (reviewed in ⁷⁰).
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Lithium given chronically to rats with lipopolysaccharide-induced neuroinflammation also increases the brain concentration of 17-OH-DHA. Thus, there may be a synergy between aspirin and lithium in forming anti-inflammatory brain DHA metabolites.

Aspirin appears effective in preliminary studies of mood disorders.

Pharmaco-epidemiological data in BD supportive of these hypotheses were published by Stolk et al.⁷⁰. Using the Netherlands based PHARMO Record Linkage System (which connects pharmacy dispensing records to hospital discharge records of > two million individuals since 1985), these researchers tested whether non-steroidal anti-inflammatory drugs (NSAIDs) or glucocorticoids would ameliorate bipolar symptoms. The target sample consisted of 5,145 patients receiving lithium (mean age = 48.6 ± 15 yrs; mean duration of lithium use=847 days), based upon the assumption that lithium treatment is relatively specific to individuals with BD. The main outcome measure was a calculated incidence density (ID) of medication events (change in the type or numbers of psychotropic medications prescribed, or increase [$>30\%$] in the psychotropic drug dose). Subjects receiving low-dose (≤ 80 mg/day) aspirin were 17% less likely to have a medication event, a finding that remained significant after adjusting for age, sex, chronic disease score and health care utilization. This effect was selective for low-dose ASA. In contrast, high-dose aspirin or non-selective NSAIDs (i.e., regimens expected to inhibit both COX-1 and -2), selective COX-2 inhibitors and glucocorticoids did not produce a statistically significant protection. Instead, the co-administration of non-selective NSAIDs and glucocorticoids was associated with statistically significant increases in medication events, suggesting destabilization of bipolar illness. The finding that low-dose aspirin decreased the number of medication events was particularly noteworthy since aspirin does not significantly augment serum lithium levels in contrast to selective COX-2 inhibitors which can raise lithium levels⁷¹. These preliminary observations thus appeared consistent with the hypothesis that COX-1 inhibitors can reduce neuroinflammatory processes and thus benefit BD patients.

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3 Notably, the observation that beneficial effects in BD were conferred by low-dose ASA,
4 but not by nonselective COX inhibitors, COX-2 inhibitors or glucocorticoids, appeared
5 inconsistent with the hypothesis that drugs that down-regulate AA cascade activity in
6 general hold therapeutic potential in BD. Thus the putative neuroprotective effects
7 associated with COX-1 inhibition may contribute specifically to the benefits of low-dose
8 aspirin in BD observed by Stolk et al. For example, as reviewed above, aspirin and
9 lithium may exert synergistic effects in forming anti-inflammatory brain DHA
10 metabolites (reviewed in ⁷⁰).

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20 Other data suggest that aspirin exerts antidepressant effects within the context of MDD or
21 cardiovascular illness. Mendlewicz et al.⁷² examined the effect of aspirin augmentation of
22 conventional antidepressant pharmacotherapy in 24 patients with MDD who had proven
23 non-responsive after 4 weeks of SSRI treatment. Participants were treated openly during
24 the subsequent 4 weeks with aspirin 160 mg/day in addition to their SSRI regimen. The
25 combined administration of SSRI plus aspirin was associated with a response rate of
26 52.4%. Remission was achieved in 43% of the total sample and 82% of the responder
27 sample. In the responder group, a significant improvement was observed within week 1
28 and this benefit persisted through day 28. In another study Ketterer et al.⁷³ reported that
29 in 174 males undergoing coronary angiography (of whom 99 were taking low-dose
30 aspirin), aspirin use was associated with less depression and anxiety symptoms.

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40 In contrast, a preliminary study of the selective COX-2 inhibitor, celecoxib, was negative
41 in bipolar depression⁷⁴, potentially compatible with the negative results of COX-2
42 inhibitors reported by Stolk et al.⁷⁰. In a double-blind, randomized, add-on clinical trial of
43 celecoxib in patients (n = 28) studied during a depressed or mixed episode of BD, no
44 significant difference was observed between the celecoxib and placebo add-on groups at
45 study endpoint⁷⁴. These results contrasted with those obtained using celecoxib in unipolar
46 depression, however. In MDD, celecoxib augmentation of either reboxetine⁷⁵ or
47 fluoxetine⁷⁶ was associated with a significant therapeutic effect on depressive symptoms
48 in randomized, double-blind, add-on clinical trials.

METHODS AND ANALYSIS

Participants

One hundred and twenty male or female outpatients between 18 and 55 years of age, who meet DSM-IV-TR criteria for BD (type I or II) and for a current major depressive episode will be recruited. The depressive syndrome must have been present for at least 4 weeks and the minimum threshold for depression severity will be set at a 17-item HAM-D score ≥ 18 . Subjects will provide written informed consent as approved by the Western Institutional Review Board.

Concurrent Medications

At study entry type I BD subjects must have been taking a stable dose of a mood-stabilizing medication (lithium, valproate, carbamazepine, lamotrigine, antipsychotic agents), for at least 4 weeks, dosed clinically to target the therapeutic range. Type II BD subjects will be included irrespective of whether they present on a mood stabilizer. To investigate the utility of this augmentation strategy in the population for whom minocycline is most likely to prove therapeutically relevant, volunteers receiving stable doses of mood stabilizing, antipsychotic, antidepressant, and/or anxiolytic drugs for at least 4 weeks will be included. However, volunteers who currently are receiving more than 4 psychotropic medications in a daily regimen will be excluded, since this condition may signify a more brittle or complex clinical state. Subjects may remain in psychotherapy or have no psychosocial intervention. Volunteers will be excluded if they currently are receiving medications likely to have adverse interactions with minocycline or aspirin, including [NSAIDS](#), warfarin, digoxin, penicillins, and isotretinoin products.

For participants who enter the study, the preferred strategy will be for subjects to maintain the same regimen of concurrent medications throughout the six week study so that only the study drug regimen will be altered per protocol. Nevertheless, changes to concurrent medications will not affect study status, so long as the medication change does

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3 not target a depressive or manic symptom. If changes to concurrent medication regimens
4 are clinically required to address worsening depressive symptoms or the development of
5 manic symptoms, then the subject will be dropped from the study.
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10 **Study Design**

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14 Patients will participate in a randomized, double-blind, placebo-controlled, trial with a 2
15 x 2 design. As adjuncts to existing treatment, subjects will receive placebo-minocycline
16 plus placebo-aspirin, active-minocycline plus placebo-aspirin, placebo-minocycline plus
17 active-aspirin, or active-minocycline plus active-aspirin. The randomization sequences
18 will be determined by a research staff-member who is not obtaining clinical information
19 from the research subject and will be assigned by subject number at consenting. [A
20 restricted randomization \(permuted block randomization\) method will be used in which
21 subjects are randomly allocated to each block \(n=30\) to ensure that equal numbers of
22 participants receive each drug/placebo combination. In order to ensure that experimental
23 group assignment is not skewed across the two trial sites, the study progress will be
24 monitored by individuals who are not involved in the data collection, and in the case of
25 “drift”, adjustments will be made as necessary.](#)
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37 The trial will be conducted over 6 weeks and will comprise 7 assessment sessions (figure
38 1). The subject will be seen at the prescribed time intervals within a window of two
39 business days on either side of visit target date to complete the specified visits.
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44 At each session, a clinical assessment will be conducted using the rating scales listed
45 below, and treatment side-effects will be assessed and rated for severity. To preserve the
46 rater blind, the research staff member who conducts the clinical ratings will not be the
47 research staff member who assesses the presence of side effects, and will remain blind to
48 the information pertaining to side effects. Subjects who experience severe adverse effects
49 or who develop treatment-associated hypomania or mania will be dropped from the
50 study, instructed to discontinue the study medication, and referred for appropriate clinical
51 management of these adverse events.
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5 The primary outcome measure will be the change in the Montgomery-Asberg Depression
6 Rating Scale (MADRS) scores [at the seventh assessment session \(week 6\)](#).
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10 Medication

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14 This pilot proof-of-concept study will adhere to the dosing limits and route of
15 administration for the FDA indications for minocycline's and aspirin's use in other
16 conditions (thus an IND is not required). A fixed dose design will be followed, and all
17 medications will be administered via the p.o. route. The pilot data extant for both study
18 drugs supports an onset of improvement within two weeks, so the six week study
19 duration is expected to provide sufficient time to detect an antidepressant effect, to
20 provide information about the persistence of the antidepressant effect over about one
21 month from the anticipated onset of effect, and to minimize dropouts.
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30 For minocycline the starting dose will be 100 mg b.i.d. (total daily dose=200 mg). This
31 dose of 100 mg b.i.d. has been shown by a substantial literature to produce consistent
32 anti-inflammatory effects in rheumatoid arthritis and other inflammatory disorders. This
33 also is the dose used in a recent schizophrenia treatment trial⁵⁸. The associated placebo
34 capsules match the appearance of the 100 mg minocycline capsule.
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40 The starting dose of aspirin will be 81 mg p.o. b.i.d. This dose is sufficient to inhibit
41 COX-1, and appeared beneficial in stabilizing the course of BD in the pharmaco-
42 epidemiological study of Stolk et al.⁷⁰. When aspirin is used as an anti-platelet drug once
43 daily dosing is sufficient since anucleate platelets do not produce enough COX-1 to
44 overcome the irreversible inhibition of COX-1 within a 24-hour period. In contrast, in
45 nucleated cells COX-1 is replenished, so more frequent dosing is required to persistently
46 inhibit COX-1. Thus we will administer the dose in a b.i.d. regimen, according to the
47 guidelines described above. A total daily dose of 160 mg was administered in the
48 preliminary study which reported that aspirin significantly augmented the antidepressant
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3 effects of fluoxetine in MDD⁷². The relevant placebo matches the appearance of the
4 aspirin tablet.
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9 Participants will be advised that one of the study drugs may reduce the efficacy of oral
10 contraceptives, and to avoid taking the study drugs within 3 hours of iron products or of
11 antacids containing calcium, magnesium or aluminum. They also will be advised that one
12 study drug can increase their risk for bleeding during surgical procedure or if combined
13 with other drugs or herbal preparations that reduce hemostasis.
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17 18 19 **Compensation**

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22 Participants will be compensated for participation in the amount of \$300.00.
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26 27 **Treatment Compliance**

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30 To enhance compliance, study participants will be given an information sheet to take
31 home detailing the procedure to be followed in the case of a missed dose, and requesting
32 that this information be recorded for the investigators. The number of capsules and tablets
33 remaining in each supply given to the patients will also be counted to evaluate treatment
34 compliance. In cases where treatment compliance is poor, subjects will be excluded from
35 the data analysis, using conventional criteria for defining adequate compliance in a
36 clinical trial.
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47 48 **Psychiatric Assessment and Clinical Ratings**

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51 Patients will be evaluated and followed in the outpatient clinics at LIBR or Oklahoma
52 University School of Community Medicine in Tulsa, OK, or at the University of Kansas
53 Medical Center Research Institute (KUMCRI) in Wichita, KS. The diagnosis of BD will
54 be established using DSM-IV-TR criteria on the basis of an unstructured interview
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3 conducted by a psychiatrist and the MINI-Plus administered by trained psychiatric
4 interviewers. The following rating scales will be administered: MADRS, Quick Inventory
5 of Depressive Symptomatology (QUIDS; 16 item), Hamilton Anxiety Rating Scale
6 (HAM-A), Young Mania Rating Scale (YMRS), Universal Fagerstrom (to assess nicotine
7 use), Hollingshead socioeconomic scale, Sheehan Disability Scale (SDS) and the Family
8 Interview for Genetic Studies (FIGS). Medical assessment will include a physical
9 examination, electrocardiogram, complete blood count (CBC), electrolytes and liver-
10 function assays (SMA 20), thyroid panel, and urinalysis, serum drug and pregnancy tests
11 at study entry and study completion. At each follow-up session, the MADRS, HAM-A,
12 YMRS, and Clinical Global Impressions (CGI) scale will be repeated. Physical and
13 psychiatric symptoms will be evaluated and recorded in order to measure the side-effect
14 profiles of minocycline and aspirin. Participants will be questioned about adverse
15 reactions, including dizziness, photosensitivity, hyperpigmentation, gastrointestinal
16 distress or bleeding at each assessment and will be withdrawn from the study if medically
17 necessary. Vital signs will be measured at entry and at each session.
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32 **Immune System Measures**

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35 The activity of peripheral cytokines correlates with inflammatory processes in the CNS.
36 Peripheral cytokines cross the BBB, and can propagate signals across the BBB in the
37 form of small, freely diffusible lipophilic molecules such as prostaglandins, which induce
38 the production of cytokines from glia⁷⁷. The measurement of peripheral markers of
39 inflammation thus serves as a valid, if indirect assessment of CNS inflammation.
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46 To explore predictors and correlates of treatment outcome, blood will be sampled for
47 testing plasma and whole blood peripheral blood monocyte (PBM) based markers of
48 inflammation at baseline and study end. These markers will include 10 cytokine proteins
49 (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IFN γ , and TNF, high sensitivity
50 (hs) CRP, and RNA expression of candidate genes from PBMCs. Candidate genes
51 include IL-6, TNF, and IRF5 (a factor that mediates monocyte polarization). The 10
52 inflammation-related cytokines and the PBMC mRNA will be assayed from plasma at
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3 baseline and study end. We selected the markers IL-6, TNF and CRP because they are the
4 most widely implicated in mood disorders. The other cytokines included in the cytokine
5 bead array assays are measured simultaneously with IL-6 and have all been implicated in
6 the general regulation of inflammation. A meta-analysis of >100 studies found that IL-6
7 and CRP each were significantly elevated in depressed patients with standardized mean
8 difference scores (d) of 0.71 and 0.26, respectively⁷⁸. The associations remained
9 significant after adjustment for body-mass index (BMI) and smoking. Moreover, IL-6 has
10 been shown to modulate HPA axis function by inducing CRH release,
11 adrenocorticotrophic hormone synthesis, and corticosteroid production⁷⁹. CRP production
12 is induced by the proinflammatory cytokines, IL-1, IL-6, and IL-17, and is thus a non-
13 specific marker of systemic inflammation.
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25 Three blood samples will be transported to the immunology lab in the Department of
26 Surgery at the University of Oklahoma College of Medicine for each participant at each
27 of the sampling time-points (sessions 1 and 7). One sample will be centrifuged to obtain
28 plasma which will be stored at -80°C until analyzed. Serum CRP, IL-6, TNF, and the
29 other cytokines listed above will be assayed in duplicate with ELISA (CRP high-
30 sensitivity kit, R & D Systems, Oxford, UK) or enhanced cytokine bead array flex kits
31 (Becton Dickinson) using the manufacturer's reagents and standards. The other two
32 samples will be used to isolate PBMCs and plasma and will be frozen until processed.
33 Monocytes will be isolated from the PBMCs in order to assess mRNA levels similar to
34 the method used by Padmos et al.³². This procedure utilizes monoclonal antibodies
35 directed against human CD14 to isolate monocytes in peripheral blood monocyte cell
36 suspensions. A magnetic cell sorting system will be used for the separation of the
37 monocytes and flow cytometry will be used to gauge the purity of the population. Once
38 purity is established, total RNA will be isolated from the monocytes using an RNeasy kits
39 (Qiagen) according to the manufacturer's directions. RNA will then be reverse
40 transcribed to cDNA using standard commercial kits. rtPCR reactions will be performed
41 using the Dynamo Sybr Green HS Master Mix (New England Biolabs) and custom
42 primers will be synthesized by a commercial laboratory. Real-time rtPCR reactions will
43 be run using a Cepheid Smart Cyclo II or similar instrument. Additional aliquots of
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3 serum and plasma will be stored so that other inflammatory markers can be tested in the
4 future using Luminex bead arrays and/or additional available technologies.
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8 9 **Source of Compounds Tested**

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12 Minocycline and aspirin are available on a generic basis, and are manufactured within the
13 USA by several companies. The identity of the active medicines and placebos will be
14 blinded using placebos that match the appearance of the active drugs. The medications
15 and placebos have been formulated by Wedgewood Pharmacy, Swedesboro, NJ. The
16 study minocycline capsule and chewable aspirin tablet are identical in appearance to their
17 corresponding placebos.
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24 25 **Outcome Measures and Data Analysis**

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28 Antidepressant response will be evaluated by assessing changes in MADRS scores at
29 assessment session # 7 (i.e. 6 weeks). Our a priori hypothesis is that minocycline and/or
30 aspirin plus existing medication will exert greater antidepressant effects than placebo plus
31 existing medication by study completion. Assuming that there are equal numbers of
32 subjects in each treatment group, this hypothesis will be statistically assessed using a
33 group (for the four treatment cells)-by-session (1 vs. 7) repeated measures analysis of
34 variance (ANOVA). If the ANOVA statistic is significant, between- and within-group t
35 tests will be used in planned comparisons to identify the nature of the effect leading to the
36 significant overall ANOVA statistic. We expect to find a significant group-by-session
37 interaction, attributable to a greater reduction in MADRS scores in the minocycline and
38 aspirin groups compared to the placebo group between session 1 and session 7. If there is
39 an imbalance in the number of subjects across groups, (e.g., due to differential dropout
40 rates during the first treatment week), the data analysis will be conducted with a mixed-
41 effects model.
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55 A Mixed Effect Model Repeated Measure (MMRM)⁸⁰ will be used to impute missing
56 data points as this method has been shown to be superior to last observation carried
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3 [forward \(LOCF\) which can inflate the Type I error rates⁸¹. The LOCF and observed cases](#)
4 [\(OC\) approaches to data imputation will be used *post-hoc* to provide further confirmation](#)
5 [of the results obtained under the MMRM analysis.](#)
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10 In order to test whether the putative antidepressant effects of minocycline or aspirin have
11 a rapid onset, as a *post hoc* analysis the ANOVA will be repeated using MADRS ratings
12 from the assessment that follows the first week of exposure to active drug versus the
13 corresponding change under placebo; i.e. session 2. *Post hoc* tests will be performed to
14 assess the significance of changes in the secondary clinical outcome measures (QUIDS
15 16, HAM-A, YMRS, CGI-I).
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21 The rate of completion in the [four](#) cells also will be considered an outcome measure. The
22 completion rate in the minocycline [and/or aspirin](#) arms may be influenced more by
23 dropouts due to side effects while the completion rate in the placebo group may be
24 influenced more by dropouts due to non-response. [Two different measures of completion](#)
25 [rate will be obtained: completion of week 1 of the study \(baseline to week 1\) and](#)
26 [completion of the study \(baseline to week 6\). Differences between the groups in](#)
27 [completion rates will be assessed with an ANOVA.](#)
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37 We will test the hypothesis that minocycline and aspirin reduce inflammation (e.g. CRP,
38 IL-6, IL-6 mRNA) more than placebo using statistical analyses similar to those described
39 above. If the assay results are normally distributed then a group-by-session repeated-
40 measures ANOVA with CRP, IL-6 and nine other cytokine levels as dependent variables,
41 and BMI, smoking status, and time of blood draw as covariates, will be used to assess
42 anti-inflammatory effects of minocycline and aspirin. [Mixed-effect models will be used if](#)
43 [necessary.](#) If the CRP or inflammatory cytokine data are not normally distributed
44 (Kolmogorov-Smirnov test) or if the equality of statistical variance assumption across
45 assessments is violated (Levene's test), then Friedman's ANOVA will be used to test for
46 CRP or inflammatory cytokine differences between groups. If the Friedman's ANOVA
47 statistic is significant, Wilcoxon sign-ranked tests will be used for post-hoc analysis of
48 group differences. Nonspecific factors that influence CRP and inflammatory cytokine
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3 levels include time of day, presence of infection, treatment with anti-inflammatory
4 medications, smoking, obesity, and alcohol abuse. We will attempt to control for these
5 potential confounds by measuring BMI and recording NSAID and nicotine use
6 (Universal Fagerstrom scale), and by excluding individuals who have recently abused
7 substances or who have intercurrent infections. The serum CRP concentration shows
8 minimal diurnal variability in adults⁸² but IL-6 and other cytokine levels vary across time
9 of day⁸³. To minimize cytokine measurement variability due to circadian fluctuations, we
10 will schedule patient assessment sessions at the same time each day. Since this may not
11 always be possible, we will record the time of day that each blood-draw is made, divide
12 the day into quartiles: 7am-10am, 10am-12pm; 12pm-3pm, and 3pm-6pm, and use these
13 data as a covariate in the statistical analyses.
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25 To test whether baseline levels of CRP and inflammatory cytokines predict response to
26 minocycline or aspirin, we will subclassify the participants using conventional criteria⁸⁴
27 as achieving full response ($\geq 50\%$ reduction in MADRS score from baseline), partial
28 response ($< 50\%$ but $\geq 25\%$ reduction), or nonresponse ($< 25\%$ reduction). Patients
29 achieving remission (post-treatment MADRS score ≤ 10) will also be identified. A non-
30 parametric alternative to the ANOVA statistic, the Mann-Whitney test, will be used to
31 compare remitted and non-remitted groups in baseline levels of inflammatory cytokines
32 and CRP if the data are not normally distributed. Ideally, the impact of baseline levels of
33 inflammation on treatment response would be tested more rigorously using a formal
34 stratified design. However, in order to conduct a stratified trial with for example, 8
35 experimental groups (4 x high versus low inflammation), the sample size of the study
36 would have to be doubled, which would significantly increase costs and decrease
37 feasibility. Nevertheless, this stratification approach would be important to consider for
38 future studies if promising results are obtained in this clinical trial.
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50 51 **Statistical Power**

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55 A recent meta-analysis of 96 antidepressant treatment studies found that the average
56 effect size of a placebo treatment is 1.69 compared with 2.50 for an antidepressant
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3 treatment⁸⁵. We calculated that in order to detect [an effect size](#) of 0.81 ([i.e. the difference](#)
4 [between 2.50 and 1.69](#)) with an 80% probability (2-sided test, $\alpha=0.05$), we will require a
5 sample size of 26 subjects per group
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8 (http://hedwig.mgh.harvard.edu/sample_size/size.html). Thus given our sample size of 30
9 per group we should have sufficient power to test Specific Aim 1, allowing for a 13%
10 drop-out rate [during week 1 of the study \(dropouts after completion of study week 1 will](#)
11 [be included in the analysis under the MMRM approach described above\)](#).
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18 As discussed above, a recent meta-analysis⁷⁸ of cross-sectional studies of serum-derived
19 IL-6 and CRP in depression calculated effect sizes of 0.71 for IL-6 and 0.26 for CRP.
20 Based on these effect sizes a sample size of 26 would yield >80% probability of detecting
21 significant depression-related changes in IL-6, but only a 60% probability of detecting a
22 depression-related change in CRP. There are 3 reasons why we believe that these CRP
23 power estimations are not applicable to this study. Firstly, the effect sizes derived from
24 the meta-analysis are based on cross-sectional studies. Given the effect of variables such
25 as smoking, diet, exercise, and BMI on proinflammatory cytokines, a within-subjects
26 design is likely to reduce non-depression-related sources of variance, and substantially
27 increase statistical power. Secondly, we are not only examining the effect of mood on IL-
28 6 and CRP levels, but are treating patients with minocycline and aspirin, drugs known to
29 possess anti-inflammatory properties. We therefore suggest that our proposed study is
30 likely adequately powered to detect any true changes in plasma IL-6, CRP, and the other
31 inflammatory cytokines across treatment blocks.
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44 Regarding IL-6 mRNA gene expression in peripheral blood monocytes, Padmos et al.³²
45 reported a 38-fold increase in IL-6 mRNA levels in unmedicated patients with BD
46 compared with HC. Since minocycline reduces IL-6 levels (see above) we expect our
47 study to have very high power to detect differences between groups, as well as changes in
48 response to minocycline. The simultaneous detection of nine other inflammation-related
49 cytokines, in addition to IL-6 (using newer more sensitive technology) will provide much
50 finer resolution of the effects on inflammatory cascades than that measured in previous
51 studies.
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ETHICS AND DISSEMINATION

Gender/Minority/Pediatric Inclusion for Research

Women and Minorities will be included in the study without prejudice according to their representation in the study population. Participants will be recruited from the greater metropolitan areas of Tulsa, OK and Wichita, KS and efforts will be made to ensure that our subject population resembles the gender, ethnic and racial composition of these areas.

Exclusion Criteria

The following exclusion criteria apply: 1) inability to provide informed consent; 2) age of onset of BD > 40 years; 3) serious risk of suicide; 4) current delusions or hallucinations sufficient to interfere with the capacity to provide informed consent; 5) current manic symptoms [depressed BD patients with concurrent manic symptoms have been found to be more likely to experience adverse reactions in antidepressant treatment trials⁸⁶]; 6) medical illness including as hepatic impairment, renal dysfunction, bleeding diatheses (e.g., hemophilia), cerebrovascular disease or heart disease, hypertension that is inadequately controlled by medication, diabetes mellitus, or known peptic ulcer disease; 7) abuse of drugs or alcohol within the preceding 6 months, or substance dependence within the last 5 years; 8) daily alcoholic beverage consumption equivalent to ≥ 3 oz. of alcohol; 9) asthma or known allergies or hypersensitivities to tetracycline antibiotics, aspirin or other NSAIDs; 10) current use of drugs that could increase the risks associated with aspirin or minocycline administration, namely other antibiotic medications, other NSAIDs or anticoagulants (e.g., warfarin), acetazolamide, or methotrexate; 11) known HIV or other chronic infection including, but not limited to viral hepatitis. 12) Pregnant or nursing women, and women who are attempting to conceive during the 6 week study period, will also be excluded.

Specimens, Records, and Data Collection

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5 A physician, registered nurse, or trained phlebotomist will utilize a sterile technique to
6 draw 60 ml of blood by venipuncture. Participants will also be asked to submit a urine
7 sample. A physician, registered nurse, or trained technician will collect EKG data from
8 the subject in a private exam room.
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12 13 14 **Recruitment and Consent Procedure** 15

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18 Volunteers will be recruited from the community as well as from the clinical services at
19 the Laureate Psychiatric Clinic and Hospital and the Oklahoma University School of
20 Community Medicine in Tulsa, OK, and from the clinical services affiliated with the
21 KUMCRI. Volunteers may be referred from sources that include physicians, newspaper
22 advertising, self-help organizations, self-referral, and WIRB approved flyers posted at
23 local universities, schools, churches and grocery stores. Participants may be pre-screened
24 through screening protocols based at LIBR or KUCRI. We plan to recruit a total of 120
25 participants.
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33 All participant interactions including consenting will be conducted in private interview /
34 exam rooms. These rooms are secured from public areas via combination locked doors
35 that are only accessible to authorized personnel. Prospective participants will receive an
36 explanation of the objectives, procedures, and hazards of this protocol that is appropriate
37 to their level of understanding. The right of the subject to decline to participate or to
38 withdraw from the study at any time will be made clear.
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46 Non-English speaking participants will not be recruited.
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50 After the consent form is verbally explained to the participant, and any questions have
51 been answered, the researcher will leave the room to allow the participant to read the
52 consent form thoroughly. Family members will be allowed to be present and to discuss
53 the consenting process with the participant. After the consent is read, the researcher will
54 return and answer any additional questions the participant may have. The researcher will
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3 remind the subject that participation is strictly voluntary and that they have the right to
4 withdraw at any time. Participants will be asked to arrive 30 minutes early in order to
5 have sufficient time for the consenting process.
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10 **Subject Risks**

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14 The risks of behavioral testing are minimal. The risks of blood drawing are also minimal.
15 Possible mild side effects of the blood draw include mild pain or bruising at the site of
16 the venipuncture.
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21 Minocycline has been used a broad-spectrum antibiotic for many years in doses up to 400
22 mg/day³⁴. It has been used on a chronic basis to treat acne and rheumatoid arthritis, often
23 for many years, in hundreds of thousands of patients. The most commonly encountered
24 side effects are upset stomach, diarrhea, dizziness, drowsiness, ataxia, vertigo, headache
25 and vomiting. Prolonged use can be associated with pigmentation of the skin, gums or
26 teeth. Between 1975 and 2006, the World Health Organization Collaborating Center for
27 International Drug Monitoring listed 122 cases of adverse drug reactions to intravenous
28 minocycline; most commonly, abnormal hepatic function and thrombocytopenia³⁴. These
29 included cases of serious liver injury, including irreversible drug-induced hepatitis and
30 fulminant hepatic failure that was fatal in two cases, thought to be due to triggering or
31 unmasking autoimmune hepatitis. One case of autoimmune-related glomerulonephritis
32 has been reported. The role of oral minocycline in precipitating these conditions has not
33 been clearly established. Minocycline also has been associated with idiopathic
34 intracranial hypertension (pseudotumor cerebri). Long-term trials have shown that
35 minocycline is well tolerated. In a 2-year trial of minocycline (200 mg/day) for RA, 3 of
36 30 patients withdrew due to finger-nail discoloration, dizziness, or erythematous rash⁵⁴.
37 Of 11 patients with HD treated with minocycline (100 mg/day) for 2 years, one
38 complained of nausea in the first 3 weeks, and two of sedation⁵³, while in a 6-month trial
39 of minocycline for ALS, the mean tolerated dose was 387 mg/day and the most common
40 adverse effects were gastrointestinal⁸⁷. Five of 36 patients with schizophrenia withdrew
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3 from a 6-month trial of minocycline (200 mg/day) due to indigestion (n=2), pigmentation
4 (n=2), or a suicide attempt (n=1)⁵⁸.
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9 Low dose aspirin has been safely used in many millions of patients on a worldwide scale
10 for its role as an anti-thrombotic and thrombolytic. A meta-analysis of >100 randomized
11 trials in high-risk patients indicated that low-dose ASA reduced cardiovascular death by
12 15% and prevented nonfatal vascular events by about 30%⁸⁸. These data stand in striking
13 contrast to the data obtained in COX-2 inhibitors, which can increase cardiovascular risk.
14 In clinical trials of several COX-2 selective and nonselective NSAIDs of up to three years
15 duration have shown an increased risk of serious cardiovascular (CV) thrombotic events,
16 myocardial infarction, and stroke, which have in many cases been fatal⁸⁹. Patients with
17 known CV disease or risk factors for CV disease are at greater risk for such events during
18 chronic treatment with COX-2 inhibitors. Evidence from human pharmacology and
19 genetics, genetically manipulated rodents, and other animal models and randomized trials
20 indicates that this is consequent to suppression of COX-2-dependent cardioprotective
21 prostaglandins, particularly prostacyclin⁹⁰.
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33 Aspirin does not cause a generalized bleeding abnormality unless given to patients with
34 an underlying hemostatic defect (e.g., hemophilia, uremia, or that induced by
35 anticoagulant therapy). Aspirin-induced impairment of primary hemostasis cannot be
36 separated from its antithrombotic effect and is similar at all doses ≥ 75 mg/d⁹¹. The risk
37 of intracranial bleeding is exceedingly rare (<0.1% in high risk populations), but is higher
38 in individuals with cerebrovascular disease⁸⁸. Hypertension that is inadequately
39 controlled by medication often is considered a contraindication to aspirin because of the
40 concern that possible benefits in the prevention of cardiovascular events may be
41 counterbalanced by an increased risk of cerebral bleeding. However, hypertensive
42 patients whose blood pressure is well-controlled appear protected from myocardial
43 infarction by aspirin therapy without an increase in the number of cerebral hemorrhages
44 or strokes⁹². Moreover, aspirin therapy does not affect blood pressure or the response of
45 hypertension to antihypertensive agents^{91 93}.
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3 NSAIDs as a class can cause serious gastrointestinal (GI) adverse events including
4 inflammation, bleeding, ulceration, and perforation of the stomach, small intestine, or
5 large intestine, which rarely have proven fatal. In controlled clinical trials the percentage
6 of patients reporting one or more gastrointestinal complaints has ranged from 4% to
7 16%⁹¹. The mechanism underlying this adverse effect appears attributable to the
8 inhibition of COX-1. Thus, the incidence of GI side effects has been higher for NSAIDs
9 with more potent effects at COX-1, such as aspirin and indomethacin. For example, in
10 controlled trials the incidence of GI side effects for aspirin and indomethacin have been
11 about twice as high as that for ibuprofen, a nonselective COX inhibitor, in equally
12 effective doses for arthritis. Nevertheless, the incidence of GI side effects associated with
13 aspirin is dose-dependent, and thus is markedly lower when using aspirin in the low dose
14 range planned for the current study. Notably, the risk of GI bleeding is not reduced by
15 using the enterically coated aspirin formulations, but is thought to be lower during
16 concomitant use of omeprazole⁹¹. The effects of warfarin and NSAIDs on GI bleeding are
17 synergistic, such that the users of both drugs together have a risk of serious GI bleeding
18 higher than users of either drug alone. Fortunately, the risk of GI bleeding, which reflects
19 the inhibition of prostaglandins in the stomach (from systemic rather than local exposure)
20 is much smaller when using low-dose as opposed to high-dose aspirin.
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37 Low-dose aspirin has not been reported to alter renal function, and does not reduce
38 effectiveness of ACE inhibitors for HTN (in contrast to other NSAIDs)^{93 94}. However,
39 aspirin can inhibit the renal clearance of acetazolamide and methotrexate potentially
40 leading to increased blood concentrations of and toxicity from these agents. Salicylate
41 can displace other drugs which are protein-bound, especially phenytoin and valproic acid,
42 increasing their free drug concentrations in plasma. This may increase side effects,
43 toxicity and/or efficacy for displaced drugs. If the BD subjects are currently receiving
44 valproic acid preparations (e.g., divalproex) then the plasma levels of these agents will be
45 monitored for potential changes.
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55 Aspirin may cause a severe allergic reaction that may include: hives, asthma (wheezing),
56 facial swelling, shock. Aspirin overdose can be fatal at 30 g or higher.
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5 In sum, we believe that our two-by-two design is appropriate for trials involving
6 experimental drugs that already have been well-studied with respect to toxicity, as is the
7 case with aspirin and minocycline. A parallel arm design, as opposed to a 2 x 2 factorial
8 design, would be more clearly informative in the case of an experimental drug for which
9 the toxicity and drug interaction potential have not been thoroughly studied in human
10 subjects
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16 17 18 **PHI Protection** 19

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21 Paper copies of consents, screening forms, the Research Privacy Form, and any other
22 forms, testing results or papers containing Protected Health Information (PHI) will be
23 stored in a secured medical records room with access granted only to authorized
24 personnel.
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30 Electronic data that contain PHI will be managed in accordance with ISO 27000 series
31 information security standards with policies developed from current NIST guidelines (SP
32 800-66) for HIPAA and HITECH compliance. Specific controls implemented to protect
33 PHI are derived from NIST 800-122, and include (but not limited to):
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- 37 1) Access Enforcement (AC-3) – Individual user accounts, role based access control,
38 access control lists;
- 39 2) Separation of Duties (AC-5) – de-identification of data as appropriate,
40 acquire/analyze/manage firewall;
- 41 3) Least Privilege (AC-6) – to ensure PHI data is only available to persons with
42 established need for access;
- 43 4) Remote Access (AC-17) – Secure VPN, encrypted end devices;
- 44 5) Access Control for Mobile Devices (AC-19) – Password login, remote destruction
45 capabilities;
- 46 6) Auditable Events (AU-2) + Monitoring: Log detailed server and network
47 information, alert for problems;
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- 7) Analysis, and Reporting (AU-6) – Procedures to audit system records for inappropriate activity.
- 8) User Identification and Authentication (IA-2) – username/secure password and two factor authentication will be required when appropriate.
- 9) Media Access, Marking, Storage, and Transport (MP-2,3,4,5) – Records will be asset tagged and marked to their PHI status, PHI data will be secured and managed by professional system administrators, and will be transported via encryption (VPN, USB, File);
- 10) Media Sanitization (MP-6) – Data will be destroyed by SFHS in accordance with their policies and procedures;
- 11) Transmission Confidentiality (SC-9) – Encryption will be used when needed for all avenues of data transmission (wireless, network, etc.).

To protect subject confidentiality, blood samples will be anonymized as follows:

1. Last name: All participants will be assigned the last name “LIBR.”
2. First name: The first name will be a secure alpha cryptographic hash based on LIBR user ID. This technique is the gold standard in computer security for one-way correlation of data.

Benefits versus Risks

The participant may benefit from participation if either study drug produces an antidepressant effect. Participants will also receive a free clinical evaluation; more frequent treatment visits than are typical in practice, diligent follow-up in terms of symptoms and side effects, and physical and psychiatric monitoring during the study. The risks of delaying alternative treatments are minimal in relation to the potential long-term benefits to the subjects and the importance of knowledge that may reasonably result. The importance of the knowledge that will likely be gained from this study clearly exceeds the associated potential risks.

Alternative Treatment

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3 It is possible that some patients may feel better with talk therapy. Participating in any
4 type of talk therapy with their psychiatrist or psychologist does not require dropping out
5 of this study. Subjects will be encouraged to contact the study investigators, particularly
6 the physician in the study, with any questions they may have regarding alternatives to
7 treatment through this research study. The study investigators will assist in referring the
8 subject to another physician for treatment after their participation in the study has ended.
9 Physical and psychological testing, blood draws, urine samples, and EKG data provide no
10 known risks to persons other than those listed in the exclusion criteria whereas the
11 combinatory power of these measures may provide information relevant to understanding
12 the pathophysiology of bipolar disorder.
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22 **Data and Safety Monitoring Plan**

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26 This study involves more than minimal risk. The study progress will be overseen by a
27 Data, Safety and Monitoring Board (DSMB). The DSMB is composed of three members
28 who will meet in person or per telephone at least once every 6 months to review relevant
29 study data including adverse events and dropout rates.
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35 Any unanticipated adverse events will be reported immediately to the IRB of record and
36 to the LIBR Human Protection Administrator. Any adverse events will be included in the
37 annual IRB report.
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41 **Dissemination of Results**

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45 The study results will be presented at national and/or international biomedical scientific
46 meetings and published in peer-reviewed journals.
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50 **REGISTRATION**

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3 In accordance with the recommendations of the International Committee of Medical
4 Journal Editors⁹⁵, the proposed trial is registered in a public registry
5 (www.clinicaltrials.gov Identifier: NCT01429272).
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10 11 **Figure Legend**

12 13 14 **Figure 1: Schematic of Study Design**

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18 *Legend: Each session number (total of 7) is encircled, with the timing between sessions*
19 *indicated in weeks with a 2 business day window on either side of visit target date to*
20 *complete the visit. Session 1 is the baseline (green star) and session 7 is the study end*
21 *(purple star). Peripheral blood will be sampled at baseline and study end to assay*
22 *markers of inflammation. The study duration is 6 weeks.*
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29 30 **Author Contributions**

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33 [All authors made a significant contribution to the conception and design of the study](#)
34 [protocol.](#) The protocol was written by Drs. Savitz and W. Drevets and was critically
35 reviewed by Drs. Preskorn, Teague, D. Drevets, and Yates. [All authors gave approval for](#)
36 [the publication.](#)
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42 43 **Funding Statement**

44
45 This work was funded by the Stanley Medical Research Institute, grant number 10T-
46 1401. [The SMRI assisted with the study design but plays no role in the collection,](#)
47 [management, analysis, and interpretation of data; writing of the report; or the decision to](#)
48 [submit the report for publication.](#)
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55 56 **Competing Interests**

None of the authors [has a](#) conflict of interest to declare.

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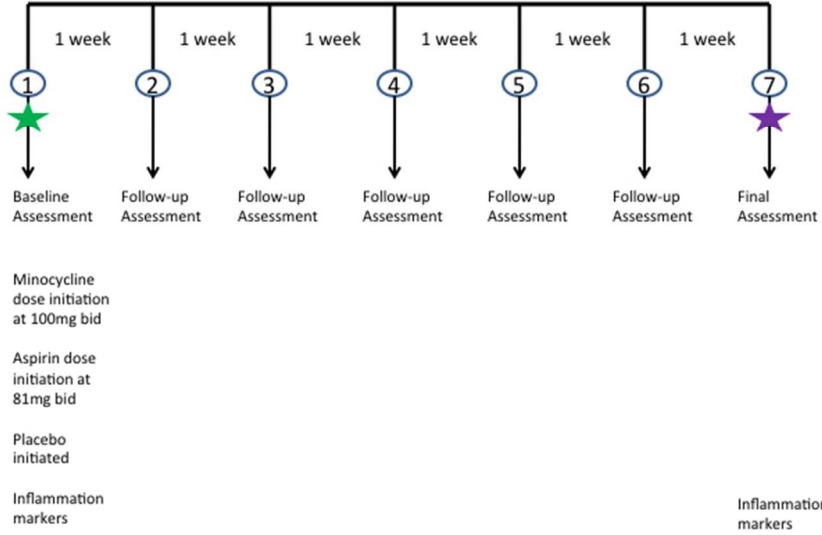
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University of Kansas Medical Center Research Institute, 8911 East Orme, Wichita, Kansas 67207

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Robert A Taylor for

Theodore D. Schultz, J.D., Chairman

8/23/2011

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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-13
	2b	Specific objectives or hypotheses	20
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	14-15
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	13-14
	4b	Settings and locations where the data were collected	17
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	15-16
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	20-21
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	21
	7b	When applicable, explanation of any interim analyses and stopping guidelines	30
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	15
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	15
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	15
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	15

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2		assessing outcomes) and how	
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4		11b If relevant, description of the similarity of interventions	
5	Statistical methods	12a Statistical methods used to compare groups for primary and secondary outcomes	20-21
6		12b Methods for additional analyses, such as subgroup analyses and adjusted analyses	20-21
7			
8	Results		
9	Participant flow (a	13a For each group, the numbers of participants who were randomly assigned, received intended treatment, and	
10	diagram is strongly	were analysed for the primary outcome	
11	recommended)	13b For each group, losses and exclusions after randomisation, together with reasons	
12	Recruitment	14a Dates defining the periods of recruitment and follow-up	
13		14b Why the trial ended or was stopped	
14			
15	Baseline data	15 A table showing baseline demographic and clinical characteristics for each group	
16	Numbers analysed	16 For each group, number of participants (denominator) included in each analysis and whether the analysis was	
17		by original assigned groups	
18			
19	Outcomes and	17a For each primary and secondary outcome, results for each group, and the estimated effect size and its	
20	estimation	precision (such as 95% confidence interval)	
21		17b For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
22	Ancillary analyses	18 Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing	
23		pre-specified from exploratory	
24			
25	Harms	19 All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
26			
27	Discussion		
28	Limitations	20 Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
29	Generalisability	21 Generalisability (external validity, applicability) of the trial findings	
30	Interpretation	22 Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
31			
32	Other information		
33	Registration	23 Registration number and name of trial registry	1, 30
34	Protocol	24 Where the full trial protocol can be accessed, if available	19
35	Funding	25 Sources of funding and other support (such as supply of drugs), role of funders	
36			

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38 *We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also

39 recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials.

40 Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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**MINOCYCLINE AND ASPIRIN IN THE TREATMENT OF
BIPOLAR DEPRESSION: a protocol for a proof-of-concept
randomized, double-blind, placebo-controlled, 2x2, clinical
trial**

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**MINOCYCLINE AND ASPIRIN IN THE TREATMENT OF
BIPOLAR DEPRESSION: a protocol for a proof-of-concept
randomized, double-blind, placebo-controlled, 2x2, clinical trial**

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ABSTRACT

Introduction: New medication classes are needed to improve treatment effectiveness in the depressed phase of bipolar disorder (BD). Extant evidence suggests that BD is characterized by neural changes such as dendritic remodeling and glial and neuronal cell loss. These changes have been hypothesized to result from chronic inflammation. The principal aims of the proposed research is to evaluate the antidepressant efficacy in bipolar depression of minocycline, a drug with neuroprotective and immune-modulating properties, and of aspirin, at doses expected to selectively inhibit cyclooxygenase 1 (COX-1). **Methods and Analysis:** One hundred and twenty outpatients between 18 and 55 years of age, who meet DSM-IV-TR criteria for BD (type I or II) and for a current major depressive episode will be recruited to take part in a randomized, double-blind, placebo-controlled, parallel-group, proof-of-concept, clinical trial following a 2 x 2 design. As adjuncts to existing treatment, subjects will be randomized to receive one of four treatment combinations: placebo-minocycline plus placebo-aspirin, active-minocycline plus placebo-aspirin, placebo-minocycline plus active-aspirin, or active-minocycline plus active-aspirin. The dose of minocycline and aspirin is 100mg bid and 81mg bid, respectively. Antidepressant response will be evaluated by assessing changes in the Montgomery-Asberg Depression Rating Scale (MADRS) scores between baseline and the end of the 6 week trial. As secondary outcome measures, the anti-inflammatory effects of minocycline and aspirin will be tested by measuring pre-and-post treatment levels of CRP and inflammatory cytokines. **Ethics and Dissemination:** Minocycline has been widely used as an antibiotic in doses up to 400 mg/day. Low dose aspirin has been safely used on a worldwide scale for its role as an anti-thrombotic and thrombolytic. The study progress will be overseen by a Data, Safety and Monitoring Board which will meet once every 6 months. Results of the study will be published in peer-reviewed publications. **Registration:** Clinical Trials.gov: NCT01429272.

INTRODUCTION

The treatment of bipolar depression remains a major challenge for psychiatry. The US FDA has not approved any of the ~25 standard antidepressants for the treatment of bipolar depression, partly because these agents have not been robustly effective in BD patients¹. Thus, currently approved treatments for bipolar depression include lithium, quetiapine, and the combination of olanzapine and fluoxetine². Other treatments used include lamotrigine, conventional antidepressant agents, other atypical antipsychotics, pramipexole or riluzole (reviewed in ³). Unfortunately, the effectiveness of these options also is limited. For example, in a placebo-controlled study in which subjects receiving lithium were randomized to receive either standard antidepressant pharmacotherapy (paroxetine or imipramine) or placebo, those receiving lithium plus an antidepressant did not show a significant improvement over those receiving lithium plus placebo⁴. Similarly, in the STEP-BD trial, 42 of 179 subjects (23.5%) receiving a mood stabilizer plus adjunctive antidepressant drug treatment had a durable recovery, which did not differ significantly from 51 of 187 subjects (27.3%) receiving mood stabilizer plus placebo. Mallinger et al. reported a similar durable recovery rate in BD depressives treated with mood stabilizer plus paroxetine (27%), but found a higher rate for adjunctive monoamine oxidase inhibitors (MAOIs; 53%)⁵, consistent with the findings of previous studies comparing MAOIs vs imipramine^{6,7}. Unfortunately MAOIs are commonly unacceptable to patients.

New classes of antidepressant drugs are needed for bipolar depression. Existing agents exert their primary actions on monoaminergic systems. The efficacy of these agents contributed to the monoamine-deficiency hypothesis of depression, which continues to receive empirical support. Nevertheless, the field is in the early stages of a paradigm shift driven by evidence of dendritic remodeling and neuronal atrophy in animal models of depression, and of reductions in gray matter (GM) volume, and glial cell loss at *postmortem* in BD⁸. The neurotrophic effects of lithium, coupled with longitudinal studies demonstrating volumetric changes over time, raise the possibility that mood disorders are underpinned by a neurotoxic process^{8,9}. The final common pathway through

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3 which neurotoxic agents exert their effect is hypothesized to involve excess glutamatergic
4 signaling¹⁰.
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9 The glutamatergic model of mood disorders is based on the premise that excessive
10 stimulation of NMDA-glutamatergic receptors, results in neuronal atrophy and apoptosis
11 of glial and/or neuronal cells, and *ipso facto*, depression. Evidence for this hypothesis
12 derives from multiple sources. In preclinical models, riluzole, which inhibits neuronal
13 release of glutamate, ceftriaxone, which increases glutamate reuptake, and NMDA
14 receptor antagonists such as ketamine, ameliorate behavioral analogs of depression¹¹. In
15 addition, rats bred to be genetically sensitive to stress show differential expression of
16 NMDA receptors¹², and behavioral analogs of depression are abrogated in NMDA
17 receptor subunit knockout mice¹³. In humans, increased serum levels of glutamate that
18 resolve with antidepressant treatment were reported in MDD, and extended to the CSF
19 post mortem¹¹. Polymorphisms of the metabotropic glutamate receptor genes, GRM2 and
20 GRM3, and a haplotype of the glutamic acid decarboxylase (GAD2) gene were
21 associated with MDD¹⁴. Finally, ketamine induced a rapid, sustained antidepressant
22 effect in BD^{15 16} and riluzole showed promising results in treatment-resistant depression¹⁵
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One potential cause of the disruption in glutamatergic signaling in BD is dysregulation of
the immune system. Increased levels of proinflammatory cytokines such as interleukin 6
(IL-6), IL-1 β , interferon alpha (IFN α), tumor necrosis factor alpha (TNF- α)
prostaglandinE2 (PGE2), and chemokine ligand 2 (CCL2) are consistently observed in
the blood and CSF of patients with mood disorders, both at baseline and after exposure to
stressors^{17 18}. Elevated serum levels of (pro-inflammatory) positive acute-phase proteins
(e.g., haptoglobin, α 1-antitrypsin, ceruloplasmin, C-reactive protein), but reduced levels
of negative acute-phase proteins (e.g., albumin and retinal-binding protein) also are
reported in mood disorders¹⁹⁻²¹. Further, treatment of hepatitis C with IFN α is known to
induce the major depressive syndrome and/or manic symptoms in approximately 40% of
patients, and the efficacy of conventional antidepressant drugs is associated with a
reduction in inflammation¹⁸. Moreover, anti-tumor necrosis factor (TNF) therapy (for

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3 psoriasis) can improve mood²². Since proinflammatory cytokines can alter brain function,
4 these data are compatible with evidence that an activated inflammatory response system
5 exists in mood disorders which plays a role in their pathophysiology²³⁻²⁶.
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10 The over-activity of the hypothalamic-pituitary-adrenal axis in mood disorders may play
11 a role in inflammation, since hypersecretion of corticotrophin-releasing hormone (CRH)
12 activates the transcription factor, nuclear factor kappa B (NF-κB). NF-κB regulates the
13 expression of proinflammatory cytokines in immune cells in the CNS and periphery, and
14 the expression of genes involved in apoptosis²⁷. In addition, NF-κB may result in the
15 expression of the class 1 major histocompatibility complex (MHC I), labeling cells for
16 removal by cytotoxic T-cells²⁷. Usually, cortisol suppresses this inflammatory response,
17 but chronic stress appears to desensitize the glucocorticoid receptor (GR) and by
18 extension, the anti-inflammatory effects of cortisol²⁷. Cytokines play a role in
19 desensitizing the system to cortisol. For example, IL1 and TNF-α retard dexamethasone-
20 induced translocation of the GR receptor from the cytoplasm to the nucleus²⁸.
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31 The immunologic and glutamatergic models of BD are complementary because a
32 proinflammatory state is one potential cause of excitotoxicity²⁷. Peripheral inflammatory
33 signals activate microglia in the brain, inducing an inflammatory cascade of cytokines
34 and free radicals. Cytokines and reactive oxygen and nitrogen species exert a direct toxic,
35 apoptotic effect on oligodendrocytes. Potentially through the loss of oligodendrocytes,
36 oxidative stress can lead to demyelination. Such a process conceivably may account for
37 the reduction in oligodendroglia found *postmortem* in the prefrontal cortex²⁹ in mood
38 disorders. The inflammatory milieu also compromises astrocyte function, leading to
39 down-regulation of glutamate transporters and impaired glutamate reuptake into
40 astrocytes, further amplifying inflammatory signaling²⁷.
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51 In addition, cytokines such as interleukin 1 (IL-1), IL-6, and TNF-α activate indoleamine
52 2, 3-dioxygenase (IDO). IDO catalyzes the breakdown of tryptophan, the amino-acid
53 precursor of serotonin, and an important regulator of T-cell function, into kynurenine
54 (Kyn)³⁰. Activation of the Kyn pathway shunts tryptophan away from 5-HT synthesis,
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3 putatively reducing serotonergic transmission. Kyn is in turn metabolized into quinolinic
4 acid (Quin), a potent NMDA receptor agonist, and neuromodulator involved in lipid
5 peroxidation, which can induce neuronal damage via oxidative stress and overstimulation
6 of NMDA receptors³⁰. Consistent with inflammation-related shunt towards Kyn
7 metabolism, the plasma tryptophan-Kyn ratio was found to correlate inversely with
8 striatal total choline (a putative cell membrane turnover biomarker) in adolescents with
9 melancholic depression³¹.
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18 The mRNA transcripts for proinflammatory genes appear particularly sensitive for
19 discriminating BD patients. Microarray gene expression profiles in purified CD14+
20 monocytes from whole blood of BD subjects, offspring of BD parents, and healthy
21 controls (HC) displayed a distinct mRNA signature representing genes from
22 inflammatory and inflammation-related pathways³². The signature showed >80%
23 sensitivity and specificity in BD subjects who were not receiving lithium or antipsychotic
24 drugs (n=11), and in affected offspring of a BD parent (n=13, of whom 10 had only
25 manifested depression). A positive signature also was present in 17 of 38 unaffected
26 offspring (45%) versus 13 of 70 healthy children (19%). Cross-sectional comparisons
27 suggested lithium and antipsychotic drugs—but not conventional antidepressant drugs--
28 down-regulated expression of most inflammatory genes. Thus, when medicated and
29 unmedicated subjects were considered together only 23 of 42 BD patients (55%) had a
30 positive signature versus 7 of 38 HCs (18%). Notably, the IL6 mRNA level remained
31 elevated in medicated BD subjects and did not differ significantly from unmedicated
32 subjects (table 1), suggesting that this assay identifies a proinflammatory diathesis even
33 in treated cases.
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Table 1: Magnitude of difference in mRNA expression between mood disordered and healthy control (HC) samples from Padmos et al.³², showing selected transcripts in unmedicated subjects vs HCs, relative to that of medicated BD subjects.

Gene Symbol	Unmedicated BD vs HC		Medicated BD vs HC		Affected offspring# vs HC	
	fold change	p-value	fold change	p-value	fold change	p-value
PDE4B	13.73*	<.001	3.42	<.001	5.79	<.001
IL6	37.92	.005	9.56	.006	935.7	<.001
CCL20	55.49	.006	6.02	.10	400.1	<.001

Legend: * - difference significant between unmedicated vs medicated BD samples; # - affected with respect to having manifested either a depressive or a manic episode
 Sample sizes: unmedicated BD n=11, medicated BD n=31, affected offspring n=13, HCs n=25 for comparisons against BD adults, n=70 for comparisons of offspring. Abbrev: BD – bipolar disorder; HC – healthy control; PDE4B - phosphodiesterase type 4B; IL6 - interleukin 6; CCL20-chemokine ligand 20

Minocycline is a second-generation tetracycline that may prevent both glutamate-induced excitotoxicity and cytokine-induced inflammation in the CNS and periphery.

Minocycline has high lipophilicity enabling efficient transfer across the blood brain barrier (BBB)³³ - its concentration in CSF reaches 11–56% of plasma concentrations³⁴. Minocycline inhibits the microglial-mediated release of proinflammatory cytokines IL-1 β , TNF- α , IL-6, and p38³⁵, while promoting release of the anti-inflammatory cytokine,

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3 IL-10³⁴. Moreover, minocycline inhibits matrix metalloproteinases which process
4 cytokines such as TNF- α and IL-1 β into their biologically active forms³⁵. Minocycline is
5 also an effective scavenger of proapoptotic reactive oxygen species and protects against
6 excitotoxicity by preventing glutamate-induced activation of nitric oxide synthase
7 (NOS)³⁶. Nitric oxide facilitates glutamate release from presynaptic neurons and inhibits
8 glial glutamate transporters, amplifying glutamatergic signaling, and contributing to
9 excitotoxic cell death¹⁰. Minocycline also upregulates a key molecular factor in the
10 apoptosis pathway, B-cell CLL/lymphoma 2 (BCL-2)³⁷, an effect shared by lithium,
11 valproate³⁸ and certain antidepressant drugs³⁹. BCL-2 represses apoptosis induced by
12 cytotoxic insults⁴⁰. Conceivably, minocycline may additionally reduce inflammation
13 indirectly by blocking the translocation of bacteria across the intestinal barrier. In mice
14 exposed to a social stressor, bacteria translocated across the intestinal barrier stimulating
15 the release of circulating cytokines such as IL6, and increasing microbicidal activity via
16 inducible NOS⁴¹. Additionally, stress induced a change in the community structure of the
17 microflora in the cecum with a decrease the relative abundance of bacteria in the genus
18 Bacteroides and an increase the relative abundance of bacteria in the genus Clostridium.
19 Notably, these effects were blocked by pretreatment with a broad spectrum antibiotic⁴¹.

35 **Minocycline has neuroprotective and anti-inflammatory properties.**

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38 Minocycline prevents glutamate-induced apoptosis of neurons *in vitro*⁴², prevents
39 ischemia-induced activation of microglia in gerbils⁴³, increases hippocampal neuron
40 survival⁴⁴, reduces lesion-volume and improves neurological function in mice with
41 traumatic brain injury⁴⁵ and in fragile X syndrome⁴⁶, reduces pro-inflammatory cytokine
42 expression and improves neurological function and locomotor activity in rats with spinal
43 cord injury⁴⁷, attenuates MDMA-induced neurotoxicity of serotonin and dopamine
44 systems in the cerebral cortex and hippocampus of mice⁴⁸, reduces inflammation in a rat-
45 model of rheumatoid arthritis (RA)⁴⁹, and delays disease progression and demyelination
46 in rodent models of encephalitis⁵⁰, amyotrophic lateral sclerosis (ALS)⁵¹ and
47 Huntington's Disease (HD)⁵². Based on these data, minocycline was employed, and has
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3 shown promise as, a therapeutic agent in human diseases including HD⁵³, rheumatoid
4 arthritis (RA)⁵⁴, and stroke⁵⁵.

7 **Minocycline has been used to treat psychiatric disorders.**

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11 Miyaoka et al.⁵⁶ discussed 2 patients with catatonic schizophrenia who benefited from
12 minocycline. This group then conducted a 4-week trial with minocycline (150 mg/day) in
13 22 patients with schizophrenia to evaluate its efficacy as an adjunct to antipsychotic
14 drugs⁵⁷. Patients showed a significant improvement in positive and negative symptoms.
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16 Levkovitz et al.⁵⁸ recently studied 54 patients with early-stage schizophrenia treated for 6
17 months with antipsychotic medication and either minocycline (200 mg/day) or placebo in
18 a double-blind trial. Minocycline was associated with a reduction in negative symptoms
19 and improved attention/ memory.
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27 The efficacy of minocycline has not been formally tested in mood disorders. In rodents,
28 minocycline reduced immobility during the forced-swim test⁵⁹, and co-administration of
29 minocycline synergized the antidepressant-like actions of desipramine (but not
30 fluoxetine)⁶⁰. Minocycline also abrogated the depression-like behavior of rodents
31 exposed to lipopolysaccharide (LPS)⁶¹. Levine et al.⁶² presented the case of a 66-year old
32 woman with severe BD, who observed that the tetracycline she took for an infection
33 alleviated her depression. When her depression returned post-treatment, minocycline was
34 reinitiated (150 mg/day). After one week her HAM-D score fell from 25 to 8.
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42 **Aspirin (Acetyl-salicylic acid, ASA) also holds potential efficacy in bipolar disorder.**

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46 The second aim of this study is to assess the antidepressant efficacy of ASA in bipolar
47 depression. Using a 2 x 2 design we will obtain data providing estimates of the effect size
48 of ASA relative to placebo, ASA relative to minocycline, and ASA in combination with
49 minocycline relative to placebo. These data also will explore the specificity of any effect
50 found for minocycline. The clinical use of low dose ASA primarily has been driven by its
51 role as an anti-thrombotic and thrombolytic. Given the exaggerated death rate from
52 cardiovascular events in BD, this action potentially is advantageous in the management
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3 of BD. Nevertheless, the recent literature also supports a role for low dose ASA in the
4 management of the mood disorder itself, specifically in the amelioration of depressive
5 symptoms.
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10 The mechanism of ASA relates to its capacity to inactivate irreversibly the
11 cyclooxygenase (COX) activity of prostaglandin (PG) H-synthase-1 and PGH-synthase 2
12 (referred to as COX-1 and COX-2, respectively). Although ASA has a short half-life (15
13 to 20 min) ASA's permanent inhibition of COX-1 allows once daily dosing for anucleate
14 platelets. In contrast, because nucleated cells rapidly regenerate this enzyme a shorter
15 dosing interval is required to persistently impact COX activity in cells that mediate
16 inflammatory processes. Moreover, ASA is 50- to 100-fold more potent in inhibiting
17 platelet COX-1 than monocyte COX-2 activity⁶³, so there is nearly a 100-fold variation in
18 the daily dose of aspirin, as higher doses are used to target COX-2 in the management of
19 treating peripheral inflammation (e.g., arthritis) or pain. As reviewed below, preliminary
20 evidence obtained in BD suggests beneficial effects are achieved using ASA in low
21 doses, where aspirin would inhibit COX-1, but not COX-2.
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33 **Aspirin has neuroprotective and anti-inflammatory properties.**

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37 In the brain, recent data indicate that genetic manipulation of COX-1 and COX-2
38 differentially modulate leukocyte recruitment during neuroinflammation, and suggest that
39 reduction of COX-1 activity is neuroprotective, whereas reduction in COX-2 activity is
40 detrimental, during a primary neuroinflammatory response (reviewed in ⁶⁴). Choi et al.⁶⁴
41 propose that these distinct roles reflect the predominant localization of COX-1 in
42 microglia, which play a major role in mediating neuroinflammation, in contrast to the
43 predominant localization of COX-2 in pyramidal neurons. For example, Choi et al.⁶⁵
44 examined the effects of COX-1 or COX-2 deficiency on intracerebroventricular
45 lipopolysaccharide (LPS)-induced neuroinflammation by comparing COX-1 (-/-) and
46 COX-2 (-/-) knockout mice to wild-type (WT) (+/+) control animals. After LPS,
47 leukocyte infiltration and inflammatory response were attenuated in the COX-1 (-/-) mice
48 but increased in the COX-2 (-/-) mice, compared with WT controls. In another study,
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3 Choi et al.⁶⁶ examined the effect of COX-1 genetic deletion on the inflammatory
4 response and neurodegeneration induced by β -amyloid, and found that in COX-1 (-/-)
5 mice, the A β 1-42-induced inflammatory response and associated neuronal damage
6 were attenuated compared to WT mice. Compatible with these results, in
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pharmacoepidemiological studies investigating whether chronic NSAID use reduced the risk of developing Alzheimer's disease (AD), indomethacin, a preferential COX-1 inhibitor, showed beneficial effects, while COX-2 selective inhibitors, failed to show any beneficial effect in AD patients with mild to severe cognitive impairment. These data suggest the hypothesis that inhibition of COX-1 activity may be a valid therapeutic strategy to reduce the cerebral inflammatory response and neurodegeneration in neuropsychiatric diseases in which neuroinflammatory components play a role in pathophysiology.

Other researchers hypothesized that NSAIDs would be beneficial in BD more specifically because of their ability to down-regulate activity in the brain arachidonic acid (AA) cascade by via interfering with phospholipase A2 (PLA2) and/or COX function. In rodents Rapoport and colleagues⁶⁷⁻⁶⁹ demonstrated that conventional mood stabilizers decrease the AA turnover in phospholipids and the expression of PLA2 and/or COX enzymes. The PLA2 and COX enzymes catalyze, respectively, release of AA from membrane phospholipid and AA conversion to eicosanoids such as prostaglandin E2 and thromboxane B2. The AA cascade is involved in neuroreceptor-initiated signaling and can be pathologically upregulated by neuroinflammation and excitotoxicity.

Nevertheless, aspirin has additional mechanisms that may underlie benefits in neuropsychiatric illness. While low-dose aspirin down-regulates AA cascade activity via inhibition of COX-1 activity, in higher doses it also down-regulates COX-2 gene transcription, increases levels of lipoxigenase-derived eicosanoids such as the anti-inflammatory lipoxin A4, and acetylates COX-2 protein to a modified enzyme that can convert unesterified AA to anti-inflammatory mediators such as 15-epi-lipoxin A4 (reviewed in ⁷⁰). The acylated enzyme also can convert docosahexaenoic acid (DHA) to 17-(R)-OH-DHA, which, like its metabolites di(R)-OH-DHA (neuroprotectin (R) D1)

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3 and tri(R)-OH-DHA (resolvin (R) D1), is highly anti-inflammatory (reviewed in ⁷⁰).
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5 Lithium given chronically to rats with lipopolysaccharide-induced neuroinflammation
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7 also increases the brain concentration of 17-OH-DHA. Thus, there may be a synergy
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9 between aspirin and lithium in forming anti-inflammatory brain DHA metabolites.

10 11 **Aspirin appears effective in preliminary studies of mood disorders.**

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16 Pharmacological-epidemiological data in BD supportive of these hypotheses were published by
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18 Stolk et al.⁷⁰. Using the Netherlands based PHARMO Record Linkage System (which
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20 connects pharmacy dispensing records to hospital discharge records of > two million
21
22 individuals since 1985), these researchers tested whether non-steroidal anti-inflammatory
23
24 drugs (NSAIDs) or glucocorticoids would ameliorate bipolar symptoms. The target
25
26 sample consisted of 5,145 patients receiving lithium (mean age = 48.6 ± 15 yrs; mean
27
28 duration of lithium use=847 days), based upon the assumption that lithium treatment is
29
30 relatively specific to individuals with BD. The main outcome measure was a calculated
31
32 incidence density (ID) of medication events (change in the type or numbers of
33
34 psychotropic medications prescribed, or increase [$>30\%$] in the psychotropic drug dose).
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36 Subjects receiving low-dose (≤ 80 mg/day) aspirin were 17% less likely to have a
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38 medication event, a finding that remained significant after adjusting for age, sex, chronic
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40 disease score and health care utilization. This effect was selective for low-dose ASA. In
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42 contrast, high-dose aspirin or non-selective NSAIDs (i.e., regimens expected to inhibit
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44 both COX-1 and -2), selective COX-2 inhibitors and glucocorticoids did not produce a
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46 statistically significant protection. Instead, the co-administration of non-selective
47
48 NSAIDs and glucocorticoids was associated with statistically significant increases in
49
50 medication events, suggesting destabilization of bipolar illness. The finding that low-dose
51
52 aspirin decreased the number of medication events was particularly noteworthy since
53
54 aspirin does not significantly augment serum lithium levels in contrast to selective COX-
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56 2 inhibitors which can raise lithium levels⁷¹. These preliminary observations thus
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58 appeared consistent with the hypothesis that COX-1 inhibitors can reduce
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60 neuroinflammatory processes and thus benefit BD patients.

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3 Notably, the observation that beneficial effects in BD were conferred by low-dose ASA,
4 but not by nonselective COX inhibitors, COX-2 inhibitors or glucocorticoids, appeared
5 inconsistent with the hypothesis that drugs that down-regulate AA cascade activity in
6 general hold therapeutic potential in BD. Thus the putative neuroprotective effects
7 associated with COX-1 inhibition may contribute specifically to the benefits of low-dose
8 aspirin in BD observed by Stolk et al. For example, as reviewed above, aspirin and
9 lithium may exert synergistic effects in forming anti-inflammatory brain DHA
10 metabolites (reviewed in ⁷⁰).

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20 Other data suggest that aspirin exerts antidepressant effects within the context of MDD or
21 cardiovascular illness. Mendlewicz et al.⁷² examined the effect of aspirin augmentation of
22 conventional antidepressant pharmacotherapy in 24 patients with MDD who had proven
23 non-responsive after 4 weeks of SSRI treatment. Participants were treated openly during
24 the subsequent 4 weeks with aspirin 160 mg/day in addition to their SSRI regimen. The
25 combined administration of SSRI plus aspirin was associated with a response rate of
26 52.4%. Remission was achieved in 43% of the total sample and 82% of the responder
27 sample. In the responder group, a significant improvement was observed within week 1
28 and this benefit persisted through day 28. In another study Ketterer et al.⁷³ reported that
29 in 174 males undergoing coronary angiography (of whom 99 were taking low-dose
30 aspirin), aspirin use was associated with less depression and anxiety symptoms.

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40 In contrast, a preliminary study of the selective COX-2 inhibitor, celecoxib, was negative
41 in bipolar depression⁷⁴, potentially compatible with the negative results of COX-2
42 inhibitors reported by Stolk et al.⁷⁰. In a double-blind, randomized, add-on clinical trial of
43 celecoxib in patients (n = 28) studied during a depressed or mixed episode of BD, no
44 significant difference was observed between the celecoxib and placebo add-on groups at
45 study endpoint⁷⁴. These results contrasted with those obtained using celecoxib in unipolar
46 depression, however. In MDD, celecoxib augmentation of either reboxetine⁷⁵ or
47 fluoxetine⁷⁶ was associated with a significant therapeutic effect on depressive symptoms
48 in randomized, double-blind, add-on clinical trials.

METHODS AND ANALYSIS

Participants

One hundred and twenty male or female outpatients between 18 and 55 years of age, who meet DSM-IV-TR criteria for BD (type I or II) and for a current major depressive episode will be recruited. The depressive syndrome must have been present for at least 4 weeks and the minimum threshold for depression severity will be set at a 17-item HAM-D score ≥ 18 . Subjects will provide written informed consent as approved by the Western Institutional Review Board.

Concurrent Medications

At study entry type I BD subjects must have been taking a stable dose of a mood-stabilizing medication (lithium, valproate, carbamazepine, lamotrigine, antipsychotic agents), for at least 4 weeks, dosed clinically to target the therapeutic range. Type II BD subjects will be included irrespective of whether they present on a mood stabilizer. To investigate the utility of this augmentation strategy in the population for whom minocycline is most likely to prove therapeutically relevant, volunteers receiving stable doses of mood stabilizing, antipsychotic, antidepressant, and/or anxiolytic drugs for at least 4 weeks will be included. However, volunteers who currently are receiving more than 4 psychotropic medications in a daily regimen will be excluded, since this condition may signify a more brittle or complex clinical state. Subjects may remain in psychotherapy or have no psychosocial intervention. Volunteers will be excluded if they currently are receiving medications likely to have adverse interactions with minocycline or aspirin, including NSAIDs, warfarin, digoxin, penicillins, and isotretinoin products.

For participants who enter the study, the preferred strategy will be for subjects to maintain the same regimen of concurrent medications throughout the six week study so that only the study drug regimen will be altered per protocol. Nevertheless, changes to concurrent medications will not affect study status, so long as the medication change does

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3 not target a depressive or manic symptom. If changes to concurrent medication regimens
4 are clinically required to address worsening depressive symptoms or the development of
5 manic symptoms, then the subject will be dropped from the study.
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10 **Study Design**

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14 Patients will participate in a randomized, double-blind, placebo-controlled, trial with a 2
15 x 2 design. As adjuncts to existing treatment, subjects will receive placebo-minocycline
16 plus placebo-aspirin, active-minocycline plus placebo-aspirin, placebo-minocycline plus
17 active-aspirin, or active-minocycline plus active-aspirin. The randomization sequences
18 will be determined by a research staff-member who is not obtaining clinical information
19 from the research subject and will be assigned by subject number at consenting. A
20 restricted randomization (permuted block randomization) method will be used in which
21 subjects are randomly allocated to each block (n=30) to ensure that equal numbers of
22 participants receive each drug/placebo combination. In order to ensure that experimental
23 group assignment is not skewed across the two trial sites, the study progress will be
24 monitored by individuals who are not involved in the data collection, and in the case of
25 “drift”, adjustments will be made as necessary.
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37 The trial will be conducted over 6 weeks and will comprise 7 assessment sessions (figure
38 1). The subject will be seen at the prescribed time intervals within a window of two
39 business days on either side of visit target date to complete the specified visits.
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44 At each session, a clinical assessment will be conducted using the rating scales listed
45 below, and treatment side-effects will be assessed and rated for severity. To preserve the
46 rater blind, the research staff member who conducts the clinical ratings will not be the
47 research staff member who assesses the presence of side effects, and will remain blind to
48 the information pertaining to side effects. Subjects who experience severe adverse effects
49 or who develop treatment-associated hypomania or mania will be dropped from the
50 study, instructed to discontinue the study medication, and referred for appropriate clinical
51 management of these adverse events.
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5 The primary outcome measure will be the change in the Montgomery-Asberg Depression
6 Rating Scale (MADRS) scores at the seventh assessment session (week 6).
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10 Medication

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14 This pilot proof-of-concept study will adhere to the dosing limits and route of
15 administration for the FDA indications for minocycline's and aspirin's use in other
16 conditions (thus an IND is not required). A fixed dose design will be followed, and all
17 medications will be administered via the p.o. route. The pilot data extant for both study
18 drugs supports an onset of improvement within two weeks, so the six week study
19 duration is expected to provide sufficient time to detect an antidepressant effect, to
20 provide information about the persistence of the antidepressant effect over about one
21 month from the anticipated onset of effect, and to minimize dropouts.
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30 For minocycline the starting dose will be 100 mg b.i.d. (total daily dose=200 mg). This
31 dose of 100 mg b.i.d. has been shown by a substantial literature to produce consistent
32 anti-inflammatory effects in rheumatoid arthritis and other inflammatory disorders. This
33 also is the dose used in a recent schizophrenia treatment trial⁵⁸. The associated placebo
34 capsules match the appearance of the 100 mg minocycline capsule.
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40 The starting dose of aspirin will be 81 mg p.o. b.i.d. This dose is sufficient to inhibit
41 COX-1, and appeared beneficial in stabilizing the course of BD in the pharmaco-
42 epidemiological study of Stolk et al.⁷⁰. When aspirin is used as an anti-platelet drug once
43 daily dosing is sufficient since anucleate platelets do not produce enough COX-1 to
44 overcome the irreversible inhibition of COX-1 within a 24-hour period. In contrast, in
45 nucleated cells COX-1 is replenished, so more frequent dosing is required to persistently
46 inhibit COX-1. Thus we will administer the dose in a b.i.d. regimen, according to the
47 guidelines described above. A total daily dose of 160 mg was administered in the
48 preliminary study which reported that aspirin significantly augmented the antidepressant
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3 effects of fluoxetine in MDD⁷². The relevant placebo matches the appearance of the
4 aspirin tablet.
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9 Participants will be advised that one of the study drugs may reduce the efficacy of oral
10 contraceptives, and to avoid taking the study drugs within 3 hours of iron products or of
11 antacids containing calcium, magnesium or aluminum. They also will be advised that one
12 study drug can increase their risk for bleeding during surgical procedure or if combined
13 with other drugs or herbal preparations that reduce hemostasis.
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17 18 19 **Compensation**

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22 Participants will be compensated for participation in the amount of \$300.00.
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26 27 **Treatment Compliance**

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30 To enhance compliance, study participants will be given an information sheet to take
31 home detailing the procedure to be followed in the case of a missed dose, and requesting
32 that this information be recorded for the investigators. The number of capsules and tablets
33 remaining in each supply given to the patients will also be counted to evaluate treatment
34 compliance. In cases where treatment compliance is poor, subjects will be excluded from
35 the data analysis, using conventional criteria for defining adequate compliance in a
36 clinical trial.
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48 49 **Psychiatric Assessment and Clinical Ratings**

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51 Patients will be evaluated and followed in the outpatient clinics at LIBR or Oklahoma
52 University School of Community Medicine in Tulsa, OK, or at the University of Kansas
53 Medical Center Research Institute (KUMCRI) in Wichita, KS. The diagnosis of BD will
54 be established using DSM-IV-TR criteria on the basis of an unstructured interview
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3 conducted by a psychiatrist and the MINI-Plus administered by trained psychiatric
4 interviewers. The following rating scales will be administered: MADRS, Quick Inventory
5 of Depressive Symptomatology (QUIDS; 16 item), Hamilton Anxiety Rating Scale
6 (HAM-A), Young Mania Rating Scale (YMRS), Universal Fagerstrom (to assess nicotine
7 use), Hollingshead socioeconomic scale, Sheehan Disability Scale (SDS) and the Family
8 Interview for Genetic Studies (FIGS). Medical assessment will include a physical
9 examination, electrocardiogram, complete blood count (CBC), electrolytes and liver-
10 function assays (SMA 20), thyroid panel, and urinalysis, serum drug and pregnancy tests
11 at study entry and study completion. At each follow-up session, the MADRS, HAM-A,
12 YMRS, and Clinical Global Impressions (CGI) scale will be repeated. Physical and
13 psychiatric symptoms will be evaluated and recorded in order to measure the side-effect
14 profiles of minocycline and aspirin. Participants will be questioned about adverse
15 reactions, including dizziness, photosensitivity, hyperpigmentation, gastrointestinal
16 distress or bleeding at each assessment and will be withdrawn from the study if medically
17 necessary. Vital signs will be measured at entry and at each session.
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32 **Immune System Measures**

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35 The activity of peripheral cytokines correlates with inflammatory processes in the CNS.
36 Peripheral cytokines cross the BBB, and can propagate signals across the BBB in the
37 form of small, freely diffusible lipophilic molecules such as prostaglandins, which induce
38 the production of cytokines from glia⁷⁷. The measurement of peripheral markers of
39 inflammation thus serves as a valid, if indirect assessment of CNS inflammation.
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46 To explore predictors and correlates of treatment outcome, blood will be sampled for
47 testing plasma and whole blood peripheral blood monocyte (PBM) based markers of
48 inflammation at baseline and study end. These markers will include 10 cytokine proteins
49 (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IFN γ , and TNF, high sensitivity
50 (hs) CRP, and RNA expression of candidate genes from PBMCs. Candidate genes
51 include IL-6, TNF, and IRF5 (a factor that mediates monocyte polarization). The 10
52 inflammation-related cytokines and the PBMC mRNA will be assayed from plasma at
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3 baseline and study end. We selected the markers IL-6, TNF and CRP because they are the
4 most widely implicated in mood disorders. The other cytokines included in the cytokine
5 bead array assays are measured simultaneously with IL-6 and have all been implicated in
6 the general regulation of inflammation. A meta-analysis of >100 studies found that IL-6
7 and CRP each were significantly elevated in depressed patients with standardized mean
8 difference scores (d) of 0.71 and 0.26, respectively⁷⁸. The associations remained
9 significant after adjustment for body-mass index (BMI) and smoking. Moreover, IL-6 has
10 been shown to modulate HPA axis function by inducing CRH release,
11 adrenocorticotrophic hormone synthesis, and corticosteroid production⁷⁹. CRP production
12 is induced by the proinflammatory cytokines, IL-1, IL-6, and IL-17, and is thus a non-
13 specific marker of systemic inflammation.
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24 Three blood samples will be transported to the immunology lab in the Department of
25 Surgery at the University of Oklahoma College of Medicine for each participant at each
26 of the sampling time-points (sessions 1 and 7). One sample will be centrifuged to obtain
27 plasma which will be stored at -80°C until analyzed. Serum CRP, IL-6, TNF, and the
28 other cytokines listed above will be assayed in duplicate with ELISA (CRP high-
29 sensitivity kit, R & D Systems, Oxford, UK) or enhanced cytokine bead array flex kits
30 (Becton Dickinson) using the manufacturer's reagents and standards. The other two
31 samples will be used to isolate PBMCs and plasma and will be frozen until processed.
32 Monocytes will be isolated from the PBMCs in order to assess mRNA levels similar to
33 the method used by Padmos et al.³². This procedure utilizes monoclonal antibodies
34 directed against human CD14 to isolate monocytes in peripheral blood monocyte cell
35 suspensions. A magnetic cell sorting system will be used for the separation of the
36 monocytes and flow cytometry will be used to gauge the purity of the population. Once
37 purity is established, total RNA will be isolated from the monocytes using an RNeasy kits
38 (Qiagen) according to the manufacturer's directions. RNA will then be reverse
39 transcribed to cDNA using standard commercial kits. rtPCR reactions will be performed
40 using the Dynamo Sybr Green HS Master Mix (New England Biolabs) and custom
41 primers will be synthesized by a commercial laboratory. Real-time rtPCR reactions will
42 be run using a Cepheid Smart Cyclyer II or similar instrument. Additional aliquots of
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3 serum and plasma will be stored so that other inflammatory markers can be tested in the
4 future using Luminex bead arrays and/or additional available technologies.
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8 9 **Source of Compounds Tested**

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12 Minocycline and aspirin are available on a generic basis, and are manufactured within the
13 USA by several companies. The identity of the active medicines and placebos will be
14 blinded using placebos that match the appearance of the active drugs. The medications
15 and placebos have been formulated by Wedgewood Pharmacy, Swedesboro, NJ. The
16 study minocycline capsule and chewable aspirin tablet are identical in appearance to their
17 corresponding placebos.
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23 24 **Outcome Measures and Data Analysis**

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28 Antidepressant response will be evaluated by assessing changes in MADRS scores at
29 assessment session # 7 (i.e. 6 weeks). Our *a priori* hypothesis is that minocycline and/or
30 aspirin plus existing medication will exert greater antidepressant effects than placebo plus
31 existing medication by study completion. Assuming that there are equal numbers of
32 subjects in each treatment group, this hypothesis will be statistically assessed using a
33 group (for the four treatment cells)-by-session (1 vs. 7) repeated measures analysis of
34 variance (ANOVA). If the ANOVA statistic is significant, between- and within-group t
35 tests will be used in planned comparisons to identify the nature of the effect leading to the
36 significant overall ANOVA statistic. We expect to find a significant group-by-session
37 interaction, attributable to a greater reduction in MADRS scores in the minocycline and
38 aspirin groups compared to the placebo group between session 1 and session 7. If there is
39 an imbalance in the number of subjects across groups, (e.g., due to differential dropout
40 rates during the first treatment week), the data analysis will be conducted with a mixed-
41 effects model.
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54 | A Mixed Effect Model Repeated Measure (MMRM)⁸⁰ will be used to [impute-derive](#)
55 missing data points as this method has been shown to be superior to last observation
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3 carried forward (LOCF) which can inflate the Type I error rates⁸¹. The LOCF and
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5 observed cases (OC) approaches to data imputation will be used *post-hoc* to provide
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7 further confirmation of the results obtained under the MMRM analysis.
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11 In order to test whether the putative antidepressant effects of minocycline or aspirin have
12
13 a rapid onset, as a *post hoc* analysis the ANOVA will be repeated using MADRS ratings
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15 from the assessment that follows the first week of exposure to active drug versus the
16
17 corresponding change under placebo; i.e. session 2. *Post hoc* tests will be performed to
18
19 assess the significance of changes in the secondary clinical outcome measures (QUIDS
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21 16, HAM-A, YMRS, CGI-I).
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24 The rate of completion in the four cells also will be considered an outcome measure. The
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26 completion rate in the minocycline and/or aspirin arms may be influenced more by
27
28 dropouts due to side effects while the completion rate in the placebo group may be
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30 influenced more by dropouts due to non-response. Two different measures of completion
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32 rate will be obtained: completion of week 1 of the study (baseline to week 1) and
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34 completion of the study (baseline to week 6). Differences between the groups in
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36 completion rates will be assessed with a [chi-squared test or a logistic regression.](#)
37
38 [ANOVA.](#)
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41 We will test the hypothesis that minocycline and aspirin reduce inflammation (e.g. CRP,
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43 IL-6, IL-6 mRNA) more than placebo using statistical analyses similar to those described
44
45 above. If the assay results are normally distributed then a group-by-session repeated-
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47 measures ANOVA with CRP, IL-6 and nine other cytokine levels as dependent variables,
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49 and BMI, smoking status, and time of blood draw as covariates, will be used to assess
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51 anti-inflammatory effects of minocycline and aspirin. Mixed-effect models will be used if
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53 necessary. If the CRP or inflammatory cytokine data are not normally distributed
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55 (Kolmogorov-Smirnov test) or if the equality of statistical variance assumption across
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57 assessments is violated (Levene's test), then Friedman's ANOVA will be used to test for
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59 CRP or inflammatory cytokine differences between groups. If the Friedman's ANOVA
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61 statistic is significant, Wilcoxon sign-ranked tests will be used for post-hoc analysis of

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3 group differences. Nonspecific factors that influence CRP and inflammatory cytokine
4 levels include time of day, presence of infection, treatment with anti-inflammatory
5 medications, smoking, obesity, and alcohol abuse. We will attempt to control for these
6 potential confounds by measuring BMI and recording NSAID and nicotine use
7 (Universal Fagerstrom scale), and by excluding individuals who have recently abused
8 substances or who have intercurrent infections. The serum CRP concentration shows
9 minimal diurnal variability in adults⁸² but IL-6 and other cytokine levels vary across time
10 of day⁸³. To minimize cytokine measurement variability due to circadian fluctuations, we
11 will schedule patient assessment sessions at the same time each day. Since this may not
12 always be possible, we will record the time of day that each blood-draw is made, divide
13 the day into quartiles: 7am-10am, 10am-12pm; 12pm-3pm, and 3pm-6pm, and use these
14 data as a covariate in the statistical analyses.
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26 To test whether baseline levels of CRP and inflammatory cytokines predict response to
27 minocycline or aspirin, we will subclassify the participants using conventional criteria⁸⁴
28 as achieving full response ($\geq 50\%$ reduction in MADRS score from baseline), partial
29 response ($< 50\%$ but $\geq 25\%$ reduction), or nonresponse ($< 25\%$ reduction). Patients
30 achieving remission (post-treatment MADRS score ≤ 10) will also be identified. A non-
31 parametric alternative to the ANOVA statistic, the Mann-Whitney test, will be used to
32 compare remitted and non-remitted groups in baseline levels of inflammatory cytokines
33 and CRP if the data are not normally distributed. Ideally, the impact of baseline levels of
34 inflammation on treatment response would be tested more rigorously using a formal
35 stratified design. However, in order to conduct a stratified trial with for example, 8
36 experimental groups (4 x high versus low inflammation), the sample size of the study
37 would have to be doubled, which would significantly increase costs and decrease
38 feasibility. Nevertheless, this stratification approach would be important to consider for
39 future studies if promising results are obtained in this clinical trial.
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51 52 53 **Statistical Power** 54 55 56 57 58 59 60

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3 A recent meta-analysis of 96 antidepressant treatment studies found that the average
4 effect size of a placebo treatment is 1.69 compared with 2.50 for an antidepressant
5 treatment⁸⁵. We calculated that in order to detect an effect size of 0.81 (i.e. the difference
6 between 2.50 and 1.69) with an 80% probability (2-sided test, $\alpha=0.05$), we will require a
7 sample size of 26 subjects per group
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12 (http://hedwig.mgh.harvard.edu/sample_size/size.html). This effect size may correspond
13 to approximately 3 points on the MADRS. Thus given our sample size of 30 per group
14 we should have sufficient power to test Specific Aim 1, allowing for a 13% drop-out rate
15 during week 1 of the study (dropouts after completion of study week 1 will be included in
16 the analysis under the MMRM approach described above).
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23 As discussed above, a recent meta-analysis⁷⁸ of cross-sectional studies of serum-derived
24 IL-6 and CRP in depression calculated effect sizes of 0.71 for IL-6 and 0.26 for CRP.
25 Based on these effect sizes a sample size of 26 would yield >80% probability of detecting
26 significant depression-related changes in IL-6, but only a 60% probability of detecting a
27 depression-related change in CRP. There are 3 reasons why we believe that these CRP
28 power estimations are not applicable to this study. Firstly, the effect sizes derived from
29 the meta-analysis are based on cross-sectional studies. Given the effect of variables such
30 as smoking, diet, exercise, and BMI on proinflammatory cytokines, a within-subjects
31 design is likely to reduce non-depression-related sources of variance, and substantially
32 increase statistical power. Secondly, we are not only examining the effect of mood on IL-
33 6 and CRP levels, but are treating patients with minocycline and aspirin, drugs known to
34 possess anti-inflammatory properties. We therefore suggest that our proposed study is
35 likely adequately powered to detect any true changes in plasma IL-6, CRP, and the other
36 inflammatory cytokines across treatment blocks.
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49 Regarding IL-6 mRNA gene expression in peripheral blood monocytes, Padmos et al.³²
50 reported a 38-fold increase in IL-6 mRNA levels in unmedicated patients with BD
51 compared with HC. Since minocycline reduces IL-6 levels (see above) we expect our
52 study to have very high power to detect differences between groups, as well as changes in
53 response to minocycline. The simultaneous detection of nine other inflammation-related
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3 cytokines, in addition to IL-6 (using newer more sensitive technology) will provide much
4 finer resolution of the effects on inflammatory cascades than that measured in previous
5 studies.
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10 **ETHICS AND DISSEMINATION**

11 **Gender/Minority/Pediatric Inclusion for Research**

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18 Women and Minorities will be included in the study without prejudice according to their
19 representation in the study population. Participants will be recruited from the greater
20 metropolitan areas of Tulsa, OK and Wichita, KS and efforts will be made to ensure that
21 our subject population resembles the gender, ethnic and racial composition of these areas.
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26 **Exclusion Criteria**

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31 The following exclusion criteria apply: 1) inability to provide informed consent; 2) age of
32 onset of BD>40 years; 3) serious risk of suicide; 4) current delusions or hallucinations
33 sufficient to interfere with the capacity to provide informed consent; 5) current manic
34 symptoms [depressed BD patients with concurrent manic symptoms have been found to
35 be more likely to experience adverse reactions in antidepressant treatment trials⁸⁶]; 6)
36 medical illness including as hepatic impairment, renal dysfunction, bleeding diatheses
37 (e.g., hemophilia), cerebrovascular disease or heart disease, hypertension that is
38 inadequately controlled by medication, diabetes mellitus, or known peptic ulcer disease;
39 7) abuse of drugs or alcohol within the preceding 6 months, or substance dependence
40 within the last 5 years; 8) daily alcoholic beverage consumption equivalent to ≥ 3 oz. of
41 alcohol; 9) asthma or known allergies or hypersensitivities to tetracycline antibiotics,
42 aspirin or other NSAIDs; 10) current use of drugs that could increase the risks associated
43 with aspirin or minocycline administration, namely other antibiotic medications, other
44 NSAIDs or anticoagulants (e.g., warfarin), acetazolamide, or methotrexate; 11) known
45 HIV or other chronic infection including, but not limited to viral hepatitis. 12) Pregnant
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3 or nursing women, and women who are attempting to conceive during the 6 week study
4 period, will also be excluded.
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8 9 **Specimens, Records, and Data Collection**

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11 A physician, registered nurse, or trained phlebotomist will utilize a sterile technique to
12 draw 60 ml of blood by venipuncture. Participants will also be asked to submit a urine
13 sample. A physician, registered nurse, or trained technician will collect EKG data from
14 the subject in a private exam room.
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19 20 21 **Recruitment and Consent Procedure**

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Volunteers will be recruited from the community as well as from the clinical services at
the Laureate Psychiatric Clinic and Hospital and the Oklahoma University School of
Community Medicine in Tulsa, OK, and from the clinical services affiliated with the
KUMCRI. Volunteers may be referred from sources that include physicians, newspaper
advertising, self-help organizations, self-referral, and WIRB approved flyers posted at
local universities, schools, churches and grocery stores. Participants may be pre-screened
through screening protocols based at LIBR or KUCRI. We plan to recruit a total of 120
participants.

All participant interactions including consenting will be conducted in private interview /
exam rooms. These rooms are secured from public areas via combination locked doors
that are only accessible to authorized personnel. Prospective participants will receive an
explanation of the objectives, procedures, and hazards of this protocol that is appropriate
to their level of understanding. The right of the subject to decline to participate or to
withdraw from the study at any time will be made clear.

Non-English speaking participants will not be recruited.

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3 After the consent form is verbally explained to the participant, and any questions have
4 been answered, the researcher will leave the room to allow the participant to read the
5 consent form thoroughly. Family members will be allowed to be present and to discuss
6 the consenting process with the participant. After the consent is read, the researcher will
7 return and answer any additional questions the participant may have. The researcher will
8 remind the subject that participation is strictly voluntary and that they have the right to
9 withdraw at any time. Participants will be asked to arrive 30 minutes early in order to
10 have sufficient time for the consenting process.
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20 **Subject Risks**

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23 The risks of behavioral testing are minimal. The risks of blood drawing are also minimal.
24 Possible mild side effects of the blood draw include mild pain or bruising at the site of
25 the venipuncture.
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30 Minocycline has been used a broad-spectrum antibiotic for many years in doses up to 400
31 mg/day³⁴. It has been used on a chronic basis to treat acne and rheumatoid arthritis, often
32 for many years, in hundreds of thousands of patients. The most commonly encountered
33 side effects are upset stomach, diarrhea, dizziness, drowsiness, ataxia, vertigo, headache
34 and vomiting. Prolonged use can be associated with pigmentation of the skin, gums or
35 teeth. Between 1975 and 2006, the World Health Organization Collaborating Center for
36 International Drug Monitoring listed 122 cases of adverse drug reactions to intravenous
37 minocycline; most commonly, abnormal hepatic function and thrombocytopenia³⁴. These
38 included cases of serious liver injury, including irreversible drug-induced hepatitis and
39 fulminant hepatic failure that was fatal in two cases, thought to be due to triggering or
40 unmasking autoimmune hepatitis. One case of autoimmune-related glomerulonephritis
41 has been reported. The role of oral minocycline in precipitating these conditions has not
42 been clearly established. Minocycline also has been associated with idiopathic
43 intracranial hypertension (pseudotumor cerebri). Long-term trials have shown that
44 minocycline is well tolerated. In a 2-year trial of minocycline (200 mg/day) for RA, 3 of
45 30 patients withdrew due to finger-nail discoloration, dizziness, or erythematous rash⁵⁴.
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3 Of 11 patients with HD treated with minocycline (100 mg/day) for 2 years, one
4 complained of nausea in the first 3 weeks, and two of sedation⁵³, while in a 6-month trial
5 of minocycline for ALS, the mean tolerated dose was 387 mg/day and the most common
6 adverse effects were gastrointestinal⁸⁷. Five of 36 patients with schizophrenia withdrew
7 from a 6-month trial of minocycline (200 mg/day) due to indigestion (n=2), pigmentation
8 (n=2), or a suicide attempt (n=1)⁵⁸.

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16 Low dose aspirin has been safely used in many millions of patients on a worldwide scale
17 for its role as an anti-thrombotic and thrombolytic. A meta-analysis of >100 randomized
18 trials in high-risk patients indicated that low-dose ASA reduced cardiovascular death by
19 15% and prevented nonfatal vascular events by about 30%⁸⁸. These data stand in striking
20 contrast to the data obtained in COX-2 inhibitors, which can increase cardiovascular risk.
21 In clinical trials of several COX-2 selective and nonselective NSAIDs of up to three years
22 duration have shown an increased risk of serious cardiovascular (CV) thrombotic events,
23 myocardial infarction, and stroke, which have in many cases been fatal⁸⁹. Patients with
24 known CV disease or risk factors for CV disease are at greater risk for such events during
25 chronic treatment with COX-2 inhibitors. Evidence from human pharmacology and
26 genetics, genetically manipulated rodents, and other animal models and randomized trials
27 indicates that this is consequent to suppression of COX-2-dependent cardioprotective
28 prostaglandins, particularly prostacyclin⁹⁰.

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40 Aspirin does not cause a generalized bleeding abnormality unless given to patients with
41 an underlying hemostatic defect (e.g., hemophilia, uremia, or that induced by
42 anticoagulant therapy). Aspirin-induced impairment of primary hemostasis cannot be
43 separated from its antithrombotic effect and is similar at all doses ≥ 75 mg/d⁹¹. The risk
44 of intracranial bleeding is exceedingly rare (<0.1% in high risk populations), but is higher
45 in individuals with cerebrovascular disease⁸⁸. Hypertension that is inadequately
46 controlled by medication often is considered a contraindication to aspirin because of the
47 concern that possible benefits in the prevention of cardiovascular events may be
48 counterbalanced by an increased risk of cerebral bleeding. However, hypertensive
49 patients whose blood pressure is well-controlled appear protected from myocardial
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3 infarction by aspirin therapy without an increase in the number of cerebral hemorrhages
4 or strokes⁹². Moreover, aspirin therapy does not affect blood pressure or the response of
5 hypertension to antihypertensive agents^{91 93}.
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10 NSAIDs as a class can cause serious gastrointestinal (GI) adverse events including
11 inflammation, bleeding, ulceration, and perforation of the stomach, small intestine, or
12 large intestine, which rarely have proven fatal. In controlled clinical trials the percentage
13 of patients reporting one or more gastrointestinal complaints has ranged from 4% to
14 16%⁹¹. The mechanism underlying this adverse effect appears attributable to the
15 inhibition of COX-1. Thus, the incidence of GI side effects has been higher for NSAIDs
16 with more potent effects at COX-1, such as aspirin and indomethacin. For example, in
17 controlled trials the incidence of GI side effects for aspirin and indomethacin have been
18 about twice as high as that for ibuprofen, a nonselective COX inhibitor, in equally
19 effective doses for arthritis. Nevertheless, the incidence of GI side effects associated with
20 aspirin is dose-dependent, and thus is markedly lower when using aspirin in the low dose
21 range planned for the current study. Notably, the risk of GI bleeding is not reduced by
22 using the enterically coated aspirin formulations, but is thought to be lower during
23 concomitant use of omeprazole⁹¹. The effects of warfarin and NSAIDs on GI bleeding are
24 synergistic, such that the users of both drugs together have a risk of serious GI bleeding
25 higher than users of either drug alone. Fortunately, the risk of GI bleeding, which reflects
26 the inhibition of prostaglandins in the stomach (from systemic rather than local exposure)
27 is much smaller when using low-dose as opposed to high-dose aspirin.
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44 Low-dose aspirin has not been reported to alter renal function, and does not reduce
45 effectiveness of ACE inhibitors for HTN (in contrast to other NSAIDs)^{93 94}. However,
46 aspirin can inhibit the renal clearance of acetazolamide and methotrexate potentially
47 leading to increased blood concentrations of and toxicity from these agents. Salicylate
48 can displace other drugs which are protein-bound, especially phenytoin and valproic acid,
49 increasing their free drug concentrations in plasma. This may increase side effects,
50 toxicity and/or efficacy for displaced drugs. If the BD subjects are currently receiving
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3 valproic acid preparations (e.g., divalproex) then the plasma levels of these agents will be
4 monitored for potential changes.
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8 Aspirin may cause a severe allergic reaction that may include: hives, asthma (wheezing),
9 facial swelling, shock. Aspirin overdose can be fatal at 30 g or higher.
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13 In sum, we believe that our two-by-two design is appropriate for trials involving
14 experimental drugs that already have been well-studied with respect to toxicity, as is the
15 case with aspirin and minocycline. A parallel arm design, as opposed to a 2 x 2 factorial
16 design, would be more clearly informative in the case of an experimental drug for which
17 the toxicity and drug interaction potential have not been thoroughly studied in human
18 subjects
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24 25 26 **PHI Protection**

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28 Paper copies of consents, screening forms, the Research Privacy Form, and any other
29 forms, testing results or papers containing Protected Health Information (PHI) will be
30 stored in a secured medical records room with access granted only to authorized
31 personnel.
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38 Electronic data that contain PHI will be managed in accordance with ISO 27000 series
39 information security standards with policies developed from current NIST guidelines (SP
40 800-66) for HIPAA and HITECH compliance. Specific controls implemented to protect
41 PHI are derived from NIST 800-122, and include (but not limited to):
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- 45 1) Access Enforcement (AC-3) – Individual user accounts, role based access control,
46 access control lists;
- 47 2) Separation of Duties (AC-5) – de-identification of data as appropriate,
48 acquire/analyze/manage firewall;
- 49 3) Least Privilege (AC-6) – to ensure PHI data is only available to persons with
50 established need for access;
- 51 4) Remote Access (AC-17) – Secure VPN, encrypted end devices;
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- 5) Access Control for Mobile Devices (AC-19) – Password login, remote destruction capabilities;
- 6) Auditable Events (AU-2) + Monitoring: Log detailed server and network information, alert for problems;
- 7) Analysis, and Reporting (AU-6) – Procedures to audit system records for inappropriate activity.
- 8) User Identification and Authentication (IA-2) – username/secure password and two factor authentication will be required when appropriate.
- 9) Media Access, Marking, Storage, and Transport (MP-2,3,4,5) – Records will be asset tagged and marked to their PHI status, PHI data will be secured and managed by professional system administrators, and will be transported via encryption (VPN, USB, File);
- 10) Media Sanitization (MP-6) – Data will be destroyed by SFHS in accordance with their policies and procedures;
- 11) Transmission Confidentiality (SC-9) – Encryption will be used when needed for all avenues of data transmission (wireless, network, etc.).

To protect subject confidentiality, blood samples will be anonymized as follows:

1. Last name: All participants will be assigned the last name “LIBR.”
2. First name: The first name will be a secure alpha cryptographic hash based on LIBR user ID. This technique is the gold standard in computer security for one-way correlation of data.

Benefits versus Risks

The participant may benefit from participation if either study drug produces an antidepressant effect. Participants will also receive a free clinical evaluation; more frequent treatment visits than are typical in practice, diligent follow-up in terms of symptoms and side effects, and physical and psychiatric monitoring during the study. The risks of delaying alternative treatments are minimal in relation to the potential long-term benefits to the subjects and the importance of knowledge that may reasonably result. The

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3 importance of the knowledge that will likely be gained from this study clearly exceeds
4 the associated potential risks.
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8 9 **Alternative Treatment**

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12 It is possible that some patients may feel better with talk therapy. Participating in any
13 type of talk therapy with their psychiatrist or psychologist does not require dropping out
14 of this study. Subjects will be encouraged to contact the study investigators, particularly
15 the physician in the study, with any questions they may have regarding alternatives to
16 treatment through this research study. The study investigators will assist in referring the
17 subject to another physician for treatment after their participation in the study has ended.
18 Physical and psychological testing, blood draws, urine samples, and EKG data provide no
19 known risks to persons other than those listed in the exclusion criteria whereas the
20 combinatory power of these measures may provide information relevant to understanding
21 the pathophysiology of bipolar disorder.
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32 **Data and Safety Monitoring Plan**

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36 This study involves more than minimal risk. The study progress will be overseen by a
37 Data, Safety and Monitoring Board (DSMB). The DSMB is composed of three members
38 who will meet in person or per telephone at least once every 6 months to review relevant
39 study data including adverse events and dropout rates.
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45 Any unanticipated adverse events will be reported immediately to the IRB of record and
46 to the LIBR Human Protection Administrator. Any adverse events will be included in the
47 annual IRB report.
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50 51 **Dissemination of Results**

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55 The study results will be presented at national and/or international biomedical scientific
56 meetings and published in peer-reviewed journals.
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REGISTRATION

In accordance with the recommendations of the International Committee of Medical Journal Editors⁹⁵, the proposed trial is registered in a public registry (www.clinicaltrials.gov Identifier: NCT01429272).

Figure Legend

Figure 1: Schematic of Study Design

Legend: Each session number (total of 7) is encircled, with the timing between sessions indicated in weeks with a 2 business day window on either side of visit target date to complete the visit. Session 1 is the baseline (green star) and session 7 is the study end (purple star). Peripheral blood will be sampled at baseline and study end to assay markers of inflammation. The study duration is 6 weeks.

Author Contributions

All authors made a significant contribution to the conception and design of the study protocol. The protocol was written by Drs. Savitz and W. Drevets and was critically reviewed by Drs. Preskorn, Teague, D. Drevets, and Yates. All authors gave approval for the publication.

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Competing Interests

None of the authors has a conflict of interest to declare.

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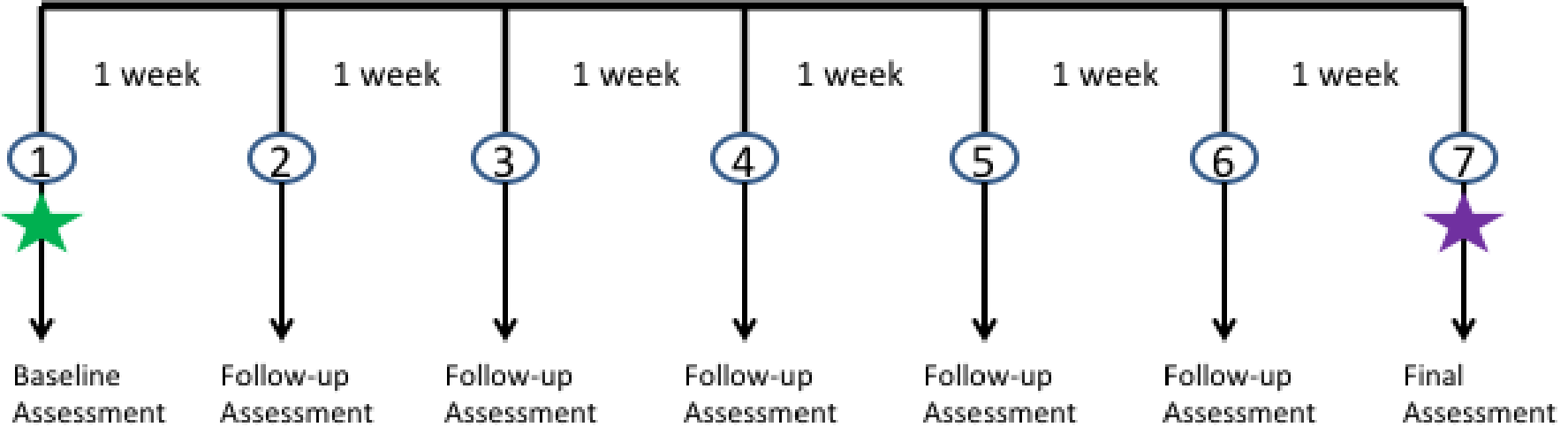
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Minocycline
dose initiation
at 100mg bid

Aspirin dose
initiation at
81mg bid

Placebo
initiated

Inflammation
markers

Inflammation
markers



CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-13
	2b	Specific objectives or hypotheses	20
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	14-15
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	13-14
	4b	Settings and locations where the data were collected	17
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	15-16
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	20-21
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	21
	7b	When applicable, explanation of any interim analyses and stopping guidelines	30
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	15
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	15
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	15
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	15

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2		assessing outcomes) and how	
3			
4		11b If relevant, description of the similarity of interventions	
5	Statistical methods	12a Statistical methods used to compare groups for primary and secondary outcomes	20-21
6		12b Methods for additional analyses, such as subgroup analyses and adjusted analyses	20-21
7			
8	Results		
9	Participant flow (a	13a For each group, the numbers of participants who were randomly assigned, received intended treatment, and	
10	diagram is strongly	were analysed for the primary outcome	
11	recommended)	13b For each group, losses and exclusions after randomisation, together with reasons	
12	Recruitment	14a Dates defining the periods of recruitment and follow-up	
13		14b Why the trial ended or was stopped	
14			
15	Baseline data	15 A table showing baseline demographic and clinical characteristics for each group	
16	Numbers analysed	16 For each group, number of participants (denominator) included in each analysis and whether the analysis was	
17		by original assigned groups	
18			
19	Outcomes and	17a For each primary and secondary outcome, results for each group, and the estimated effect size and its	
20	estimation	precision (such as 95% confidence interval)	
21		17b For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
22	Ancillary analyses	18 Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing	
23		pre-specified from exploratory	
24			
25	Harms	19 All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
26			
27	Discussion		
28	Limitations	20 Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
29	Generalisability	21 Generalisability (external validity, applicability) of the trial findings	
30	Interpretation	22 Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
31			
32	Other information		
33	Registration	23 Registration number and name of trial registry	1, 30
34	Protocol	24 Where the full trial protocol can be accessed, if available	19
35	Funding	25 Sources of funding and other support (such as supply of drugs), role of funders	
36			

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

THE FOLLOWING WERE APPROVED

INVESTIGATOR: Wayne Drevets M.D.
6655 South Yale Avenue
Tulsa, Oklahoma 74136

BOARD ACTION DATE: 08/22/2011
PANEL: 6
STUDY APPROVAL EXPIRES: 08/05/2012
STUDY NUM: 1126576
WIRB PRO NUM: 20111159
INVEST NUM: 160921
WO NUM: 1-683377-1
CONTINUING REVIEW: Annually
SITE STATUS REPORTING: Semi-Annual

SPONSOR: Laureate Institute for Brain Research (LIBR)
PROTOCOL NUM: 2011-002-00
AMD. PRO. NUM:
TITLE:
MINOCYCLINE AND ASPIRIN IN THE TREATMENT OF BIPOLAR DEPRESSION

APPROVAL INCLUDES:
Subject Information Sheet - Visit Five #9212383.0 - As Submitted
Subject Information Sheet - Visit Four #9212382.0 - As Submitted
Subject Information Sheet - Visit One #9212379.0 - As Submitted
Subject Information Sheet - Visit Six #9212384.0 - As Submitted
Subject Information Sheet - Visit Three #9212381.0 - As Submitted
Subject Information Sheet - Visit Two #9212380.0 - As Submitted
Consent Form [IN1]

WIRB APPROVAL IS GRANTED SUBJECT TO:
RE-CONSENTING INSTRUCTIONS: Subjects currently enrolled are not required to sign the enclosed version(s) of the consent form(s). All subjects who will be enrolled in the future for this study must sign the most current WIRB-approved consent form(s).

WIRB HAS APPROVED THE FOLLOWING LOCATIONS TO BE USED IN THE RESEARCH:
Laureate Institute for Brain Research, 6655 South Yale Avenue, Tulsa, Oklahoma 74136
University of Kansas Medical Center Research Institute, 8911 East Orme, Wichita, Kansas 67207

If the PI has an obligation to use another IRB for any site listed above and has not submitted a written statement from the other IRB acknowledging WIRB's review of this research, please contact WIRB's Client Services department.

IF YOU HAVE ANY QUESTIONS, CONTACT WIRB AT 1-800-562-4789

This is to certify that the information contained herein is true and correct as reflected in the records of the Western Institutional Review Board (WIRB), OHRP/FDA parent organization number IORG 0000432, IRB registration number IRB00000533. WE CERTIFY THAT WIRB IS IN FULL COMPLIANCE WITH GOOD CLINICAL PRACTICES AS DEFINED UNDER THE U.S. FOOD AND DRUG ADMINISTRATION (FDA) REGULATIONS, U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES (HHS) REGULATIONS, AND THE INTERNATIONAL CONFERENCE ON HARMONISATION (ICH) GUIDELINES.



Robert A Taylor for

Theodore D. Schultz, J.D., Chairman

8/23/2011

(Date)

This document electronically reviewed and approved by Taylor, Robert on 8/23/2011 1:46:33 PM PST. For more information call Client Services at 1-360-252-2500.
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ALL WIRB APPROVED INVESTIGATORS MUST COMPLY WITH THE FOLLOWING:

1. Conduct the research in accordance with the protocol, applicable laws and regulations, and the principles of research ethics as set forth in the Belmont Report.
2. Although a participant is not obliged to give his or her reasons for withdrawing prematurely from the clinical trial, the investigator should make a reasonable effort to ascertain the reason, while fully respecting the participant's rights.
3. Unless consent has been waived, conduct the informed consent process without coercion or undue influence, and provide the potential subject sufficient opportunity to consider whether or not to participate. (Due to the unique circumstances of research conducted at international sites outside the United States and Canada where WIRB approved materials are translated into the local language, the following requirements regarding consent forms bearing the WIRB approval stamp and regarding certification of translations are not applicable.)
 - a. Use only the most current consent form bearing the WIRB "APPROVED" stamp.
 - b. Provide non-English speaking subjects with a certified translation of the approved consent form in the subject's first language. The translation must be approved by WIRB.
 - c. Obtain pre-approval from WIRB for use of recruitment materials and other materials provided to subjects.

4. Obtain pre-approval from WIRB for changes in research.

5. Obtain pre-approval from WIRB for planned deviations and changes in research activity as follows:

If this research is federally funded or conducted under an FWA, obtain pre-approval from WIRB for all planned deviations and changes in research activity, except where necessary to eliminate apparent immediate hazards to the human subjects. OHRP considers all planned protocol deviations to be changes in research that need prior IRB review and approval.

If this research is **not** federally funded and **not** conducted under an FWA, obtain pre-approval from WIRB for any planned deviations that could adversely affect the rights, safety or welfare of subjects, or the integrity of the research data and any changes in the research activity, except where necessary to eliminate apparent immediate hazards to the human subjects. FDA has not adopted the policy that all planned protocol deviations are changes in research that need prior IRB review and approval.

Deviations necessary to eliminate apparent immediate hazards to the human subjects should be reported within 10 days.

6. Promptly report to WIRB all unanticipated problems (adverse events, protocol deviations and violations and other problems) that meet all of the following criteria:
 - a. Unexpected (in terms of nature, severity or frequency);
 - b. Related or possibly related to participation in the research; and
 - c. Suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.

Please go to www.wirb.com for complete definitions and forms for reporting.

7. Provide reports to WIRB concerning the progress of the research, when requested.
8. Ensure that prior to performing study-related duties, each member of the research study team has had training in the protection of human subjects appropriate to the processes required in the approved protocol.

Federal regulations require that WIRB conduct continuing review of approved research. You will receive Continuing Review Report forms from WIRB. These reports must be returned even though your study may not have started.

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