Study Title: A Pilot Double-Blind Randomized Placebo-Controlled Crossover Study to Investigate Rapid Antidepressant Effects of Leucine

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1. Introduction and Purpose: This randomized double-blind placebo-controlled crossover study seeks to evaluate the antidepressant effect of L-leucine, an essential amino acid, in patients with Major Depressive Disorder (MDD). Current antidepressant medications take several weeks to attain maximal therapeutic effect and are ineffective for a large percentage of patients. Hence, there is an urgent need to identify novel and rapid-acting antidepressant treatments. We hypothesize that oral intake of L-leucine will result in rapid improvement of depressive symptoms in MDD patients.

The specific aims of the study are as follows:
Aim 1: The primary aim is to evaluate the change in depressive symptoms with L-leucine (LEU) as compared to placebo (PBO) in MDD patients. Symptom change from baseline will be measured at 3 days, 7 days, and 14 days using the self-rated version of Quick Inventory of Depressive Symptomatology (QIDS-SR).

Aim 2A: A secondary aim of this study is to evaluate the change in anxiety symptoms, anhedonia (inability to experience pleasure), fatigue and quality of life with L-leucine in MDD patients. Anxiety symptom change from baseline will be measured at 3 days, 7 days, and 14 days using self-rated Generalized Anxiety Disorder 7-item (GAD-7) scale. Changes in anhedonia from baseline will be measured at 3 days, 7 days, and 14 days using Snaith Hamilton Pleasure Scale (SHAPS). Change in fatigue from baseline will be measured at 3 days, 7 days, and 14 days using Multidimensional Fatigue Inventory (MFI). Change in psychosocial functioning from baseline will be measured using the Work and Social Adjustment Scale (WSAS) at 3, 7, and 14 days.

Aim 2B: Another secondary aim of this study is to evaluate tolerability and safety of oral L-leucine in MDD patients. Tolerability and safety will be monitored using vital signs and following scales: Frequency, Intensity, and Burden of Side Effects Rating (FIBSER), Patient Adherence Questionnaire (PAQ), Systematic Assessment for Treatment Emergent Events (SAFTEE), Concise Health Risk Tracking (CHRT), and Concise Associated Symptom Tracking (CAST).

The study outcomes are as follows:
Primary outcome: Comparison of reduction in QIDS-SR after 14 days of treatment with LEU and PBO in MDD patients.

Secondary outcome 1: Percentage of MDD patients with 50% or greater reduction in depression severity after 3 days, 7 days and 14 days of LEU and PBO treatments.

Secondary outcome 2: Percentage of MDD patients with QIDS-SR score less than 5 at 3 days, 7 days and 14 days of LEU and PBO treatments.

Secondary outcome 3: Rates of adverse effects or treatment emergent side effects after 3 days, 7 days and 14 days of LEU and PBO treatments.

Secondary outcome 4: Average change in depression, anxiety, anhedonia, and fatigue symptoms and psychosocial functioning from baseline after 3, 7, and 14 days of LEU and PBO treatments.

Secondary outcome 7: Conduct exploratory analyses of potential predictors (e.g., biomarkers, duration of current episode, age, gender, baseline severity of MDD, baseline profile of depressive symptoms, baseline level of symptoms of anxiety etc.) of response to leucine.

Secondary outcome 8: Evaluate changes in pro-inflammatory biomarkers (interleukin 6 and C-reactive protein) as well as ratio of kynurenine and tryptophan in peripheral blood after L-leucine intake and how it correlates with change in depression severity.

The potential study risks include side effects associated with oral administration of L-leucine, the most common of which is transient drowsiness. The potential benefit of this study is identification of a novel and rapid-acting antidepressant agent. This is a pilot study and adequately powered double-blind randomized controlled studies would be required in future to establish the antidepressant effects of L-leucine.

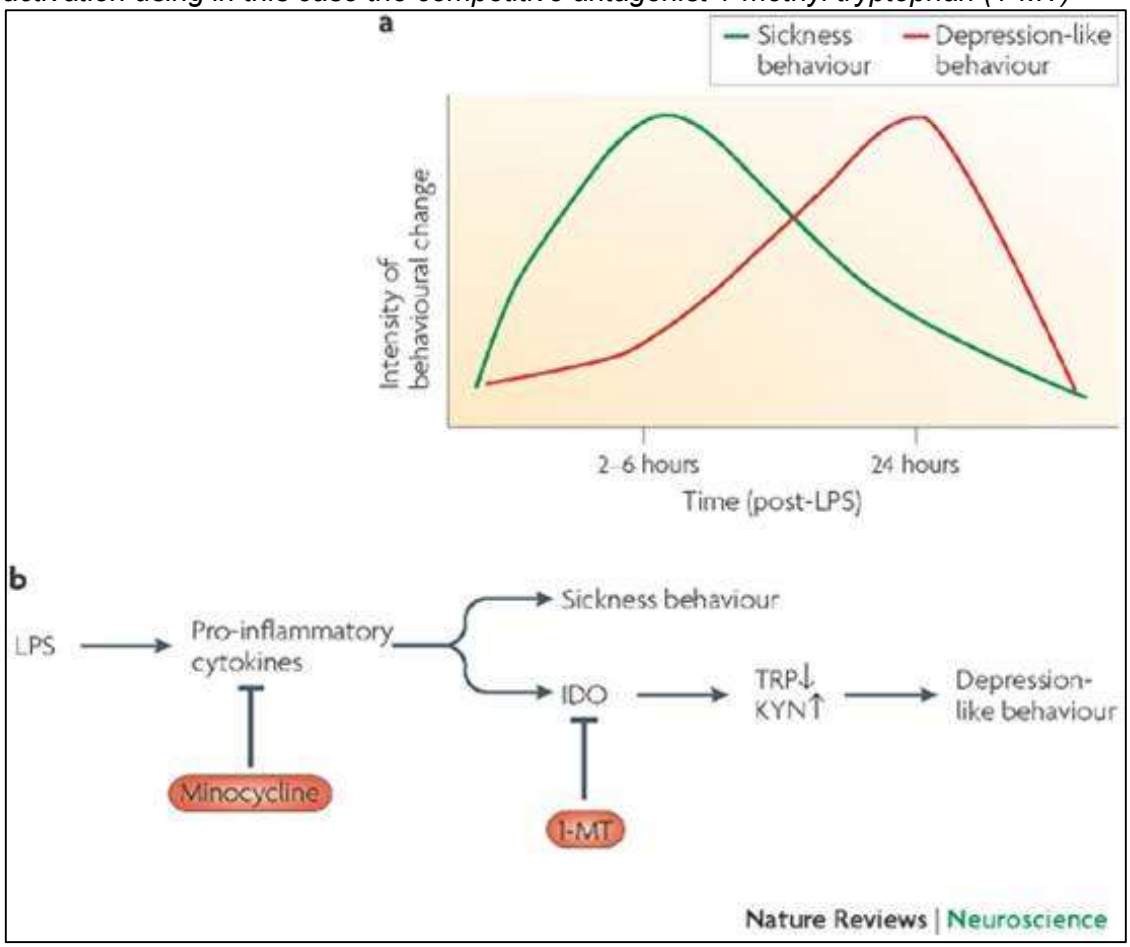
2. Background: Major depressive disorder (MDD) is a common [1, 2], chronic [3-5] and debilitating [6, 7] illness. Most MDD patients stay on ineffective medications for too long, switch treatments too early, or simply drop out of care [8-10]. Response rates to currently available antidepressant treatments are low, duration to attain therapeutic benefit is long, and treatment-emergent side effect burden is significant [11-13]. Treatments are selected based not on efficacy but on patient or provider preferences, cost, side effects, tolerability, and/or response during previous episode(s) [14]. Attempts to utilize demographic features (like gender [15], race [16], employment status [11]), illness characteristics (like baseline severity of depression [17], duration of illness [18], number of previous episodes [19], age of onset [20], family history of mood disorders [19], presence of anxious features [21], depression subtypes [22], co-morbid psychiatric disorders [16], psychosocial functioning [23]), and social factors (like marital status [19], level of social support, social status [24]) have proven to be of minimal utility in predicting differential response to currently available antidepressants [22, 25-29]. Biological markers, which are objective and can be measured externally [30, 31], are urgently needed for targeted drug development in depression. Biomarkers of inflammation are promising targets for novel antidepressant drug development.

Several lines of investigation implicate inflammation in pathophysiology of MDD [32]. Patients, who receive cytokines as treatment for their medical conditions, such as hepatitis C or malignancies, develop MDD at high rates. Studies of antidepressant medications further suggest the potential role of targeting inflammation to improve depressive symptoms. Levels of peripheral blood inflammatory cytokines are reduced with SSRI [33]. Low (<1 mg/mL) levels C-reactive protein (CRP), a marker of inflammation, at baseline has been used successfully to identify patients who would respond to escitalopram as compared to nortriptyline [34]. As compared to escitalopram, use of nortriptyline in patients with higher CRP levels at baseline was associated with greater reduction in their depression severity. Raison et al. [35] found that elevated CRP (> 5 mg/ml) at baseline was associated with a significantly greater likelihood of treatment response with infliximab, a tumor necrosis factor antagonist, as compared to placebo. Also, levels of protein p11 in natural killer (NK) cells and monocytes may be potential biomarker as Svenningsson et al. found that reduction in p11 after 1-2 weeks of citalopram treatment was significantly correlated with subsequent reduction in depression severity [36]. Janssen et al. found that antidepressants modulate cytokine functioning and directly influence treatment outcome in MDD [37]. Further, they showed that antidepressants normalize serum levels of cytokines including interleukin IL-6, IL-1 β, tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ)[37]. Maes et al. examined the effects of clomipramine, sertraline and trazadone on the stimulated production of IFN-γ and IL-10, and observed that all three antidepressants significantly decreased IFN-γ/IL-10 ratio[38].

In animal models, as discussed by Dantzer et al. below [32], depression-like behaviors persist even after return of normal motor activity and food intake when rodents are injected with the cytokine inducer lipopolysaccharide (LPS). The transition from inflammation-induced sickness to depression is mediated by activation of the tryptophan metabolizing enzyme indoleamine 2,3 dioxygenase (IDO).

IDO metabolizes tryptophan (TRP) into kynurenine (KYN) that is transported into the brain and further metabolized by activated microglia in neurotoxic kynurenine metabolites. Prevention of IDO activation by blockade of inflammation with minocycline, a 2nd generation tetracycline with potent inflammatory properties, or 1-methyl tryptophan (1-MT) that acts as a competitive antagonist of IDO abrogates inflammation-induced depression.

*Figure 3.from From inflammation to sickness and depression: when the immune system subjugates the brain. Robert Dantzer, Jason C. O'Connor, Gregory G. Freund, Rodney W. Johnson & Keith W. KelleyNature Reviews Neuroscience **9**, 46-56 (January 2008) – Note the transition from sickness to depression mediated by IDO activation and the possibility to treat depression by inhibition of IDO activation using in this case the competitive antagonist 1-methyl tryptophan (1-MT)*



Kynurenine generated by IDO needs to enter the brain to be further metabolized into neurotoxic kynurenine metabolites. The entry of kynurenine into the brain is mediated by the large amino acid transporter LAT1 (SLC7A5) that also transports branched chain amino acids including L-leucine. It is therefore theoretically possible to block the entry of kynurenine into the brain by administering L-leucine. Preclinical experiments carried out by the Dantzer's research team have demonstrated that L-leucine decreases brain kynurenine levels without altering peripheral kynurenine levels and abrogates inflammation-induced depression-like behavior in mice.

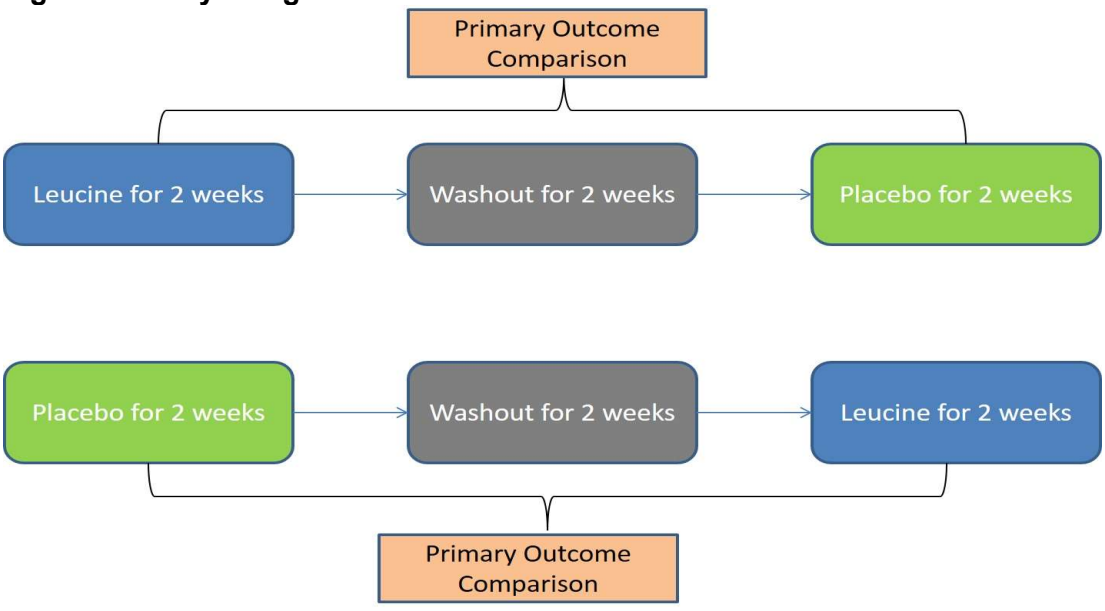
The object of the present clinical trial is to assess whether any beneficial inflammation of L-leucine supplementation observed in mice also apply in humans with inflammation-associated depression. L-leucine is an essential amino acid that can be safely used as a supplement. It has been used to try to stimulate muscle protein synthesis, promote weight loss in overweight subjects and improve glucose utilization in subjects prone to type 2 diabetes (e.g., NCT01492010, NCT00683826).

3. Concise Summary of Project: This is a pilot phase II clinical trial of L-leucine to test its efficacy in reducing depressive symptoms in MDD patients, especially those who exhibit increased inflammation. The determination of increased inflammation will be done post-hoc, so we will invite all adults with major depressive disorder seeking treatment for their depressive symptoms to participate in this study. Screening will be conducted with a brief phone screen followed by a longer screening visit for those who qualify. We anticipate that the majority of participants will be recruited through existing studies of the Center for Depression Research and Clinical Care (CDRC) (IRB protocol # 092015-049, 112015-021, 032014-068, and 072010-013). Participants will also be recruited from specialty clinics where patients typically have higher inflammation levels. Participants will provide informed consent at the screening visit prior to any study procedures. During the screening visit, all study participants will provide demographic information and complete Quick Inventory of Depressive Symptomatology Self-Rated version (QIDS-SR), Concise Health Risk Tracking (CHRT), Pain Frequency, Intensity and Burden scale (PFIBS), and Antidepressant Treatment Response Questionnaire (ATRQ). Procedures performed during the screening visit will include a blood draw, urinalysis, urine drug screen, urine pregnancy test (if applicable), physical exam and MINI Neuropsychiatric Interview (MINI). All participants who qualify will then be eligible to participate in the study and will be randomized to either receive leucine or placebo. All participants will be evaluated at days 0, 3, 7, 14, 17, 21, 28, 31, 35, and 42 (See study schedule in figure 3 below). In this cross-over study, participants will be crossed over to the second treatment after 2 weeks of washout. Medication will be provided as part of the study at no cost to participants. L-leucine is commercially available in the US as oral and intravenous dietary supplements. For this study, we will use oral L-leucine preparation (8 gm in two divided doses) mixed with an effervescent powder. The compound will be obtained from a Dallas-area pharmacy through the UTSW Research pharmacy. The pharmacy will also provide a placebo mixture (maltodextrin as the placebo ingredient) which will resemble to the compounded mixture of L-leucine and effervescent powder.

We aim to screen 80 subjects for 40 subjects to be randomized into the study as we project that about 40 subjects will fail screening or withdraw early from study prior to randomization. The study period will last 42 days (6 weeks) from the baseline visit (43-56 days from the screening visit). All collected data will be de-identified and presented in aggregate. We hypothesize that MDD subjects will have greater reduction in depression severity on leucine as compared to placebo.

Subjects may be exited from the study prior to expected completion date due to non-compliance with scheduled follow-ups or subjects may withdraw consent. Subjects may also be exited early from the study if the study physician deems the subject to be a potential danger to self or others or if the subject develops severe acute medical or psychiatric condition that would put the subject at risk.

Figure 2. Study design



4. Study Procedures:

Treatment: Participants with contraindication to treatment with L-leucine (Maple Syrup Urine Disease or known history of hypersensitivity) will not be included in this study. All study participants will be randomized in a double blind fashion to either receive L-leucine or placebo, as shown in the study design figure (Figure 2). Study physician will review pre-administration information to establish that participant is eligible for leucine administration. Participants will be requested to take the total dose of leucine in twice daily dose.

Study drug: Individual packets containing the following ingredients will be provided to participants during the course of study.

Each packet containing L-leucine will include:

1. L-leucine: 4 gram (gm)
2. Effervescent powder: 1 gm
3. Citric acid: 2 gm
4. Sweetened flavor base: gm

Total weight: 10 gm

Each packet containing maltodextrin will include:

1. Maltodextrin: 4 gm
2. Effervescent powder: 1 gm
3. Citric acid: 2 gm
4. Sweetened flavor base: gm

Total weight: 10 gm

Subjects will be provided with following instructions for taking study drug: Store packets in a cool dry dark place away from bright light or sun light. Pour contents of packet in a dry cup or glass and add 6 oz of room temperature water while gradually stirring the contents of packet. Effervescence is expected. Drink the mixture quickly before the effervescence subsides. Do not use cold water.

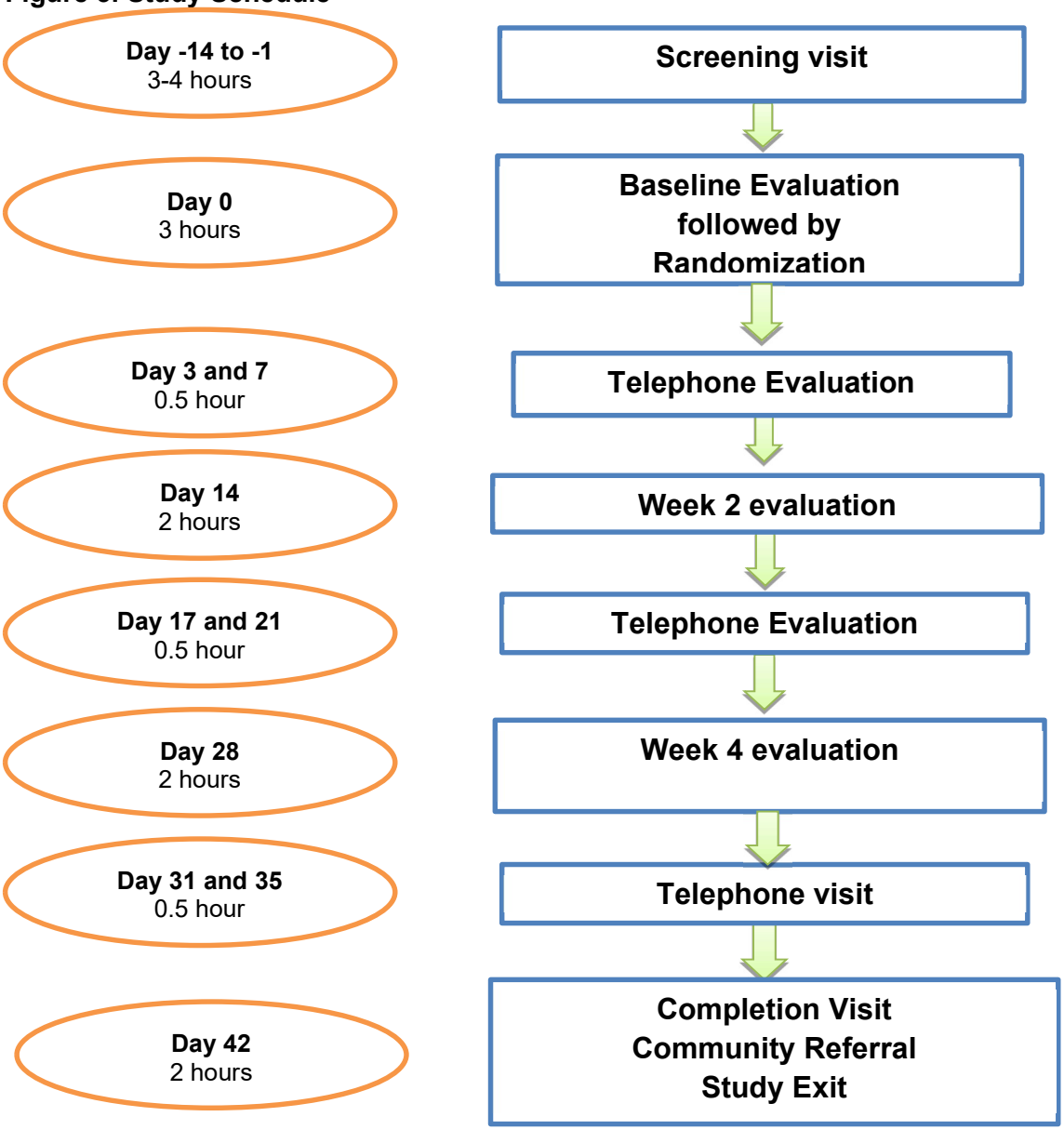
Schedule: Participants will be assessed at screening for study eligibility and those who are willing and eligible will be evaluated in ten (10) study visits on days 0, 3, 7, 14, 17, 21, 28, 31, 35, and 42. Only visits on day **0**, day **14**, day **28**, and day **42** are in-person visits. The other evaluations will be conducted by telephone. The screening visit will take 3-4 hours and include MINI Neuropsychiatric Interview (MINI). On day **0**, participants will be assessed initially to confirm eligibility for study participation and eligible participants will be randomized to either placebo or leucine in a double blind fashion. Subjects will be given a two-week supply of compounded mixture on days **0** and **28**. As days **14** to **27** is wash-out period, participants will not be given any study drug during this period. The participants will be assessed on days 3, 7, 17, 21, 31, and 35 via telephone for 20-30 minutes and will be reminded to complete the self-report forms. In-person follow-up visits will be conducted on days **14**, **28**, and **42** for approximately 2 hours in each visit. The final visit will be on day **42**. Participants may also come in at any time between visits if they request to do so, or if the study physician feels it is clinically indicated. In addition, subjects will be contacted via mail and/or phone to remind them of upcoming study visits. Please see Figure 3 for the schematic representation of study schedule.

Study visits will be at no charge, and participants will be provided $ 200.00 for study completion. If any participant does not complete the study, she/he will be paid for each in-person visit completed as follows:

Screening N/A
Visit 1 (Baseline visit) $ 60
Visit 2 and 3 (Telephone evaluation)
Visit 4 (In-person follow-up evaluation) $ 40
Visit 5 and 6 (Telephone evaluation)
Visit 7 (In-person follow-up evaluation) $ 40
Visit 8 and 9 (Telephone evaluation)
Visit 10 (Final study visit, In-person evaluation) $ 60

Assessments: Study participants will be assessed with the MINI Neuropsychiatric Interview (MINI), Antidepressant Treatment History Questionnaire (ATRQ), Quick Inventory of Depressive Symptomatology self-rated (QIDS-SR) version, Generalized Anxiety Disorder 7-item (GAD-7) scale, Snaith Hamilton Pleasure Scale (SHAPS), Work and Social Adjustment Scale (WSAS), Concise Associated Symptom Tracking (CAST) scale, Frequency, Intensity, and Burden of Side Effects Rating (FIBSER) scale, Systematic Assessment of Treatment Emergent Effects (SAFTEE), Pain Frequency, Intensity and Burden scale (PFIBS) and Concise Health Risk Tracking (CHRT) scale. Trained study personnel will establish the diagnosis of major depressive disorder using the MINI a semi-structured interview for DSM-5 diagnoses. The self-rated version of the QIDS is a well validated 16-item measures that assesses symptoms of major depressive disorder and will be used to screen for study eligibility as well as measure change in depression severity as subjects' progress through the study. The GAD-7 is a well validated clinician and self-rated instrument for assessment of anxiety symptoms. The SHAPS is a 14-item self-report instrument which measures the ability to experience pleasure. The WSAS is a 5-item self-report questionnaire of psychosocial functioning. The CHRT is a 7-item scale which will be used to monitor suicidal ideation The CAST is a 17-item self-report scale that will be used to assess mood changes associated with study medication administration. Adherence to study medication will be monitored using the PAQ.

Figure 3. Study Schedule



The MFI is a commonly used instrument to assess fatigue. The side effects burden will be monitored with FIBSER which is a 3-item self-report measure and the SAFTEE which is a 56-item comprehensive self-report for side-effects. Pain will be monitored using the PFIBS scale – a validated instrument to measure pain frequency, intensity, and burden.
For individuals meeting inclusion criteria from screening and potentially interested in participating, study personnel will review eligibility, explain the study procedures, including risks, benefits, and alternatives, answer questions and obtain informed consent. A trained research assistant will collect blood and urine samples, and measure the participant's height, weight, heart rate, and blood pressure. For baseline and follow-up visits, a study physician or trained research personnel will complete the clinician rated instruments while participants fill out self-rated instruments. Please see Table 1 and individual visit descriptions below for further details. Individuals who are not eligible to participate as well as subjects who complete or terminate study participation will be offered a referral for clinical treatment in the community.

Screening visit will last approximately 3-4 hours and will include following:

- Informed Consent.
- Assessment by study physician for suitability of study participation.
- Diagnostic assessment with the MINI Neuropsychiatric Interview (MINI).
- Medical and psychiatric history, as well as review of current medications.
- A physical exam, including height, weight, assessment of pain (using PFIBS), and vital signs (blood pressure, temperature, respiratory rate, and heart rate).
- Collection of blood sample, approximately 2 teaspoon, to test to test for hematology, liver, thyroid and kidney function, and other general health measures; urine sample for urine drug screen; and a urine pregnancy test for women capable of become pregnant.
- Collection of demographic information.
- Completion of self-report forms.

As some participants will be recruited through the Center for Depression Research and Clinical Care, we will also use diagnostic information contained in the MINI conducted as part of their participation in other research studies. Individuals, who are eligible and give consent to participate, will be scheduled for the baseline visit and randomization on visit 1. As laboratory investigations may take a few days, final decision on study eligibility will be made on baseline day 0.

Visit 1: The visit on **day 0** will take approximately 3 hours and will include:

- Vital signs and the questionnaires listed in table 1.
- Blood draw of approximately 3 teaspoons (3 x 5 ml). The blood sample will be collected in lavender/purple top (EDTA) tube, yellow top (ACD) tube (only at baseline for DNA), orange top (thrombin based clot activator) tube, and PAXGene tube. Plasma, serum, and buffy coat (leukocytes and platelets) will be aliquoted for storage.
- A urine pregnancy test for females capable of becoming pregnant will be performed.

At the end of visit 1, participants will either be provided with paper copies of following forms: QIDS-SR, GAD-7, SHAPS, WSAS, PFIBS, MFI, SAFTEE, CAST, or advised to fill out these forms through a REDCap based email survey. When using paper forms, participants will bring these on next in-person study visit.

Visit 2: On **day 3**, trained research personnel will contact the participant and remind her/him to fill out the QIDS-SR, GAD-7, SHAPS, WSAS, FIBSER, PAQ, PFIBS, MFI, SAFTEE, and CAST (paper or REDCap). This telephone evaluation will take approximately 30 minutes.

Visit 3: On **day 7**, trained research personnel will contact the participant and remind her/him to fill out the QIDS-SR, GAD-7, SHAPS, WSAS, FIBSER, PAQ, PFIBS, MFI, SAFTEE, and CAST (paper or REDCap). This telephone evaluation will take approximately 30 minutes.

Visit 4: The visit on **day 14** will take approximately 2 hours. Participants will start 2-week washout period per cross-over design (Figure 2) in a double-blind fashion. NO study drugs will be provided for this two-week period.

- Follow-up evaluations will include vital signs and the questionnaires listed in Table 1.
- Blood draw of approximately 3 teaspoons (3 x 5 ml). The blood sample will be collected in lavender/purple top (EDTA) tube, orange top (thrombin based clot activator) tube, and PAXGene tube. Plasma, serum, and buffy coat (leukocytes and platelets) will be aliquoted for storage.
- A urine pregnancy test for females capable of becoming pregnant will be performed.

At the end of visit 4, participants will either be provided with paper copies of following forms: QIDS-SR, GAD-7, SHAPS, WSAS, FIBSER, PAQ, PFIBS, MFI, SAFTEE, and CAST, or advised to fill out these forms through a REDCap based email survey.

Visit 5: On **day 17**, trained research personnel will contact the participant and remind her/him to fill out the QIDS-SR, GAD-7, SHAPS, WSAS, FIBSER, PAQ, PFIBS, MFI, SAFTEE, and CAST (paper or REDCap). This telephone evaluation will take approximately 30 minutes.

Visit 6: On **day 21**, trained research personnel will contact the participant and remind her/him to fill out the QIDS-SR, GAD-7, SHAPS, WSAS, FIBSER, PAQ, PFIBS, MFI, SAFTEE, and CAST (paper or REDCap). This telephone evaluation will take approximately 30 minutes.

Visit 7: The visit on **day 28** will take approximately 2 hours. Participants will switch to the second medication per cross-over design (Figure 2). They will be provided with two-week supply of medications.

- Follow-up evaluations will include vital signs and the questionnaires listed in table 1.
- Blood draw of approximately 3 teaspoon (3x5 mL). The blood sample will be collected in lavender/purple top (EDTA) tube, yellow top (ACD) tube (only at baseline for DNA), orange top (thrombin based clot activator) tube, and PAXGene tube. Plasma, serum, and buffy coat (leukocytes and platelets) will be aliquoted for storage.
- A urine pregnancy test for females capable of becoming pregnant will be performed.

At the end of visit 7, participants will either be provided with paper copies of following forms: QIDS-SR, GAD-7, SHAPS, WSAS, FIBSER, PAQ, PFIBS, MFI, SAFTEE, and CAST, or advised to fill out these forms through a REDCap based email survey.

Visit 8: On **day 31**, trained research personnel will contact the participant and remind her/him to fill out the QIDS-SR, GAD-7, SHAPS, WSAS, FIBSER, PAQ, PFIBS, MFI, SAFTEE, and CAST (paper or REDCap). This telephone evaluation will take approximately 30 minutes.

Visit 9: On **day 35**, trained research personnel will contact the participant and remind her/him to fill out the QIDS-SR, GAD-7, SHAPS, WSAS, FIBSER, PAQ, PFIBS, MFI, SAFTEE, and CAST (paper or REDCap). This telephone evaluation will take approximately 30 minutes.

Visit 10: The visit on **day 42** will take approximately 2 hours. The evaluations on day 42 will be conducted per table 1. Blood sample of approximate 3 teaspoons (3x5 mL) will be collected in lavender/purple top (EDTA) tube, orange top (thrombin based clot activator) tube, and PAXGene tube. Plasma, serum, and buffy coat (leukocytes and platelets) will be aliquoted for storage. This will be final study visit and participants will be given referrals for treatment in the community, if interested.

Sample Collection: A single sample of 10 mL of blood will be collected at the screening visit for routine laboratory work up including measurement of blood urea nitrogen (BUN) and creatinine as leucine has a predominant urinary excretion. We will draw serum from peripheral venous blood drawn by appropriately trained staff. At the screening visit, a urine sample for urinalysis, urine drug screen, and urine pregnancy test for female participants will be collected. Blood and urine samples will be analyzed by Quest Diagnostics through contract with the UTSW CTRC. Blood samples will also be

collected on days 0, 14, 28, and 42 to identify biomarkers of response to leucine. Whole blood will be collected in EDTA (purple/lavender top), ACD (yellow top; only once at baseline), thrombin based clot activator (orange top) and PAXGene tubes; plasma, WBCs buffy coat, and serum aliquots will be isolated from whole blood and stored at -70 C.

Table 1. Visit Schedule

	Screening (Day -14 to -1)	Baseline or Day 0	Day 3	Day 7	Day 14	Day 17	Day 21	Day 28	Day 31	Day 35	Day 42
MINI	X										
ATRQ	X										
Clinical interview	X										
Physical Exam	X										
Heart Rate	X										
Blood Pressure	X										
Urine Drug Screen	X										
Urine Preg Test	X	X			X			X			
Contraceptive history form	X	X									
Study Physician Assessment	X	X			X			X			X
Blood Draw	X	X			X			X			X
PFIBS	X	X			X			X			X
CHRT P	X	X			X			X			X
CHRT C	X	X			X			X			X
QIDS-SR	X	X	X	X	X	X	X	X	X	X	X
GAD-7	X	X	X	X	X	X	X	X	X	X	X
SHAPS	X	X	X	X	X	X	X	X	X	X	X
MFI	X	X	X	X	X	X	X	X	X	X	X
WSAS	X	X	X	X	X	X	X	X	X	X	X
CAST	X	X	X	X	X	X	X	X	X	X	X
FIBSER		X	X	X	X	X	X	X	X	X	X
PAQ		X	X	X	X	X	X	X	X	X	X
SAFTE		X	X	X	X	X	X	X	X	X	X

5. Sub-Study Procedures:
Biomarkers – We will collect blood samples on days 0, 14, 28 and 42. Exploratory proteomic or microarray analyses may be conducted to evaluate change in protein or mRNA expression with leucine. Participation in the main study includes participation in the sub-study. Part of sample that is left-over from the sub-study will also be banked for future research. Participants have to option to decline participation in the banking of blood samples for future research studies

6. Criteria for Inclusion of Subjects: Participants meeting the following criteria will be enrolled in the study:
1. Adults, age 18-64 years.
2. Able to complete assessments and interview in English and Spanish.
3. Provide written informed consent.
4. Outpatients or inpatients with a current primary diagnosis of nonpsychotic major depressive disorder per the MINI.
5. QIDS-SR score of ≥ 14 at screening visit and baseline assessment at Day 0 visit
6. Stable antidepressant dose of no more than one antidepressant medication for 4 weeks and no anticipated changes during the study period.
7. Stable doses of all concomitant medications for over 6 weeks.
6. No more than two failed antidepressant trials of adequate dose and duration, as defined by ATRQ, in the current episode.

7. Criteria for Exclusion of Subjects: Participants meeting the following criteria will be excluded from the study:

1. Psychiatric co-morbidity posing safety risk, including current suicidality or psychosis as assessed on clinical interview.
2. Pregnant or breastfeeding or plan to become pregnant over the ensuing 2 months following study entry or are sexually active and not using adequate contraception
3. Meets DSM-5 criteria for substance dependence in the last 6 months or substance abuse in the last 2 months (except for nicotine).
4. History (lifetime) of psychotic depressive, schizophrenia, bipolar (I, II or NOS), schizoaffective or other Axis I psychotic disorders
5. Have an unstable general medical condition (GMC) that will likely require hospitalization or deemed terminal (life expectancy less than 6 months).
6. Require medications for their GMCs that interact with L-leucine (e.g. sildenafil).
7. Receiving or have received during the index episode vagus nerve stimulation, ECT, or rTMS, or other somatic antidepressant treatment
8. Taking thyroid medication for hypothyroidism (but may be included if they have been stable on the thyroid medication for 3 months).
9. Therapy that is depression specific, such as CBT or Interpersonal Psychotherapy of Depression. Participants can participate if they are receiving psychotherapy that is not targeting the symptoms of depression, such as supportive therapy or marital therapy.
10. Hypersensitivity to L-leucine
11. Have Maple Syrup Urine Disease.

8. Sources of Research Material: Data obtained in this study will be used specifically for research purposes. Sources of material will include data collected from patients' screening and study participation including demographics; psychiatric history and examination; physical examination; heart rate; blood pressure; laboratory investigations; contraceptive history; urine pregnancy test for females; urine drug screen; blood sample collected for biomarker studies; and information from the following scales: MINI, ATRQ, PFIBS, QIDS-SR, GAD-7, SHAPS, WSAS, MFI, PAQ, CHRT, CAST, FIBSER, and SAFTEE.
Samples: At screening, we will collect 10 mL peripheral venous blood for evaluation of overall heath and urine for urine drug screen and pregnancy test in women who are capable of becoming pregnant. We will also collect 3 teaspoons (3 x 5 ml) of blood each on days 0, 14, 28 and 42 (total amount of blood 12 teaspoons (12 x 5 ml)). Blood will be collected in EDTA (purple/lavendar-top), ACD (yellow-top, only once at baseline for DNA), thrombin based clot activator (orange top) and PAXGene tubes.

9. Recruitment Methods and Consenting Process: We plan to randomize a maximum of 40 participants into this study, for which we expect to consent 80 participants. Participants will be recruited from the UTSW Family Medicine Clinic, the UTSW General Internal Medicine Clinic, the UTSW Employee Assistance Program, the UTSW psychiatry inpatient unit, and from sites off-campus. The PI and study psychiatrist will meet with MDs and clinic managers at these sites to explain the study goals, inclusion and exclusion criteria, and study process prior to recruitment. We will provide the MDs with written information about the study and the characteristics desired in referrals, along with a form that can be used by MDs to communicate a referral to the research team. Patients seen by physicians at these sites with a likely diagnosis of major depressive disorder and a desire to seek treatment will be referred to the study team, given information about the study, and provided the phone number for the study team. Most of the participants are expected to come through the CDRC standard recruitment process (IRB protocol # 092015-049, 112015-021, 032014-068, and 072010-013). In this sense they will already be patients in the clinic under a research protocol and not a part of the principal investigator's clinical practice. Previous participants in research at the CDRC who have provided consent for future contact will also be invited to participate in this study. Participants may also be recruited after screening of existing records at UTSW and clinics affiliated with CDRC. Participants may also be recruited from IRB approved recruitment database and UTSW Volunteer Research Participant Registry.

As part of the CDRC standard recruitment process, there may be a phone screening in which the study will be described, and the essential inclusion and exclusion criteria explained. A brief phone screen asking about medical and psychiatric history, including current and past medications taken for depression, will be done. We will provide all needed information about the study to help potential subjects decide if they are interested in participating. Subjects who are interested in participating will then be seen by research personnel and given an opportunity to provide informed consent.

Before asking potential participants to sign the informed consent form, research personnel will:

- Explain the purpose and requirements of the study. It is important that potential participants understand the time commitment involved in research visits and research outcomes assessments.
- Provide the consent form to the potential participant for him/her to read.
- Review the consent form with the potential participant, going over the major points.
- Ask the potential participant if s/he has any questions.
- Answers any questions.

10. Potential Risks:

Loss of Confidentiality: Any time information is collected; there is a potential risk of loss of confidentiality. Every effort will be made to maintain confidentiality, and only the personnel having direct subject contact will have subjects' identifiable information.

Stress: The process of seeking treatment for their illness could be stressful for some participants. Participants will also be made aware that a slight risk for psychological discomfort exists when assessing depression. Some questions may be uncomfortable for some participants to answer.

Unforeseen Risks: If new, unanticipated risks arise during the study, participants will be notified promptly and be allowed to reconsider their participation in the study.

Risks of blood draw: Risks of blood draw are minimal, and include pain, anxiety, bleeding and bruising at the puncture site. Infection is possible but infrequent. Some participants may also experience dizziness or fainting. Participants may have some anxiety or discomfort associated with the blood draw.

Risks of urine collection: Risks of urine collection are minimal. There may be some inconvenience and potential for embarrassment associated with urine collection.

Loss of Privacy: Discussion of psychological health and psychiatric symptoms in public area may lead to loss of privacy.

Risks of medication:

Risks of taking L-leucine: Excessive intake of L-leucine (greater than 750mg/kg body weight) may result in hypoglycemia [39]. L-leucine when ingested up to 200 mg/kg dose (for adult weighing 75 kg, this dose will equal 15 gm) has no effect on plasma insulin or glucose [40]. While not reported in human beings, animal models suggest that very high doses of L-leucine (in rodent models at 15 g/kg) may lead to pellagra like symptoms due to disruption of tryptophan metabolism [41]. L-leucine also has a synergistic effect on action of phosphodiesterase 5 (PDE5) inhibitor and may lead to interaction with medications such as sildenafil, hence there may be risks associated with taking Leucine and other medications. Other effects of L-leucine include weight loss and increased muscle mass. There is also the potential risk of allergic reaction.

Overdose of L-leucine (greater than 750mg/kg) can cause low blood sugar and pellagra like symptoms. Signs and symptoms of low blood sugar include: shakiness, dizziness, sweating, hunger, irritability or moodiness, anxiety or nervousness, headache, tiredness, muscle weakness, blurred vision, confusion, convulsion or seizures, unconsciousness, and if untreated then it can potentially lead to death. Pellagra like symptoms include: high sensitivity to sun light, aggression, mental confusion, diarrhea, swelling of tongue, inflammation of tongue (glossitis) or skin (dermatitis), peripheral nerve damage, enlarged or weakened heart, and dementia.

Risk of taking maltodextrin (placebo): allergic reaction, unexplained weight gain, bloating, flatulence. As maltodextrin does not have an antidepressant effect, participants may experience worsening of symptoms.

As with all drug treatments for depression there is a risk of symptoms (depression) remaining the same or worsening.
Risks to Sperm, Embryo, Fetus or Breast-fed Infant: The risk to sperm, embryo, fetus or breast-fed infant with Leucine supplementation is unknown, hence non-use of adequate contraception is an exclusion criterion.

11. Subject Safety and Data Monitoring:
The risks and benefits of leucine, specific study procedures, and the study as a whole will be explained to participants. After a thorough history, subjects will undergo careful physical, psychiatric, and laboratory examinations to assure the clinical appropriateness and safety of their participation. Clinical monitoring by the study physician will ensure the appropriateness and safety of their continued participation. Subjects will be provided counseling, if necessary.

Except for specific medications that can lead to interactions with L-leucine and those medications listed as exclusionary criteria, concomitant medications are allowed. Research personnel will assess participants at each outpatient study visit for illness stability, medication side effects, and subject tolerance of study drug. All data collected will be systematically reviewed by study physicians to ensure the safety of participants, with primary responsibility for data monitoring held by the Co-Investigator/study physician under the regular guidance and oversight of the PI. The Co-I/study physician will review all accrued data in a timely manner, including participant visit reports, attrition, adverse and unanticipated events, protocol deviations/violations and any event/new data that may change the assessed ratio of risk to benefit for study participants. The PI will be notified immediately regarding any urgent matters requiring his attention. At each visit subsequent to study medication administration, participants will complete a self-report form indicating any side effects to the study treatment. The clinician side-effect report will be completed as well. Side effects will also be evaluated by the study physician. All reportable serious adverse events (SAE's) will be reported to the IRB within 24 hours. Since this study will not have a DSMB, all accrued data and safety information will be reviewed with the PI at least quarterly during the active study period.

Depression symptoms will be monitored at each visit, with the risk of suicide thoroughly assessed prior to discharge from research clinic. All data forms completed by the participants will be reviewed for content by the study personnel before participant leaves the clinic. This process will ensure that nothing is overlooked in the evaluation process, including any indication of suicidality. All data collected at each visit will be reviewed by the study physician ASAP so that monitoring of study data for any risk consistently occurs in real time throughout the study. At any time when the research personnel learn that the participant is at risk for suicide, appropriate suicide risk management procedure will be implemented. The risk management procedure will include the following: Participants who report risk of self-harm at any point of contact will be thoroughly evaluated by the study physician, for intent, plan and means for suicide if the participant is in the clinic. If the participant is deemed an acute risk for suicide, the physician will direct the measures necessary to get the participant to safety, using clinic specific crisis management procedures. If the research personnel identify that the participant may be at acute risk during a phone conversation, the study physician will be informed immediately for a complete evaluation of situation. Measures to keep the participant safe, including the option of calling 911 for police assistance will be implemented as based on the assessment of risk, and consistent with the Depression Center's crisis management procedures. Research personnel will also report to emergency treatment providers any information relevant to treatment decision making, including any evidence of suicidality.

Participants who discontinue the assigned study treatment (e.g., if condition gets worse or serious side effects develop) will be withdrawn from the study. Participants will be discontinued from the study if any adverse event, laboratory result, or illness indicates that therapy provided by the study is not in the best interest of the participant. Female participants will be withdrawn if they become pregnant during the time between screening and final study visit. Investigators will provide 24-hour

availability by phone. Appropriate treatment, including hospitalization, will be determined for participants who experience adverse events during the study as necessary.

Data will be collected either electronically via REDCap or on paper forms either by the study physicians or research staff. We will use Redcap to the extent possible to enter and store data. Redcap offers a secure, web-based platform for data collection and storage. Study participants may enter survey responses in REDCap, decreasing the need for paper charts. In the case the REDCap cannot be used, a double data entry procedure using Microsoft Excel will be used. The first entry will be performed by the study physician and the second by an appropriately trained research assistant. Using an Excel macro, the two data sets will be compared, and discrepancies will be addressed by the study physician by checking the original paper records against the database. The PI will have the final authority for determining the correct value and if this cannot be clearly determined then the value will be declared to be missing from the dataset.

Blood Draw: Participants will have blood drawn by appropriately trained research personnel at the research clinic. Should reasonable attempts to draw blood using standard procedures fail, the participant will be withdrawn. Should complications of blood draw arise; participants will receive appropriate referral after assessment by a study physician.

12. Procedures to Maintain Confidentiality:

Initially, participant-identifying information will be necessary to link data sources on each subject. Once all data has been obtained, entered and checked for accuracy, the following procedure will be used to protect confidentiality:

- Paper charts used to collect clinical data on participants will be kept in locked cabinets, inside offices whose doors will be locked when not in use. Only authorized study personnel will have access to the locked files. Copies of executed consent forms will be kept with patient charts. A master log which includes ID number and identifying information will be stored and locked separately from other data. Please note that all identifying information and the master log will be stored in double locked conditions when not in use.

- To the extent possible, data entry and storage will be completed in REDCap, a University-sponsored secure web-based platform to minimize the storage of data on individual workstations and in paper charts. All electronic data will be kept on a secure UTSW network server.

- Clinical data will be transferred to a computerized database using a study ID number. The database will not contain any identifying information about participants, and the link between identifying information and study ID number will be kept only in the locked master log. The database files will be password protected and only authorized study personnel will have access.

- The study ID number will be the sole identifier used on participants' blood samples and on the data generated from their analysis. Blood samples will be sent with study name and id number as the only identifiers. Personnel in the labs analyzing the blood samples will not have access to identifying information. Laboratory results will be entered in the study database using the study ID as the sole identifier, and any paper reports with identifying information will be stored separately and securely.

- No participant names will be used in any publications.

13. Potential Benefits: Participants in the study will receive comprehensive evaluation for their mood disorder. The study medication may also improve their depression. Participants will also receive referrals for psychiatric care in the community when they complete the study or if they exit the study prior to completion. There is potential benefit to future patients if leucine is found to be a rapid-acting antidepressant medication.

14. Biostatistics: Mixed effects repeated measures analysis of variance appropriate for a cross-over design will be used to compare continuous data from baseline to those at days 3, 7, 14, 17, 21, 28, 31, 35, and 42; binary data will be analyzed by a generalized linear mixed-effects model also comparing baseline to days 3, 7, 14, 17, 21, 28, 31, 35, and 42. Assumptions specific to a cross-over design (i.e., absence of period and order effects) will be tested. We will use descriptive analyses to report response at 3 days, response defined as greater than 50% reduction in depression severity as measured by HDRS. In a cross-over study design, effect size of 0.5 is considered larger than moderate and the total sample size needed to test our primary outcome with 80% power is 34. Hence, our planned sample size of 40 ensures adequate power to detect a difference of moderate effect size. We will also use descriptive analyses to report side effects with oral administration of L-leucine.

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