A MULTISITE, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED 12-WEEK STUDY EVALUATING THE EFFICACY, SAFETY, AND TOLERABILITY OF ADJUNCTIVE INFLIXIMAB FOR THE TREATMENT OF BIPOLAR I/II DEPRESSION

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RATIONALE

Disturbances in the immuno-inflammatory system have been implicated in the etiology, pathophysiology, phenomenology and comorbidity of Bipolar Disorders (BD). For example, preclinical and clinical studies implicate inflammatory networks in the etiology of 'sickness behaviour', a syndrome phenotypically similar to a major depressive episode. Furthermore, increased rates of medical comorbidity (e.g. obesity, abdominal obesity, type 2 diabetes mellitus, metabolic syndrome, and cardiovascular disease) relative to age-and sex-matched healthy volunteers, point towards common underlying pathophysiology (1). Moreover, preliminary evidence from genome-wide association studies, suggests common susceptibility loci for BD and several inflammatory-mediated chronic medical disorders (2).

Elevated levels of both circulating and cerebral spinal fluid pro-inflammatory cytokines have been consistently replicated in individuals with mood disorders. Elevated levels of Tumor Necrosis Factor-alpha (TNF-α) and its receptors, TNF-R1 and TNF-R2, are amongst the most consistently identified pro-inflammatory cytokine abnormalities in BD, across depressive, manic and euthymic states. Preliminary evidence suggests that increased inflammation may be associated with a more severe illness presentation and illness progression (3;4). Additional evidence also suggests that monocytes of individuals with BD have increased inflammatory gene expression, which may confer a discriminating mRNA signature in offspring of individuals with BD (5). Specifically, evidence suggests that susceptibility to BD may be associated with TNFA*2 allele and the –G308A polymorphism of the TNFα gene (6). Disturbances in inflammation however, are not evident in all individuals, as mixed results have been reported. The foregoing degree of inter-individual variability suggests that immuno-inflammatory mechanisms are salient to a subset of individuals with BD.

Conventional mood stabilizers (e.g. lithium. antipsychotics, anticonvulsants) act in varying capacities to down-regulate the production of pro-inflammatory cytokine mRNA and protein gene expression (5;7). In keeping with this view, double-blind, randomized, placebo-controlled studies have documented significant antidepressant effects following adjunctive treatment with the anti-inflammatory agent, celecoxib, in individuals with BD and MDD (8-10). Although favorable results have been reported with non-steroidal anti-inflammatory drugs (NSAIDs), not all studies have been positive and the effect sizes are of questionable clinical significance. It could be hypothesized that antagonism of TNF-α, notably in individuals who manifest signs of inflammation, may exert more specific and superior efficacy in mitigating symptoms of bipolar depression.

Tumor Necrosis Factor Alpha

Tumor necrosis factor alpha is a 157 amino acid pro-inflammatory cytokine synthesized and secreted by disparate cells types including macrophages, lymphocytes, astrocytes, and microglia. Its expression is increased in response to injury as well as inflammatory and infectious stimuli. Tumor necrosis factor alpha serves a homeostatic function in both innate and adaptive immune responses, and is capable of exerting salutary, as well as detrimental effects on immune function. For example, acute modest elevations of TNF-α concentrations augment host defense mechanisms, while chronically elevated concentrations may contribute to allostatic load (11).

Tumor necrosis factor alpha is released from cells as a soluble cytokine (sTNF, a homotrimer of 17 kDa) after being enzymatically cleaved from its cell surface-bound precursor [transmembrane TNF (tmTNF), a homotrimer of 26-kDa monomers] by TNFα-converting enzyme (TACE). Both sTNF and tmTNF ligands are biologically active and exert their effects by binding to TNF receptors: TNF-R1 and TNF-R2. Both TNF receptors are membrane glycoproteins that specifically bind TNF-α and lymphotoxin alpha-3 (11;12). TNF-R1 mediates apoptosis, cytokine production, activation of transcription factors (e.g. NF-kB), as well as inflammatory gene activation. Activation of TNF-R1 has been shown to trigger a dual signaling cascade leading to apoptosis, proliferation, differentiation, or survival of different cell types. Reverse signaling can be initiated by TNF-R2 or TNF-α antagonist binding to cell surface tmTNF, resulting in cytokine suppression or apoptosis.

Postmortem studies indicate that individuals with BD exhibit regional abnormalities in the size, shape, and density of neurons and glia. Tumor necrosis factor alpha may via activation of caspase 3 mediate the observed regional abnormalities in BD. Cellular and biochemical studies indicate that TNF-α binding to its canonical receptors subsequently activates an intracellular cascade that results in recruitment of the TNF receptor associated death domain. This adapter protein subsequently recruits Fas-associated death domain that leads to caspase-3 activation (11). Caspase-3 activation is part of the 'pro-apoptotic' machinery that possibly mediates neuronal and glial apoptosis in BD. Taken together, these observations provide a framework for hypothesizing that activated pro-inflammatory cytokines are integral to the neurotoxic process in BD.

Anti-TNF Antagonists

There are several commercially available biologic agents that specifically inhibit TNF-α, and have approved indications for several inflammatory-based diseases, including rheumatoid arthritis, Crohn's disease, ankylosing spondylitis, psoratic arthritis, and ulcerative colitis (12). There are no commercially available biosimilar agents to the available TNF antagonists in North America. Infliximab is a chimeric monoclonal antibody that has recently been shown to mitigate depressive symptoms in individuals treated for other medical conditions, with only preliminary evidence for its efficacy in a subgroup of individuals with treatment resistant depression.

Infliximab is a chimeric IgG1k monoclonal antibody for TNF-α with a molecular weight of 150 kDa. It is composed of human constant and murine variable regions of the antibody. Infliximab is supplied as a sterile, white, lyophilized powder for intravenous injection; following reconstitution with 10 mL of sterile water for injection with a pH of approximately 7.2. Each single-use vile contains 100 mg of infliximab, 50 mg sucrose, 0.5 mg polysorbate 80, 2.2 mg monobasic sodium phosphate, monohydrate, and 6.1 mg d dibasic sodium phosphate, dehydrate, with no added preservatives (12). Infliximab binds with high affinity to the soluble and transmembrane forms of TNF- α and inhibits binding of TNF- α with its receptors (12). Treatment with infliximab reduces the access of inflammatory cells into inflamed areas in particular inflamed joints or intestine areas in patients with rheumatoid arthritis and Crohn's disease, respectively. Decreases in serum IL-6 and C-reactive protein have also been reported following infliximab treatment (13).

Single intravenous (i.v.) infusion of infliximab results in a linear increase between the maximum serum concentration and dose of administration (3–20 mg/kg). Infliximab is primarily distributed in the vascular compartment. Its volume of distribution at steady state is independent of dose. Single dose infusion of 3–10 mg/kg indicates that the median terminal half-life is 8.0–9.5 days. Repeated infusions of infliximab at 2 and 6 weeks result in predictable concentration-time profiles following each treatment. No systemic accumulation occurs at 4- or 8-week intervals with 3–10 mg/kg dosing. At 8 weeks, mean serum concentrations of infliximab range from 0.5 to 6.0 mcg/mL (12).

TNF Antagonists in Depression

Several lines of trans-disciplinary evidence indicate that TNF antagonists improve measures of depression in individuals with psoriasis, rheumatoid arthritis, Crohn's disease and ankylosing spondylitis. Treatment with TNF antagonists has been associated with lower rates of MDD and anxiety disorders in individuals with rheumatoid arthritis, as compared to individuals who did not receive these medications (14). In a 6-week, open-label study, infliximab (5 mg/kg administered at 0, 2 weeks and 6 weeks) was associated with reduced depressive and anxious symptom severity in individuals with ankylosing spondylitis (15). In a single-dose study of infliximab, a significantly smaller proportion of subjects met criteria for depression at 4 weeks (16% vs. 24%) as compared to baseline (16). In a single-blinded study of patients with Crohn's disease (n = 14), each of whom received placebo (i.e. saline) at baseline, followed by infliximab (5 mg/kg) at 2 weeks, demonstrated a significant effect of infliximab on depressive symptom severity at 4 weeks (17). In patients with advanced cancer (n = 17), open-label infliximab was associated with improvements in fatigue, anxiety and depression subscores (18). Taken together, the results demonstrate that infliximab at 5 mg/kg may offer therapeutic benefit for depressive symptoms associated with BD.

Studies with other TNF antagonists also provide support for their putative antidepressant properties. In an animal model of depression, 5 weeks of etanercept treatment was demonstrated to exert antidepressant-like effects on the Forced Swim Test (19). In clinical trials, 12 weeks of etanercept was demonstrated to result in a reduction of depressive symptom severity on the Hamilton Depression Rating scale (HAMD) and Beck Depression Inventory (BDI) in individuals with plaque psoriasis (20;21). Significant improvement in depressive symptom severity has also been demonstrated in individuals with Crohn's disease following adalimumab maintenance therapy (40 mg every other week) (22).

To our knowledge, there is only one published study that has evaluated the effects of infliximab in a primary depressed population. Individuals with treatment resistant depression (N=60; MDD: n=51; BD: n=9) were enrolled in a 12-week, randomized, double-blind, placebo-controlled study with infliximab (5 mg/kg). Infliximab was administered at baseline, week 2 and week 6. Infliximab was superior to placebo in mitigating depressive symptom severity on the HAMD, only in individuals who exhibited elevated inflammation at baseline [i.e. high sensitivity C-reactive protein (hsCRP) level of > 5mg/L]. This subgroup also exhibited a significantly higher response rate following treatment with infliximab as compared to placebo (62% and 33%). No significant time by drug interaction was observed in the full analysis set. Infliximab was well tolerated; the most commonly reported side effect was headaches (67%), followed by coughing and insomnia (17%), sore throat, upper respiratory infection, rash, and sinus congestion (13%), diarrhea and yeast infection (10%), panic attacks (7%) and fever (3%) (23).

Safety and toxicity concerns

The major risk associated with TNF-antagonists is an increased propensity for infections. As a result, treatment should not be initiated in patients who demonstrate evidence of acute or chronic infections. Patients must be monitored closely for clinical presentations suggestive of infection while receiving a TNF antagonist. Allergic reactions have been reported in approximately 1% of patients; serious allergic reactions are rare. Also, in rare cases patients treated with TNF-antagonists may develop antibodies against the biologic agent and very rarely develop a lupus-like syndrome. Other rare side-effects include: increased risk of serious infections, risk of demyelinating disorders (e.g. multiple sclerosis), psychosis, decrease in WBC and RBC count, and less than 1% risk of developing malignancy. Common side-effects include: mild skin reaction at the injection site (itchiness, redness, and mild swelling), nausea, abdominal pain, headache, rash and upper respiratory tract infections (such as sinusitis).

Microbial Changes *(optional participation)*

Every human harbors complex microbial communities (collectively, the human 'microbiome') that cover the skin and the body's mucosal surfaces. These communities have a profound effect on our well-being. For instance, recent studies have outlined distinct associations between gut microbial communities and obesity, inflammatory bowel disease, nationality and aging.

Emerging research on axenic and gnotobiotic mice indicates that the influence of the intestinal microbiome extends well beyond the gut to involve organs that include the skin, liver and brain. Research on an animal model of depression has also shown that oral administration of a probiotic increased plasma tryptophan levels and decreased serotonin metabolite concentrations in the frontal cortex and decreased dopamine metabolite concentrations in the amygdaloid cortex. To date, there is no data on the nature of the microbiome of patients with BD.
As part of this investigation into the efficacy of Infliximab, we will also look at the biomarker of changes in the gut microbiome.

HYPOTHESIS

Primary Hypothesis

Adjunctive treatment with intravenous infliximab (5 mg/kg) will significantly mitigate depressive symptoms in individuals with DSM-5-defined bipolar I/II depression as compared to intravenous placebo (saline).

Secondary Hypothesis

Reduction in depressive symptom severity following infliximab treatment will be mediated by reduction in the levels of pro-inflammatory mediators, notably TNF-α.
Reduction in levels of pro-inflammatory mediators, notably TNF- α, following treatment with infliximab mediates:

- reduction in depressive symptom severity
- favorable changes in cold and hot cognition
- improvements in quality of life and functioning
- alteration in central glutamate neurotransmission

OBJECTIVES

Primary Objective

The primary objective is to evaluate the efficacy of adjunctive treatment with intravenous infliximab (5 mg/kg) in mitigating depressive symptoms in individuals with DSM-5-defined bipolar I/II depression as compared to intravenous placebo (saline) as measured by the mean change in Montgomery-Asberg Depression Rating Scale (MADRS) total scores.

Secondary Objectives

Secondary objectives are (1) to evaluate the effect of infliximab/cytokine milieu on measures of cognitive function including cold cognition and hot cognition (i.e., anhedonia) (2) to evaluate the effect of infliximab/cytokine milieu on the neuroprogression in BD as measured by prefrontal N-Acetyl Aspartate and choline levels as well as hippocampal and whole brain gray matter volume using MRS and MRI, respectively (3) to evaluate the effect of treatment on quality of life and functioning as measured by 36-Item Short Form Health Survey (SF-36), Sheehan Disability

Scale (SDS) and Endicott Workplace Productivity Scale (EWPS), respectively (4) To evaluate if the gut microbial signature changes with treatment. This will be done by comparing changes pre- and post- treatment with Infliximab, as well as comparing the treatment group to the placebo group.

STUDY DESIGN

This is a phase II, 12-week, fixed dose, multisite, randomized, double-blind, placebo-controlled study of the efficacy, safety, and tolerability of adjunctive infliximab for the treatment of individuals with bipolar I/II depression.

RECRUITMENT

The two sites, Mood Disorder Psychopharmacology Unit, and Stanford University School of Medicine provide psychiatric assessment to approximately 20 new patients with BD per week. Based on experience from other interventional studies, we expect to recruit approximately 1-3 participants per month per site for this study for a period of 2 years. An attrition rate of 30 percent is expected; we anticipate that approximately 78 individual will be enrolled for a total of 60 evaluable subjects.

The study will be approved by the respective institutional review boards as well as Health Canada and FDA.

SELECTION OF SUBJECTS

Male and female outpatients (N=60; n=55 at Toronto Western Hospital and n=5 at Stanford University) between the ages of 18 and 65 who meet DSM-5 criteria for a current major depressive episode as part bipolar I/II disorder and are able to provide written informed consent will be eligible for inclusion in the study. The diagnosis of BD will be confirmed clinically according to DSM-5. At study entry, eligible subjects will be required to have a minimum Hamilton Depression Rating scale 17- item (HAMD-17) total score of $\geq$20 and Young Mania Rating Scale (YMRS) total score of <12; have previously failed a trial of a CANMAT BD guideline/FDA-approved first-line treatment for the depressive phase of BD during the index episode and/or during a prior episode. Treatment failure will be adjudicated either historically or prospectively. Female participants of childbearing potential must test negative for pregnancy and must be using adequate birth control measures (e.g., abstinence, oral contraceptives, intrauterine device, barrier method with spermicide, or surgical sterilization) throughout the study and must continue such precautions for 6 months after receiving the last study drug administration. Enrollment will be stratified based on indicators of inflammation; subjects will be randomized to treatment if they meet any **one** of the following criteria:

1. Central Obesity (ethnicity-specific waist circumference – see table below for specific values) **OR** BMI ≥30 kg/m2.

   AND

- Raised triglycerides: ≥1.7 mmol/L (150 mg/dL) or specific treatment for this lipid abnormality **OR**
- Reduced HDL-cholesterol: <1.03 mmol/L (40 mg/dL) in males; <1.29 mmol/L (50 mg/dL) in females or specific treatment for this lipid abnormality **OR**
- Raised Blood Pressure: Raised blood pressure Systolic: ≥130 mm Hg or diastolic: ≥85 mm Hg or treatment of previously diagnosed hypertension.

2. Diabetes: 8-hour fasting plasma glucose ≥ 7.0 mmol/L or Hb-A1C test ≥ 6.5% (as per the 2013 CDA diagnostic criteria) or previously diagnosed type 1 or 2 diabetes (current prescription medication for diabetes acceptable of diagnosis). Participants with child onset of diabetes will be excluded.
3. Inflammatory bowel disorder (Ulcerative Colitis, Crohn's disease).
4. Rheumatological disorders (rheumatoid arthiristis); Psoriasis.
5. Smoking cigarettes (daily - minimum of ½ pack).
6. Migraines (as per the International Headache Society guidelines) (24;25)
7. CRP ≥5 mg/L

Country/Ethnic Specific Values for Waist Circumference*

Country/Ethnic Group	Waist Circumference (as Measure of Central Obesity)
Europeans	Male ≥94 cm Female ≥80 cm
South Asians	Male ≥90 cm Female ≥80 cm
Chinese	Male ≥90 cm Female ≥80 cm
Japanese	Male ≥85 cm Female ≥90 cm

*These are pragmatic cut points and better data are required to link them to risk. Ethnicity should be the basis for classification, not the country of residence.

Participants will be excluded from the study if according to clinician judgment, they have another concurrent psychiatric disorder that requires primary clinical attention, a history of schizophrenia, active psychotic symptoms; meet criteria for substance abuse and/or dependence within 6 months; have received electroconvulsive therapy in the past 6 months, are actively suicidal or evaluated as being a suicide risk (a score of ≥3 on the HAMD-17 suicide item; a score

of ≥ 4 on the MADRS and/or according to clinical judgment using the Columbia-Suicide Severity Rating Scale). Participants will also be excluded if they have a clinically significant unstable medical illness, a history of tuberculosis (confirmed by chest radiography, a positive tuberculin skin test, or a blood test; as per Canadian TB standards) or a high risk of tuberculosis exposure; severe infections such as sepsis, abscesses, tuberculosis and opportunistic infections; viral hepatitis B (assessed using Public Health of Canada hepatitis B screening recommendation); a history of hepatitis C infection; documented or suspected human immunodeficiency virus (HIV) infection (confirmed by laboratory testing); any unstable autoimmune disorder; active fungal infection; a history of recurrent viral or bacterial infections; received within 3 months prior to screening or are expected to receive any live viral (e.g., smallpox) or live bacterial vaccinations during the trial or up to 3 months after the last administration of study agent; have had a *C.difficile* infection within the past 4 months; have a history of lymphoproliferative disease; a history of cancer, excluding basal cell or squamous cell carcinoma of the skin (fully excised with no recurrence); unstable cardiovascular, endocrinological, hematological, hepatic, renal or neurological disease determined by physical examination and laboratory testing; a concomitant diagnosis or any history of congestive heart failure; and/or are concomitantly treated with non-steroidal and steroidal anti-inflammatory medications or other biologics; have current or past exposure to anti-TNF biologics; a previous immediate hypersensitivity response, including anaphylaxis, to an immunoglobulin product (plasma-derived or recombinant, e.g., monoclonal antibody); known allergies, hypersensitivity, or intolerance to infliximab or its excipients; known allergy to murine proteins or other chimeric proteins; currently on or have used any investigational drug within 30 days prior to screening, or within 5 half-lives of the investigational agent. Females who are pregnant or breastfeeding will also be excluded from this study.

TREATMENT AND DOSING

Infliximab will be prescribed adjunctively to a conventional mood stabilizer or atypical antipsychotic agent. Eligible participants will have received conventional treatment for bipolar depression for a minimum of 4 weeks prior to randomization to infliximab or placebo. The choice and dosing of mood stabilizing agents will remain unchanged throughout the trial; allowing for adjunctive treatment with mechanistically dissimilar mood stabilizing agents, which foster recruitment.

Participants will be randomized to receive intravenous infliximab (5 mg/kg) or placebo (saline solution) at baseline, week 2 and 6 under clinical observation. Placebo will be matched to infliximab in color and consistency. The dosing and dosing schedule are identical to a previously published RCT with infliximab in MDD.

CONCOMITANT TREATMENTS:

Prohibited medications:

- Anakinra
- Abatacept
- Other biologics
- Nonsteroidal and steroidal anti-inflammatory medications (exception: 81 mg aspirin if medically indicated)

Restricted medications:

- Medications taken on a prn basis will be reviewed and may be restricted at certain time points during the study (e.g., medications that interfere with cognitive function).

SUBJECT DISCONTINUATION

Subjects may be discontinued from the study participation at any time.

Reasons for discontinuation include:

1. Voluntary discontinuation by the subject without prejudice to further treatment.
2. Worsened depression on 2 consecutive visits as measured by MADRS**.**
3. Onset of hypo/manic symptoms, i.e., YMRS >12 on 2 consecutive visits.

PROCEDURES

Participants will be enrolled at the Mood Disorders Psychopharmacology Unit, University Health Network, University of Toronto, Canada and Department of Psychiatry, Veterans' affairs Hospital, USA.

A written, signed and dated informed consent should be obtained from the subject before any study-related procedures are performed. A member of the research staff will explain the study in full detail to any prospective subject in understandable, non-medical terms. The research staff, as well as the investigator, will answer any questions raised and ensure that the prospective participant understands all the procedures and risks involved. Once the subject has had some time to consider the study (usually up to 24 hrs) and decides that they wish to participate, the study staff will confirm the subject's voluntary and informed participation in the study. A copy of the signed consent form will be given to the patient for their reference.
The subject should then be assigned a subject number.

Screening Visit

Screening procedures will be performed within -28 days of the baseline (day 0) visit, unless approved by the principal investigator.

The following information/assessments will be collected:

- Subject demography and social characteristics
    - Date of birth (mmm/yyyy), sex, race, ethnicity
    - Marital status, employment status, education
- Physical Activity History: International Physical Activity Questionnaire (IPAQ)
- Medical history: Illness characteristics will be obtained from patient interview and hospital medical records.
- Current medication(s) and the daily dosages will be recorded.
- Psychiatric history and evaluation:
    - Diagnosis of Bipolar Disorder I/II will be confirmed with the Structured Clinical Interview for DSM Disorders (SCID).
    - Comorbid psychiatric disorders will be assessed with the MINI International Neuropsychiatric Interview (MINI).
    - Active psychotic symptoms as well as substance abuse/dependence will be assessed with the MINI.
    - Symptoms of depression will be assessed using Montgomery-Asberg Depression Rating Scale (MADRS) and Hamilton Depression Rating Scale (HAMD-17).
- Other assessments
    - Symptoms of hypo/mania will be assessed using the Young Mania Rating Scale (YMRS).
    - Risk of suicide will be assessed using the Columbia Suicide Severity Rating Scale (C-SSRS).
    - Symptom severity will be assessed using the CGI-Severity.
    - Chronic stress and episodic life events will be assessed using the UCLA Life Stress Interview.
- Spontaneously reported adverse events will be recorded.
- Anthropometric measures will be recorded.
    - Height
    - Weight
- Vital signs will be recorded.
    - Blood Pressure
    - Pulse rate
- Laboratory samples will be collected.
    - TB skin test; chest x-ray (if indicated)
    - HIV test
    - Hep B test

    - Hep C test
    - Hematology: hemoglobin, hematocrit, total and differential WBC count, platelet count
    - Chemistry: blood urea nitrogen/urea, creatinine, sodium, potassium, calcium, alkaline phosphatise, total protein, albumin, AST, ALT, total bilirubin
    - Lipid panel: triglycerides, HDL, LDL, total cholesterol
    - Insulin
    - Glucose
    - Lithium
    - Valproate
    - hCG
    - Urine drug test
- participants will be given a stool collection kit to collect stool sample for microbial profile which is to be submitted within 24 hours of collection at baseline visit *(optional participation)*

Baseline / Endpoint Evaluation

The baseline visit is on day 0 of the study.

The endpoint visit is on day 84 of the study.

The following assessments will be completed in the aforementioned visits:

- Symptoms of depression will be assessed using Montgomery-Asberg Depression Rating Scale (MADRS), Hamilton Depression Rating Scale (HAMD-17) and the Quick Inventory of Depressive Symptomatology – Self Report (QIDS-SR). The HAMD-17 will be administered at baseline.
- Symptoms of hypo/mania will be assessed using YMRS and the Hypomania Check List (HCL).
- Risk of suicide will be assessed using the Columbia – Suicide Severity Rating Scale (C-SSRS).
- Symptom severity will be assessed using the CGI-Severity at baseline and endpoint whereas symptom improvement will be assessed using CGI-Improvement at endpoint.
- Quality of life will be assessed using Short Form - 36 (SF-36), Sheehan Disability Scale (SDS) and Endicott Work Place Productivity Scale (EWPS).
- The Snaith Hamilton Pleasure Scale (SHAPS) will be administered.
- History of childhood adversity will be assessed using the Childhood Trauma Questionnaire (CTQ) at baseline.
- Digit Symbol Substitution Test (DSST) from the WAIS-III and the Rey Auditory Verbal Learning Test (RAVLT) will be administered.

- The Perceived Deficits Questionnaire for Depression – 20 item will be administered.
- Diet will be assessed using the Brief Diet Questionnaire (BDQ).
- Spontaneously reported adverse events will be recorded.
- All prescription or non-prescription drugs, herbal or nutritional supplements will be recorded.
- Vital signs as well as waist circumference and weight will be measured and recorded.
- Laboratory specimens will be collected (endpoint only).
- Anatomical and functional magnetic resonance imaging (MRI) scan and a single voxel proton magnetic resonance spectroscopy (MRS) scanning will be conducted.
- Stool sample will be collected *(optional participation)*.

Follow-up Visits

All follow-up visits will be scheduled within ± 2 days of visit date to allow for variations in subject schedules, unless approved by the principal investigator. The overall treatment period in the protocol will be maintained.

The following assessments will be completed in the aforementioned visits:

- Symptoms of depression will be assessed using MADRS, the Quick Inventory of Depressive Symptomatology – Self Report (QIDS-SR)and the HAMD-17 (Baseline, Week 0 and Week 12)
- Symptoms of hypo/mania will be assessed using YMRS and the Hypomania Check List (HCL).
- Risk of suicide will be assessed using the Columbia – Suicide Severity Rating Scale (C-SSRS).
- Symptom severity and improvement will be assessed using the CGI-Severity and CGI-—Improvement, respectively, at all follow-up visits.
- Quality of life will be assessed using Short Form - 36 (SF-36) at Week 4.
- Sheehan Disability Scale (SDS) will be administered.
- The Snaith Hamilton Pleasure Scale (SHAPS) will be administered (Week 0, Week 6, Week 12)
- Digit Symbol Substitution Test (DSST) from the WAIS-III and the Rey Auditory Verbal Learning Test (RAVLT) will be administered at week 2.
- The Perceived Deficits Questionnaire for Depression – 20 item will be administered at week 2.
- Diet will be assessed using the Brief Diet Questionnaire (BDQ).
- Spontaneously reported adverse events will be recorded.
- Weight will be recorded.
- Vital signs will be measured and recorded.
- Changes to medicines will be assessed.

- Laboratory specimens will be collected (hematology and biochemistry at Week 2 and Week 6).
- Changes in physical activity will be assessed using the International Physical Activity Questionnaire (IPAQ) (Screening, Week 6 and Week 12).

- Stool sample will be collected at week 6 *(optional participation)*.

At a single time point during the study, the history of childhood and adolescent experience of neglect and abuse will be assessed using the Childhood Experience of Care and Abuse (CECA) Interview. The CECA interview will be conducted by telephone and audio-recorded. The interview and subsequent ratings will be made by highly trained and experienced research staff supervised by Dr. Kate Harkness.

Laboratory Assessments

Routine: The following laboratory tests will be completed:

- CRP (at screening)
- HIV testing (at screening)
- Hep B, C testing (at screening)
- hCG (at screening)
- Hematology: hemoglobin, hematocrit, total and differential WBC count, platelet count
- Chemistry: blood urea nitrogen/urea, creatinine, sodium, potassium, calcium, alkaline phosphatase, total protein, albumin, AST, ALT, total bilirubin
- Lipid panel: triglycerides, HDL, LDL, total cholesterol
- Insulin
- Glucose
- Lithium (if indicated)
- Valproate (if indicated)
- Urine drug screen (at screening)

Biomarkers: Blood samples for biomarker analysis will be collected at baseline, week 2, week 6, and week 12.. The samples will be collected after a 12 hour fast and centrifuged at 1000g for 15 min at 4 ^{o}C; plasma will be stored at -80 ^{o}C. Ultrasensitive ELISA will be used to measure high-sensitivity CRP and TNF-α, TNF-R1 and TNF-R2. For exploratory mechanistic analysis, inflammatory targets including inflammatory cytokines and receptors will be measured (e.g. IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13). Other exploratory targets include chemokines and receptors (e.g. MCP-1) as well as cell adhesion molecules (e.g., VCAM, NCAM, ICAM, e-selectin). Metabolic targets for evaluation include insulin, leptin, ghrelin, adiponectin and resistin levels. .

mRNA Analysis: Blood samples for mRNA analysis will be collected at baseline, week 2, week 6, and week 12. Samples will be stored at -80 ºC until analysis.

Microbiome Analysis: Fecal samples will be collected from participants and controls and extracted DNA will be analyzed by PCR and next-generation sequencing. Microbial community profiles will be generated using Illumina sequencing and paired-end assembly of 16S rRNA gene reads (one lane; 1-2 million reads per sample). This method has been used multiple times in the Neufeld lab and has been published. Importantly, no human DNA will be amplified and sequenced with this approach.

Other: Human anti-chimeric antibody (HACA) levels will also be measured at endpoint/early termination.

COMPENSATION

Subjects will receive financial compensation for participation in the study. Subjects will be reimbursed up to $50.00 per visit for travel expenses (e.g. parking, public transport) and food-related expenses.

Subjects who agree to provide stool samples will receive $10.00 at visits 2, 6, and 10.

INVESTIGATOR PRODUCT AND ADMINISTRATION

Investigational Product

Infliximab is supplied as a sterile white lyophilized powder for intravenous infusion. Each vial contains 100 mg infliximab, 500 mg sucrose, 0.5 mg polysorbate 80, 2.2 mg monobasic sodium phosphate, monohydrate and 6.1 mg dibasic sodium phosphate, dehydrate. No preservative are present.

Infliximab lyophilized concentrate for IV injection is supplied in individually-boxed single-use vials of 100 mg dosage strength.

Storage

The lyophilized product will be refrigerated at 2°C to 8°C (36°F to 46°F). Reconstitution and dilution of infliximab will take place in controlled, aseptic conditions. The infusion solution will be administered within 3 hours of reconstitution and dilution. If the diluted infliximab is not used

within 3 hours of preparation, the infusion solution may be stored for no longer than 24 hours at 2°C to 8°C.

Stool samples will be stored in a -80 ºC fridge at University of Toronto until transported in dry ice to the University of Waterloo for analysis.

SAFETY MEASURES

Tuberculin skin testing will be conducted at screening. All subjects will be screened for HIV, hepatitis B and C. Infusion will be delayed if any evidence of other infections is present at baseline until symptoms have resolved and/or appropriate treatment has been initiated. Treatment and safety at the time of infusion will be monitored by a trained Registered Nurse that has clinical trial experience with biologic agents. Safety measures will be evaluated at each visit and will include measurement of vital signs, assessment of mood symptoms and adverse event development; in addition, routine blood tests will be conducted at screening, week 2, 6, and 12. Adverse events will also be recorded at each visit by spontaneous report. The YMRS will be administered at each visit to monitor induction of hypo/manic symptomatology.

EFFICACY MEASURES

Primary Endpoints

Clinical outcome measures will be administered at baseline, week 1, 2, 3, 4, 6, 8, 10, and 12. The primary efficacy variable will be mean change from baseline to week 12 on the MADRS total score.

Secondary Endpoints

Secondary efficacy evaluations will be response (i.e. >50% reduction from baseline week 12 on the MADRS) and remission (i.e. MADRS $\leq$12) rates, as well as change in CGI Severity.

STATISTICS

Statistical Methods

Separate randomization schedules will be prepared for each site.

The intention-to-treat sample will include subjects who receive at least one infusion of infliximab or placebo and have at least one post-baseline assessment. Missing data will be estimated with the maximum likelihood method using a mixed model for repeated measures ANOVA. The mixed model repeated measures ANOVA will also be used to evaluate change

from baseline to endpoint as a function of treatment status. All tests will be two tailed with significance defined by $\alpha<0.05$.

All data collected during this study will be uploaded to the National Institute for Mental Health data respiratory.

Interim Analysis

No interim analysis will be conducted.

Sample Size Calculation and Statistical Analysis

Using the published literature with infliximab in MDD (23), it is estimated that 30 evaluable subjects per treatment arm will have better than 80% power to detect a difference in depressive symptoms between infliximab-treated and placebo-treated subjects at level of 5% significance.

ADVERSE EVENTS

Definitions

Adverse event

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

Serious adverse event

A serious adverse event is an AE occurring during any study phase (i.e., run-in, treatment, washout, follow-up), and at any dose of the investigational product, comparator or placebo, that fulfils one or more of the following criteria:

- results in death
- is immediately life-threatening
- requires in-patient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital abnormality or birth defect

- is an important medical event that may jeopardise the patient or may require medical intervention to prevent one of the outcomes listed above.

The causality of SAEs (i.e., their relationship to study treatment) will be assessed by the investigator(s). Note that SAEs that could be associated with any study procedure should also be reported. For such events the casual relationship is implied as "yes".

Recording of adverse events

AEs will be collected from baseline visit to until the end of the study. At each visit, subjects will be asked if they have had any health problems since the previous visit. All AEs will be recorded appropriately, whether or not considered related to the investigational product. This will include AEs spontaneously reported by the patient and/or observed by the staff as well as AEs reported in response to a direct question e.g. “Have you had any health problems since your last visit?”
For each AE, the following parameters be described:

- start and stop date
- action taken with regards to investigational product
- outcome
- if the AE caused the patient to discontinue
- a statement if the AE fulfils the criteria for a SAE or not
- the investigator’s assessment of the causal relationship between the event and the investigational product
- intensity of the AE
    - mild (awareness of sign or symptom, but easily tolerated)
    - moderate (discomfort sufficient to cause interference with normal activities)
    - severe (incapacitating, with inability to perform normal activities)

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be an SAE.
Symptoms associated with overdose should be reported as AEs. For further information regarding overdose, see section.
Pregnancy in itself is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication.
Follow-up of adverse events should be based upon the clinical judgement of the investigator.

Reporting of serious adverse events

Reporting of SAEs to regulatory authorities will be done by the investigator in accordance with local regulations. A copy of the report will also be sent to the manufacturer of the investigational product.

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