Confidential Information

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Transcranial Continuous and Pulse Near-Infrared Light in Depression: a Placebo-Controlled Study (ELATED-3).

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Transcranial Continuous and Pulse Near-Infrared Light in Depression: a Placebo-Controlled Study.

1. Study Summary

Protocol #:	2016P001490 (MGH)/ XXX (NYUSOM)

Study Purpose: The purpose of this study is:

- To assess the antidepressant effect of the Transcranial continuous and pulse near infrared light Therapy in subjects with major depressive disorder (MDD).
- To assess the safety and tolerability of the Transcranial continuous and pulse near infrared light Therapy in MDD subjects
- To pilot test the impact on neuroinflammation of the continuous and pulse near infrared light Therapy in MDD subjects
- To pilot test the impact on cognition of the Transcranial continuous and pulse near infrared light Therapy in MDD subjects

Trial Design: This study includes two phases of two nearly identical clinical trials, one studying the continuous light intervention and the other studying a pulsed light intervention for the treatment of depression. The two, back-to-back clinical trials differ only for the specific treatment modality of continuous and pulse near-infrared light. Each clinical trial is a double-blind, placebo-controlled, 2-arm, sequence parallel design (SPD) study of Transcranial Light Therapy (TLT) – given in addition to current antidepressant treatments for MDD subjects.

This two-site study will randomize in its first part (**PART-1**) 24 MDD subjects (at 1:2 ratio) into 2 groups: continuous light (**c-TLT**) and sham. Sham non-responders after 6 weeks will be randomized (at a 1:1 ratio) in to c-TLT and sham. Sham responders after 6 weeks will also be randomized to ensure a chance to optimize outcome and to achieve remission at a ratio 1:1 to c-TLT and sham.

In its second part (**PART-2**), which will begin following the completion of PART-1– meaning after the first 26 randomized subjects have completed their trial, the study will randomize 26 MDD subjects (at 1:2 ratio) into 2 groups: pulse light (**p-TLT**) and sham. Sham non-responders after 6 weeks will be randomized (at a 1:1 ratio)

in to p-TLT and sham. Sham responders after 6 weeks will also be randomized to ensure a chance to optimize outcome and to achieve remission at a ratio 1:1 to p-TLT and sham.

The TLT groups in **PART- 1 and 2** will receive either one of two types of TLT treatments: c-TLT or p-TLT with identical total energy delivered per session. Two sessions per week will be delivered for the duration of each phase. In both clinical trials part 1 and part 2, the sham group (n=16) will receive 2 sham treatments per week for 6 weeks. At the end of the 6 weeks the sham will be re-randomized to TLT and sham. All subjects and investigators (except the study statistician) will be blind to treatment sequence assignment.

Study Population: Patients eligible for study participation will be diagnosed with major depressive disorder (MDD) by DSM (MINI) criteria. Subjects will be between 18-70 years of age (have not had 71st birthday) on the date of screening. Subjects who will be permitted into the study include those who meet all the inclusion criteria and have none of the exclusion criteria.

Duration of Participation: Subjects will be followed in the study for 12 weeks.

Endpoints:

Safety - The safety endpoints will evaluate the TLT and sham groups for differences in tolerability through 12 weeks of follow-up.
Efficacy - The efficacy endpoint will be the antidepressant effect of TLT through week 12, as assessed using the QIDS-C scale.

Engagement of Neuro-Inflammation – The effect of TLT on inflammation will be assessed at week 6 and 12 by measuring the plasma levels of IL6, TNF-α, IL-1β as primary cytokines (compared to baseline).

Engagement of Cognitive circuitry – The effect of TLT on cognition will be assessed at week 6 and 12 by measuring pattern recognition and cognition with emotional stimuli (compared to baseline).

Entry Criteria: **Inclusion Criteria**

1. Subjects age at screening will be between 18 and 70 years old (inclusive).
2. Diagnosis of major depressive disorder (MINI)
3. HAM-D17≥14

4. CGI-S ≥4 or higher, i.e., "moderately depressed"
5. Women of child-bearing potential must use a double-barrier method for birth control (e.g. condoms plus spermicide) if sexually active.
6. Subject Informed Consent obtained in writing in compliance with local regulations prior to enrollment into this study.
7. The subject is willing to participate in this study for at least 12 weeks.
8. Subjects will need to be on stable dose(s) of antidepressants (if taking any) for at least six weeks prior to enrollment.

Exclusion Criteria:

1. The subject is pregnant or lactating.
2. The subject failed more than 2 adequate treatment with FDA-approved antidepressants during current episode per ATRQ criteria (less than 50% decrease in depressive symptomatology).
3. Structured psychotherapy focused on treating the subject's depression (i.e. CBT or IPT) is permitted if started at least 8 weeks prior to the screening visit.
4. Substance dependence or abuse in the past 3 months.
5. History of a psychotic disorder or psychotic episode (current psychotic episode per MINI assessment).
6. Bipolar affective disorder (per MINI assessment).
7. Unstable medical illness, defined as any medical illness which is not well-controlled with standard-of-care medications (e.g., insulin for diabetes mellitus, HCTZ for hypertension).
8. Active suicidal or homicidal ideation (both intention and plan are present), as determined by C-SSRS screening.
9. Cognitive impairment (MOCA<21)
10. The subject has a significant skin condition (i.e., hemangioma, scleroderma, psoriasis, rash, open wound or tattoo) on the subject's scalp that is found to be in proximity to any of the procedure sites.
11. The subject has an implant of any kind in the head (e.g. stent, clipped aneurysm, embolised AVM, implantable shunt – Hakim valve).
12. Any use of light-activated drugs (photodynamic therapy) within 14 days prior to study enrollment (in US: Visudine (verteporfin) – for age related macular degeneration; Aminolevulinic Acid- for actinic keratoses; Photofrin (porfimer sodium) – for esophageal cancer, non-small cell lung cancer; Levulan Kerastick (aminolevulinic acid HCl) – for actinic keratosis; 5-aminolevulinic acid (ALA)- for non-melanoma skin cancer)
13. Recent history of stroke (90 days).

14. The subject failed a device-based intervention FDA-approved for the treatment of depression, during the current episode (e.g. less than 50% decrease in depressive symptomatology with TMS, ECT or VNS).

List of Abbreviations

31P-MRS = Phosphorous-31 Magnetic Resonance Spectroscopy
ALA = 5-aminolevulinic acid
ASQ = Anxiety Symptoms Questionnaire
ATP = adenosine triphosphate
AVM = Arteriovenous Malformation
CADSS= Clinician Administered Dissociative States Scale
C-SSRS = Columbia-Suicide Severity Rating Scale
CFR = Code of Federal Regulations
CGI-I = Clinical Global Impressions - Improvement
CGI-S = Clinical Global Impressions – Severity
CHRT = Concise Health Risk Tracking
DCRP = Depression Clinical and Research Program
DNA = deoxyribonucleic acid
ECT= Electroconvulsive Therapy
FDA = Food and Drug Administration
HAM-D-17 = Hamilton Depression rating scale
ISO = International Standards Organization
MADRS = Montgomery-Asberg Depression Rating Scale
MOCA Montreal Cognitive Assessment
MDD = Major Depressive Disorder
MGH = Massachusetts General Hospital
NEST 1 = NEST 1 - NTS® Effectiveness and Safety Trial – 1
NEST 2 = NEST 2 - NTS® Effectiveness and Safety Trial – 2
NEURO-QOL = Quality of Life in Neurological Disorders
TSRQ = TLT Self-Report Questionnaire
NKI = Nathan S. Kline Institute for Psychiatric Research
NSMHA = North Suffolk Mental Health Associate
NYUSOM = New York University School of Medicine
OSHA = Occupational Safety and Health Administration
PBQ = Perceptions of Blinding Questionnaire
PCL-S = PTSD Checklist-Specific
PI = Principal Investigator at the site
QIDS-CR = Quick Inventory of Depressive Symptomatology-Clinician Rated Scale
SAE = Serious Adverse Event
SAFTEE-SI = Systemic Assessment for Treatment Emergent Events
SCID = Structured Clinical Interview for Diagnostic Statistical Manual-IV
SSRI = selective serotonin reuptake inhibitor

TLT = Transcranial Light Therapy
TMS = Transcranial Magnetic Stimulation
TS-1 = Treatment Sequence 1
TS-2 = Treatment Sequence 2
VNS= Vagus Nerve Stimulation

1. Introduction

Major Depressive Disorder (MDD) is a prevalent and disabling condition: 16.2 % of the US population has MDD at some point in life (Kessler et al., 2003). In the US, depression is the third leading cause of years lost because of disability or death, after cardiovascular diseases and lung and breast cancers (Michaud et al., 2006). Despite the availability of effective treatments such as antidepressants and psychotherapy, MDD frequently remains untreated, with only one fifth of the cases receiving adequate treatment (Kessler et al., 2003). Moreover, diagnostic and treatment rates for depression among Blacks and Hispanics are less than half the observed rates for Whites (Sclar et al., 2008). Hispanics and Blacks have significantly more concerns regarding the quality-of-life effects of medications than Whites (Huang et al., 2009). Among Hispanics stigma associated with receiving antidepressants is a prominent concern (Interian et al., 2007). Delayed treatment for depression, not only prolongs the associated disability, but might result in lower rates of response to antidepressants (de Diego-Adelino et al., 2009).

Current standard treatments for MDD are antidepressants and psychotherapy. Antidepressants are effective but their efficacy is often limited by adherence to treatment (Lingam and Scott, 2002). Psychosocial interventions are usually preferred in primary care (Backenstrass et al., 2006), especially among minorities (Givens et al., 2007), but they are typically not cost-effective due to the higher initial costs of providing psychotherapy (Barrett et al., 2005). In addition, counseling and psychotherapy are limited in the community by the lack of counselors who are culturally trained and proficient with specific languages (e.g. Spanish).

Because of the limitations of medication treatment and talk-therapy in the approach to depression, new treatment approaches are required especially in primary care and in the community, where several minorities are underserved. We propose a novel treatment approach for depression based on the use of the continuous and pulsed Transcranial Light Therapy (TLT). The treatment consists in exposing bilaterally the frontal brain to the TLT, which may enhance ATP production in depressed subjects.

Major Depressive Disorder has been associated with deficits in brain bioenergetic metabolism. In an experimental model of depression, the mitochondrial respiratory chain (the cellular site for energy production) was found to be inhibited by chronic stress (Rezin et al., 2008). Depressed subjects have also significantly lower production of ATP (an energy vector) in their muscle tissue and greater incidence of deletions in their mitochondrial DNA (Gardner et al., 2003). Data from magnetic resonance spectroscopy in subjects with MDD showed that response to the augmentation of a selective serotonin

reuptake inhibitor (SSRI) with triiodothyronine (a thyroid hormone) is associated with restoration of the levels of ATP in the brain (Iosifescu et al., 2008).

The TLT is a non-ionizing electromagnetic wave. The TLT is invisible, penetrates the skin and skull into brain tissue, is non-invasive (Zhang et al., 2000), is minimally dissipated as thermal energy and is mainly absorbed by specific chromophores (Mochizuki-Oda et al., 2002). The benefits of TLT are wavelength specific. A mitochondrial enzyme, the Cytochrome c oxidase, is the primary chromophore for the TLT with wavelength around 830 nm (Eells et al., 2003). The energy absorbed by the cytochrome c oxidase leads to increased adenosine triphosphate (ATP) production, through the respiratory chain. Ultimately, the increased ATP leads to increased energy metabolism for the cell, and it is hypothesized that a signaling cascade is also activated promoting cellular plasticity and cytoprotection (Eells et al., 2003).

These properties of the TLT have led to novel therapeutic applications in neurology. In acute ischemic stroke subjects, acute treatment with the TLT led to significantly better outcome as compared to sham (Lampl et al., 2007). These results were confirmed in a different cohort of stroke patients with mild to moderate severity of illness (Zivin et al., 2009). Both studies on stroke subjects showed no significant difference in rate of adverse events, as well as serious adverse events, between the TLT and sham treated subjects (Lampl et al., 2007; Zivin et al., 2009) [See section 4.17]. The TLT has also been used as a treatment of alopecia (Leavitt et al., 2009) and in animal models for methanol-induced retinal toxicity (Eells et al., 2003). The near-infrared light is already widely used for non-invasive assessment of brain function (replacing fMRI in studies of infants and young adults, under the name of Near Infrared Spectroscopy) underscoring the relatively low risk of TLT. The major risk of TLT when using a laser as the light source is associated with accidental retinal exposure, when beams are projected through the lens, with increased risk of macular lesions (Kim et al., 2007). In our study, we will have multiple protections to safeguard against this risk [See section 4.17].

Preliminary uncontrolled studies in 10 and 4 depressed subjects, respectively, have shown that the TLT is safe, effective and well tolerated (Schiffer et al., 2009; Cassano et al., 2015). Our study offers a larger sample size, a cleaner design with placebo-control (also called Sham treatment), and tests the efficacy and tolerability of repeated sessions of TLT. Because the TLT is a non-ionizing radiation, multiple sessions, likely required for the treatment of depression, are expected to be safe.

The advantage of the TLT treatment approach as compared to pharmacotherapy is that adherence can be easily monitored (since treatment is delivered at the clinic) and the patient is not required to ingest any substance. It is possible that the exposure to TLT might be more acceptable than use of medications among some minorities. As compared to talk-therapy, the TLT therapy has the advantage of not requiring providers with specific cultural expertise or second language proficiency. Our study will contribute to answer the question of whether TLT has an antidepressant effect and whether it is acceptable in minority populations, thus justifying further studies and investments.

3.1 Summary of Prior Clinical Experience

We conducted two studies on the safety and efficacy of TLT delivered to the forehead (prefrontal cortex) twice a week in subjects with MDD.

ELATED-1 was a proof of concept study of 3 weeks of TLT (Neurothera - class IV laser, wavelength 808 nm; irradiance 700 mW/cm^2; fluence of 84 J/cm^2; total energy 2.4 kJ per session for 6 sessions) (Cassano et. al. 2015). All subjects (n=4) were treated with TLT. Baseline mean HAMD-17 scores for depression decreased from 19.8±4.4 (SD) to 13±5.35 (SD) after treatment (p=0.004).

ELATED-2 was a double-blind, randomized, controlled study of 8 weeks of TLT (Omnilux New U - LED, 830 nm; 36.2 mW/cm^2; up to 65.2 J/cm^2; vs. sham for 16 sessions). At endpoint, the mean HAMD-17 for depression decreased of 11.7±7.5 points (TLT, n=9) vs. 5.3±7.0 points (sham, n=9) (p=0.04); the differences were even more pronounced in the completers sample as HAMD-17 decreased of 15.7±4.4 (TLT, n=6) points vs. 6.1±7.9 points (sham, n=7) (p=0.01).

These findings suggest that TLT could be a novel intervention for patients with MDD. Both the Laser study (ELATED-1) and the LED study (ELATED-2) were conducted by our group at MGH. ELATED-3 (the present submission) will test for the first time pulse light therapy (p-TLT) in a comparable population of MDD subjects.

The LiteCure® PhotoBioModulation-1000 (TPBM-1000) device to be used in this study does not require FDA approval. In fact, the TPBM-1000device used in this study is considered a non-significant risk device because:

- TPBM -1000 dose does not exceed the Maximum Permissible Exposure (MPE) for skin – Center for Devices and Radiological Health (CDRH), 21CFR1040.10 and 21CFR1040.11;
- TPBM-1000 is substantially equivalent to the OmniLux New U device (used in ELATED-2 at MGH), which the FDA has already categorized as a non-significant risk device;
- TPBM-1000 differs from the OmniLux New U only in that it provides an additional site of irradiation – treatment is delivered to EEG sites FP1 and FP2 in addition to F3 and F4 on the forehead;
- currently FDA-cleared LED devices are typically available over-the-counter and are considered safe to use without professional supervision.

3.2 Overview of LiteCure Laser System

LiteCure® TPBM-1000 is a new device with the flexibility to safely deliver either continuous or pulsed light: c-TLT or p-TLT, or sham. The TPBM-1000 device is configured for in-office use.

TPBM-1000 delivers NIR light output at 830 +/- 30 nm. The NIR output is invisible to the naked eye, thus, the device includes visual and/or audible indicators of light emission, and is designed to deliver light to EEG sites: Fp1, Fp2, F3 and F4, covering a total surface active treatment area, of 35.8 cm^2[(11.52 cm^2x2) + (6.38 cm^2x2)] with an average irradiance of 54.8 mW/cm^2 and an average fluence of 65.8 J/cm^2. While the average fluence (energy density) is comparable to the Omnilux New U, the treatment area is significantly less for the TPBM-1000 (Omnilux New 28.7 cm^2 x2, which results in less total energy delivered per session of 2.3 kJ (compared to up to 3.7 kJ for the Omnilux New U).

TPBM-1000 devices will be fabricated to deliver either c-TLT, p-TLT, or Sham. In simple terms, in either TLT mode the TPBM-1000 device delivers therapeutic NIR energy; in sham the device will not deliver any light energy. The apparent behavior (i.e. the performance/output of all visible and audible indicators) of the device in any of the three programmed treatment modalities will be identical, ensuring the study is blinded to the operator. In addition, because of the low average irradiances delivered in either c-TLT and p-TLT modes the patients will not experience any heating sensation ensuring the study is double-blind.

The TPBM-1000 devices consists of a source of light, mounted on a cap, attached to a console that controls the light delivery modality: c-TLT, p-TLT, or Sham.

3.3 Overview of Rationale for Cognitive Tasks, and for neuroinflammatory laboratory tests.

Cognitive Tasks

The prefrontal cortex (PFC) and dorsal lateral prefrontal cortex (DLPFC) are uniquely situated to play a critical role in learning, executive functions, attention and inhibition. Studies have shown that DLPFC exhibits significant plasticity during the learning process, in which neuronal activity changes to dynamically encode and recode procedural memories once a sensory motor association has been made. The purpose of this study is to determine whether TLT can be used to change the speed at which subjects learn and perform in cognitive tasks. TLT applied to prefrontal regions of the brain implicated in visual-motor learning processes can enhance neural plasticity and improve behavioral performance.

Neuro-inflammatory laboratory tests

Animal and clinical research suggest that the inflammatory immune system contributes to MDD. Post-mortem gene expression profiling on tissue samples from Brodmann area 10 (BA10 – prefrontal cortex) have shown that MDD is characterized by increased inflammation and apoptosis (Shelton et al., 2011). In a case-control study, Simon and colleagues (2008) found that antidepressant-naive MDD subjects had significant elevations in the following cytokines and chemokines when compared to healthy controls: MIP-1α, IL-1α, IL-1β, IL-6, IL-8, IL-10, Eotaxin, GM-CSF, and IFNγ.

Although IL-10 is an anti-inflammatory cytokine, the results suggested that the elevated levels of this IL-10 were likely induced in response to the overall elevation of pro-inflammatory cytokine levels (Simon et al., 2008). In a review of the research on inflammation in MDD, Raison, Capuron, and Miller (2006) proposed that pro-inflammatory cytokines might cause brain abnormalities that are characteristic of MDD. Indeed, animal research has shown that IL-1 mediates chronic depression in mice by suppressing hippocampal neurogenesis (Raison et al., 2006; Goshen et al., 2007).

One pro-inflammatory cytokine that may be of particular relevance to depression is CSF IL-6 (IL6 measured in cerebrospinal fluid). In a recent report, patients with MDD had significantly higher CSF IL-6 levels compared to healthy controls; CSF IL-6 levels were significantly higher than in the serum and there was no significant correlation between CSF and serum IL-6 levels (Sasayama et al., 2013). These findings are consistent with a prior report showing a positive correlation between CSF IL-6 levels and the severity of depression and suicide attempts, with the strongest correlation found in violent suicide attempters (Lindqvist et al., 2009). One report in a smaller sample of depressed patients has shown that CSF IL-6 was lower or comparable to healthy controls (Levine et al., 1999).

Near-infrared light and red light (600-1600 nm) decreased synovial IL-6 gene expression (decreased mRNA levels) in a rat model of rheumatoid arthritis (Araki et al., 2011). In another study, NIR (810 nm) used as a treatment for pain in patients with rheumatoid arthritis decreased production of the following pro-inflammatory cytokines: TNF-α, IL-1β, and IL-8 (Yamaura et al., 2009). Khuman and colleagues (2012) showed that transcranial NIR improved cognitive function and reduced neuroinflammation as measured by Iba1+ activated microglia in brain sections from mice that had suffered a traumatic brain injury. Finally, NIR (970 nm) has been found to be an effective treatment for inflammatory-type acne (Barolet and Boucher, 2010). In summary, it is reasonable to predict that transcranial NIR treatment would likewise have an anti-inflammatory effect in patients suffering from MDD.

2. Investigational Plan

4.1 Rationale

The specific aims of this study are:

1. To assess the antidepressant effect of the continuous and pulse Transcranial Light Therapy in depressed subjects.
2. To assess the safety and tolerability of the continuous and pulse Transcranial Light Therapy in depressed subjects
3. To pilot test the impact on cognitive functioning and neuro-inflammation of the continuous and pulse Transcranial Light Therapy in depressed subjects

Rationale for the use of this device in individuals with MDD:

Different devices have been used in the study of near-infrared radiation (NIR) treatment effects in humans. We decided to use a new device, created by Litecure

specifically for this study in order to allow, in the same setting, testing of both continuous and pulse NIR in a blind fashion and with adequate control (sham).

4.2 Study Design

This study is a double-blind, placebo-controlled, sequenced parallel study on the use of Transcranial continuous light therapy (PART 1 – TLT-c) and pulse light therapy (PART-2 TLT-p) as treatment for depressive symptoms in patients with Major Depressive Disorder (MDD).

This study will include up to 50 subjects randomized at a 1:2 ratio into 2 groups: Transcranial Light Treatment (TLT) and sham.

All subjects will receive 2 TLT/sham treatments per week for 12 weeks. Subjects randomized to the sham group will receive sham treatments for the first 6 weeks; during weeks 7-12 subjects in the sham group will be re-randomized to either TLT or to sham. All subjects and investigators (except the study statistician) will be blind to treatment sequence assignment.

Treatment group assignment will be decided by randomization and managed by the study statistician. Each study site (MGH and NYUSOM) will have one Litecure device, which will recognize the randomization codes generated by the study statistician. For each randomized subject a sealed envelope with treatment assignment will be available at the respective study site. Both subjects and study staff will be blind to the treatment group assignment. The Sham treatment consists in applying all the procedures for the delivery of the TLT, while actually delivering no light. The blinding of the sham will be effective because the radiation is not visible and because the active therapy (continuous or pulsed) will not produce any patient discernable difference from sham, e.g., skin warming. The sham treatment will mimic the TLT procedure. The treatment will be bilateral and applied to the frontal areas with two application sites on the left side and two on the right side [left and right forehead centered on EEG sites on F3, F4, Fp1, Fp2]. Energy is administered with a radiation wavelength of 830 nm. The duration of irradiation is 20 minutes at each application site (the 4 sites are irradiated at the same time which is equivalent to 20 minutes of total time), while the entire session is estimated as lasting 25 minutes or less (Schiffer et al., 2009) (Lampl et al., 2007, Zivin et al., 2009). The additional 5 minutes are needed to prepare the subject, to place the necessary protections (e.g. goggles), to inspect the subject's skin, to set the TLT devices and to give time to rest to the subject after the irradiation. TLT treatment will only be administered by licensed physicians (i.e. MDs) who are on study staff. All staff who deliver treatment must pass training that is approved by the MGH and/or NYUSOM Laser Safety Committee. The duration of irradiation will be decreased if clinically indicated, based on tolerability to 15 min as needed. The treatment will follow these specifications: wavelength 830 nm; average irradiance 54.8 mW/cm2; average fluence of 65.8 J/cm2 (consistently with parameters used in the ELATED-2 study at MGH with the device Omnilux New U).

Subjects diagnosed with major depressive disorder who meet all inclusion criteria, have none of the exclusion criteria, and are willing to undergo the Informed Consent process will be eligible to participate in this clinical study.

4.3 Study Population

4.3.1 Population Description

The subject population for this study will consist of 50 subjects between the ages of 18 and 70, of any ethnic background and diagnosed with major depressive disorder. The subject will meet all the inclusion criteria, have none of the exclusion criteria, and provide their written Informed Consent to participate in this clinical study. Any subject who signs informed consent will be considered enrolled into the study, although they may not participate if they do not qualify for the study. In the event that a subject's ability to comprehend and communicate is compromised (per the assessment of the Investigator), local regulations pertaining to Informed Consent signatures should be followed.

Subjects will be recruited, via screenings, advertisements and postings. Study participants will also be recruited via internet-based advertisements, e.g., Craigslist, Clinical Trials @ Partners, the study sites websites, RSVP for Health (which will also generate listserv emails to Partners employees, regarding research studies in need of volunteers), etc.

Participants who contact the respective study site staff in response to any of the aforementioned advertisements will complete phone screening questions to determine tentative eligibility for the study.

4.3.2 Use in Female Subjects of Childbearing Potential

Female subjects of childbearing potential must consent (without any element of coercion) to use a double-barrier method for birth control (e.g. condoms with spermicide) if sexually active. A pregnancy test will be performed at the Screening Visit.

4.3.3 Criteria for Enrollment – listed on pages 5-6

4.4 Subject Numbering

Subjects enrolled in the ELATED-3 study will be identified by subject numbers ~~1~~01 through 99 (to account for screen failures and drop-out before randomization).

4.5 Sample Size

The ELATED-3 Study is expected to include 50 subjects with analyzable data (at least 1 session post randomization).

4.6 Subject Confidentiality

Any information and data provided to Litecure Inc. or its designees in reference to any subject's participation in this investigation will be considered confidential. The Investigator will need to ensure that all subjects' anonymity will be maintained on all documentation submitted to Litecure Inc by completely redacting (eliminating or "blacking out") each subject's name and/or other identifying information. The identifying information will be replaced with the subject's study number and initials. The investigational site is not to provide to Litecure Inc information such as subject's telephone numbers, home address, personal identification numbers such as passport numbers, etc. Care must be taken by site research personnel when communicating with representatives from Litecure Inc in the form of telephone or electronic correspondence in not providing information that may disclose a subject's identity.

Documents associated with the study that are not intended to be submitted to Litecure Inc (e.g., signed Informed Consent Forms) must be kept in strict confidence by the Investigator. Only study site personnel, authorized Litecure Inc personnel, and regulatory authority inspectors will have access to these confidential files.

4.7 Study Visits

Study visits occur at MGH Depression Clinical and Research Program (DCRP) in Boston and at the NYUSOM Nathan S. Kline Institute (NKI) site in Orangeburg, NY.

The study involves screening, treatment, and a series of follow-up visits.

Screening Visit (Week -1)
The Principal Investigator (PI) at the site or a Sub-investigator will assess patients against the inclusion and exclusion criteria. If a patient appears to qualify and agrees to participate in the study, then the patient will undergo the informed consent process prior to initiation of any study-specific activities. Screening Case Report Forms (CRFs) will be completed and subjects who qualify to participate in the study will proceed to the first Treatment Visit 1. If the subject does not meet entry criteria, the Investigator will document the subject's ineligibility for study participation and complete the appropriate CRFs.

Treatment Visits – Visits 1-24 (Weeks 1-12)
There are 2 treatment visits per week for 12 weeks. This visit-schedule applies both to the PART-1 (TLT-c) and PART-2 (TLT-p) phase of the study. Treatment Visit 1 will consist of all subjects being randomized to the TLT and Sham groups and undergoing baseline assessments including HAM-D-17, QIDS-C, ASQ and cognitive tasks prior to treatment.

Treatment Visits 1-12 will include various assessments as detailed in Section 4.8 and in Attachment 1.

Follow-Up Visit – Visit 25

All subjects will undergo subject assessments including HAM-D-17, QIDS-C, ASQ, and cognitive tasks at follow-up Visit 25 (Week 13).

4.8 Detailed Study Visits

4.8.1 Screening Visit (Week -1)

Purpose
The purpose of the Screening Visit will be to determine the eligibility of patients for participation in the ELATED-3 Study.

Procedures
At this visit subjects will receive a psychiatric evaluation, will be consented for the study, and will be screened for presence of MDD using the MINI. The HAM-D-17, QIDS-C, SDQ and CGI will be used to assess severity of depressive symptoms, the ASQ will be used to assess anxiety symptoms, and the C-SSRS will be used to assess suicidality. The ATRQ will be used to assess resistance to treatment. The MoCA will help determining cognitive impairment that would contraindicate study participation.

Routine laboratory tests (including urine collection for Urine Analyses, Urine Tox Screen, and Urine Pregnancy Test) and complete physical exam including vitals and weight will be performed at screen; the tests will be processed by a laboratory at MGH and at NYUSOM.

About 15cc ± 5cc (15 mL) of blood is drawn for laboratory safety tests. To confirm a patient is healthy enough to participate a complete blood test with differentials, thyroid stimulating hormone test, a comprehensive14-metabolic panel test, and a c-reactive high sensitivity protein test are conducted on screening day. An additional 2-6 cc of blood will be drawn for the cytokine assays during the screen visit.

Once all tests and procedures become available for review, the Investigator will then proceed to closely re-evaluate the subject for all the inclusion and exclusion criteria.

Subjects are allowed into the study if they did not fail more than two antidepressants in the current episode. Subjects could decide to continue their antidepressant during the study or to taper it off prior to the baseline visit.

4.8.2 Treatment Visits – Visits 1-24 (Weeks 1-12)

The Treatment Sessions will occur twice weekly for twelve weeks at MGH Depression Clinical and Research Program (DCRP) in Boston and at NYUSOM Nathan S. Kline Institute (NKI), Orangeburg, NY. Subjects missing one session will be allowed to still receive their treatment within the same week. The study PI will be an active participant in the delivery of the TLT. The PI and other collaborators from MGH DCRP and at NYUSOM NKI will assist with the delivery of TLT. The device (TPBM-1000) for the

administration of the TLT will be provided by Litecure Inc. Litecure will provide training of MGH DCRP and NYUSOM NKI staff in the use of the device and will provide training certificates at the completion of the training.

The overall session is estimated to last about 25 min. Study sessions will take place in an office dedicated to TLT treatment at MGH DCRP and at NYUSOM NKI. Only the subject and the staff administering the TLT will be allowed during the session. The subject will comfortably lie down on an exam bed or sit on recliner. The sites of application of TLT (left and right forehead) will be inspected for any possible skin lesions (e.g. laceration or signs of inflammation) which would contraindicate the treatment. During treatment sessions the office will be kept completely dark and a warning sign will be hanged at the office door. The staff will be provided training on basic safety procedures relative to the use of the device.
The trained staff administering the TLT will use utmost care to never shine the light in or near the eyes of the subject safety goggles will be selected based on the TLT biophysical properties, in accordance to the Occupational Safety and Health Administration (OSHA) guidelines. The delivery of the TLT is expected to last 20 min total (simultaneous application on the left and right forehead). The subject will be asked to rest for five minutes after the delivery of TLT. The skin at the sites of the application will be inspected again prior to dismissing the subject. A log with the dates of treatment delivery per each study subject will be kept at MGH DCRP (Boston) and at NYUSOM NKI, NY.

4.8.2.1 Treatment Visit 1

During Treatment Visit 1, subjects will be randomized to either TLT or Sham. The following will be performed prior to treatment and serve as baseline values:

- HAM-D-17
- QIDS-C
- CGI
- C-SSRS
- ADVERSE EVENTS FORM
- CADSS
- Concomitant medications form
- ASQ (self-rated)
- SAFTEE-SI (self-rated)
- SDQ (self-rated)
- NEURO-QOL (self-rated)
- SFI (self-rated)
- PEG (self-rated)
- Cognitive tests

4.8.2.2 Screening Visit, Visit 13, and Visit 25

During these visits, approximately 2-6 cc of blood will be drawn to conduct cytokine assays.

Patients shall be instructed to contact the investigator or a member of his staff at any time between visits concerning adverse events or worsening of symptoms.

4.8.2.3 Treatment Visits 2-24

During Treatment Visits 2-24, subjects will undergo a treatment procedure. At Visit 7, 13 and 19 the HAMD17 scale will be administered. At Visit 10 and 22 only, subjects will undergo a TSRQ assessment post-treatment. At visits 10 and 22, subjects and raters will complete the Perception of Blindness Questionnaire. The PEG (self-rated) will be administered at visit 13.

Cognitive testing will be computerized and performed at Visits 7, 13 and 19. Weight and vitals will be recorded at visit 13. The CADSS will be administered at visit 13. During visit 10 and visit 22 the PBQ will administered. In addition to treatment, at Visits 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and 23, the following will be performed prior to treatment:

- QIDS-C
- CGI
- C-SSRS
- ADVERSE EVENTS FORM
- Concomitant medications form
- ASQ (self-rated)
- SAFTEE-SI (self-rated)
- SDQ (self-rated)
- NEURO-QOL (self-rated)
- SFI (self-rated),

Patients will be instructed to contact the investigator or a member of his staff at any time between visits concerning adverse events or worsening of symptoms.

4.8.3 Post-Treatment Visit – Visit 25, (Week 13)

After treatment completion, subjects will continue with one more clinical study visit to measure the long-term antidepressant effect of TLT. Post TLT administration, subjects will be permitted to start or change treatment with antidepressant medications, as clinically necessary (and at any time in the study if severe worsening of depressive symptoms – see 4.8.4 Termination). Cognitive testing will be performed.

At Visit 25, the following will be performed:

- Vital Signs and weight
- HAM-D-17

- QIDS-C
- CGI
- C-SSRS
- CADSS
- ADVERSE EVENTS FORM
- Concomitant medications form
- ASQ (self-rated)
- SAFTEE-SI (self-rated)
- SDQ (self-rated)
- NEURO-QOL (self-rated)
- SFI (self-rated)
- PEG (self-rated)

4.8.4 Termination

We will encourage subjects to continue enrollment in the study via follow-up visits regardless of discontinuation of the TLT/ sham. At the end of the study, we will refer the subjects to their counselor and /or psychiatrist. If the CGI-S increases more than 2 points during the study, the subject will be counseled to start or change to an FDA-approved antidepressant medication. If the CGI-S increases above score-5 or if a subject becomes actively suicidal based on the C-SSRS scale, the subject will be counseled to start (or switch if already on) an FDA-approved antidepressant medication. Subjects who are deemed actively suicidal and are in imminent danger will be escorted to the local psychiatric Emergency Department at MGH or NYUSOM for evaluation and subsequent hospitalization; they will also be terminated from the study. If the subject is appropriate for outpatient monitoring, the subject will be followed with frequent appointments outside the study. Each subject will have the Investigator's contact information as well as instructions on how to call for emergency services, if needed.

4.9 Scales & Forms

We will offer clinical interviews and study forms in English. All scales will explore the past 7 days except for M.I.N.I (life-time), TSRQ (study duration).

- **(M.I.N.I)** – The M.I.N.I. is a short structured diagnostic interview, developed jointly by psychiatrists and clinicians in the United States and Europe, for DSM and ICD psychiatric disorders. With an administration time of approximately 15 minutes, it is designed to meet the need for a short but accurate structured psychiatric interview for clinical trials. According to researchers at the National Institute of Mental Health's (NIMH) Division of Clinical and Treatment Research, the M.I.N.I. is a fully validated and more time-efficient alternative to the Structured Clinical Interview for DSM Disorders (SCID) (Sheehan et al., 1998)
- **Quick Inventory of Depressive Symptomatology- Clinician Rated Scale (QIDS-C)** – This is a brief (16-item) clinician-rated inventory of core depressive symptoms such as sleep, depressed mood, appetite, concentration, suicidal ideation, interest, energy, psychomotor retardation or agitation.

- **Hamilton Depression Rating Scale – 17 items (HAM-D-17;** Hamilton, 1960**)** – This instrument is completed by the clinician based on his/her assessment of the patient's depressive symptoms, using a structured interview and defined anchor points. The HAM-D aims to quantify the degree of depression in patients who already have a diagnosis of major depression. Questions focus on neurovegetative and other depressive symptoms experienced over the past 7 days. Several different versions of the HAM-D exist; they differ only in the number of questions included. The standard form which is generally used in research studies is the 17-item Hamilton D (HAM-D-17). Answers to questions are rated on a scale of 0-4 or 0-2, with higher scores indicating more severe pathology. Scores on the HAM-D-17 typically fall into the following ranges: a) not depressed = 0-7; b) mildly depressed = 8-13; c) moderately depressed = 14-18; d) severely depressed = 19-22; e) very severely depressed = 23 and over.
- **Symptoms of Depression Questionnaire (SDQ)** – This is a comprehensive measure of depression that includes the assessment of symptoms in the anxiety–depression spectrum. It assesses irritability, anger attacks, and anxiety symptoms together with the commonly considered symptoms of depression. Analysis of the factor structure of the SDQ identified 5 subscales, including one in the anxiety–depression spectrum, with adequate internal consistency and concurrent validity (Pedrelli et al., 2014).
- **Anxiety Symptoms Questionnaire (ASQ) –** This, is a 17-item self-report questionnaire measuring the frequency and intensity of 17 symptoms of anxiety, including nervousness, worrying, irritability, trouble relaxing, insomnia, lack of energy, difficulty concentrating, somatic symptoms, and impairment in functioning due to anxiety.
- **Clinical Global Impressions – Severity and Improvement (CGI-S, CGI-I)** – These two instruments are scored 1-7 by the clinician based on assessment of the subject's overall clinical status. They measure, based on history and scores on other instruments: (a) depressive severity (CGI-S) and (b) clinical improvement (CGI-I).
- **Columbia Suicide Severity Rating Scale (C-SSRS)** for suicidal ideation and behaviors: an instrument endorsed by the FDA for clinical trials. This instrument systematically tracks suicidal ideation and behavior (e.g., suicide attempts, wish to die, thoughts of suicide, plan and intent) (Posner et al. 2007).
- **Systematic Assessment for Treatment Emergent Events (SAFTEE-SI)** – The SAFTEE is a commonly used instrument originally developed by NIMH and adapted into a self-report instrument. The version of the scale that we plan to use is the same used by the multi-center, NIMH-sponsored CO-MED trial, and it examines in a systematic fashion all possible treatment-emergent side effects and probes specific adverse symptoms, including suicidal thoughts and behaviors, and self-injurious behavior.
- **Adverse Events Form-** the Adverse Events Form captures any adverse event (serious or otherwise) specifically related to the application of the TLT. This form will help to determine if the side effects of the TLT are too great for a participant to continue in the study.

- **Quality of Life in Neurological Disorders (Neuro-QoL Item Bank v2.0 – cognition function– short form) -** The cognitive section of the Neuro-QoL is an 8 item self-rated measure of both executive function and general concerns. It measures perceived difficulties in cognitive abilities (e.g., memory, attention, and decision making) or in the application of such abilities to everyday tasks (e.g., planning, organizing, calculating, remembering and learning).
- **Massachusetts General Hospital Sexual Functioning Inventory (MGH-SFI) -** This is a patient-rated self-report outcome measure that quantifies sexual dysfunction into 5 functional domains ("interest in sex," "sexual arousal," "ability to achieve orgasm," "ability to maintain erection" [males only], and "sexual satisfaction").
- **The TLT Self-Report Questionnaire (TSRQ)** – An open-ended questionnaire focusing on potential inconveniences and discomforts from the TLT. It will be offered after at least two TLT sessions have been delivered.
- **Concomitant medications form –** This form will be completed at every TLT study visit, including the screening visit, as a safety-monitoring tool.
- **Montreal Cognitive Assessment (MoCA**): This is a 10-item rapid screening instrument for mild cognitive dysfunction. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. This measure will be used to assess entry criteria (Nasreddine et al. 2005).
- **The Clinician Administered Dissociative Symptoms Scale (CADSS):** This is a 23-item clinician-administered scale for the assessment of dissociative symptoms. The CADSS can measure the amount of change in participants dissociations from the beginning of a study or treatment to the end. The CADSS consists of both subjective and objective questions. For a person in dissociation the experience is subjective, and although, dissociation is an individual's cognitive process, dissociation is still observable by other. It will be measured for the "past 6 weeks" at baseline, and after 6 and 12 weeks into the study (Bremner, et al., 1998).
- **The Massachusetts General Hospital (MGH) Antidepressant Treatment Response Questionnaire (ATRQ):** a scale used to determine treatment resistance in major depressive disorder (MDD). The ATRQ examines the efficacy (improvement from 0% [not improved at all] to 100% [completely improved]), and adequacy (adequate duration and dose) of any antidepressant treatment in a step-by-step procedure.
- **The Perceptions of Blinding Questionnaire (PBQ): The Perceptions of Blinding Questionnaire (PBQ):** the PBQ is a self-report questionnaire to determine the degree to which the participant believes s/he is receiving the treatment or the sham.
- **PEG Assessment:** The PEG is a self-report questionnaire with three questions used to assess pain severity and interference with daily life.

4.10 Cognitive tests

Multi-Source Interference Task : The Multi-Source Interference Task (MSIT) is a cognitive paradigm that was designed to reliably identify the cingulo-frontal-parietal cognitive/attention network (CFP network) within individual subjects (Bush et al., 2006). Among other relevant brain regions, the pre-SMA and dorsal anterior cingulate cortex are activated in this 10-15 task when performed inside of the scanner. We will use this cognitive paradigm to identify our target of stimulation on an individual-subject basis.

Briefly, the MSIT presents a string of 3 numbers to subjects, which can be different combinations of 0,1,2,3 in which 2 numbers are the same and one is different (e.g. 100, 323, 221). Subjects are asked to identify what number is different by pressing one of 3 buttons available in the response box. Some trials will be congruent (e.g. 100) and some incongruent (010), depending on the position of the number that is different. The test also involves emotional stimuli to enhance the discriminative power of the task. The emotional stimuli are images that have been used in other studies involving patients with MDD at MGH and have been approved by the IRB (i.e. protocol number 2011P002821). Because emotional stimuli are involved in the task, the clinician will provide a verbal warning prior to the task and debrief with the patient immediately following.

Analyzing the brain response to these congruent and incongruent trials has been shown to robustly identify these brain networks and regions involved in executive functions at the individual level in healthy and patient populations (Bush et al., 2006).

Pattern Recognition Task: The pattern separation task is a high throughput behavioral task that captures the input-output transformation function characteristic of pattern separation processes (Stark et al., 2013). For example, if you park your car in the same lot everyday, but not the same space, pattern separation is thought to be involved in the process of you finding your car everyday despite being in a different space; this may be dysfunctional in people with depression. In this task, patients are shown a series of every-day objects (e.g., a car, garden tool, food, etc.) and are asked to identify the objects as being indoor or outdoor objects. Immediately after this, a second part of the task is started in which the patients are shown another series of objects. They are asked to call the objects as “old” if they have seen the objects before in the task, “new,” or “similar.” As previously done by Stark and colleagues, (2013) a third of the objects in the testing phase are “old”, “similar” and “new”. Identifying a “similar’ object correctly conveys pattern separation, whereas, incorrectly identifying it as “old” conveys pattern completion. By plotting responses as a function of object similarity, we can generate an input-output transfer curve. This task will serve the purpose of rapid assessment of putative changes in pattern separation.

Voice Pattern Recognition Task: The content and quality of vocal production may be a valuable marker of health information. Vocal production, for example speech, contain rich information in both the content (linguistic) and quality (non-linguistic) characteristics of sound information.

Previous research has used vocal samples to predict important markers of cognitive and affective state. From the non-linguistic information of speech, including but not limited to the

information about the pitch, phonemes, and power spectrum of the vocal signal, researchers have been able to predict with high accuracy the cognitive load of an individual at a given moment. For example, vocal quality changes during increases in cognitive load (Le et al. 2011); cognitive load may reflect increasing difficulty of a mental task or suppressing automatic reading response to name the color of a text instead of the word of the text (MacLeod 1991). Similarly, researchers have used vocal quality to predict negative affect in the context of depression (Cummins et al. 2015). The features used to predict cognition and affect can be extracted from small samples of speech under various conditions, but to apply them for the prediction of health information, the collection of these data must be scalable and easy to use.

On a smartphone or tablet at the study site, participants will be administered a series of voice tasks via a mobile application (‘app’). The devices used for the voice tasks are study team devices, i.e. are not the participant’s personal devices. Additionally, no personal identifiers will be collected, shared, or stored through the mobile application. At study visits 1, 7, 13, 19 and 25 (screening, baseline, treatment/sham, and follow-up), participants will be given one or more voice task (see below) to be recorded on the smartphone or tablet. These tasks include:

i. A Stroop task. In the Stroop task, the participant is instructed to read out loud the color of the word presented on the screen. The research study application then presents words in succession. In a low cognitive load condition, the color of the word matches the text of the word (the word "blue" in blue-colored text), and the participant says the color. In a higher cognitive load condition, the color of the word does not match the text of the word (the word "purple" in yellow-colored text), and the participant must inhibit the automatic reading of the text in order to correctly name the color. Sometimes the text of the word is also emotionally salient ("happy" or "angry") or will vary in size, shape, color, font, duration on the screen, position on the screen, saturation, brightness, or contrast.

ii. A digit span task (DST). In the DST, participants are shown and read a sequence of numbers, ranging from 1 to 12 digits depending on difficulty condition. They are then sometimes read a brief sentence (eg. "the dog ran through the woods."). They are then asked to speak out loud the digits that were presented earlier and/or the sentence.

iii. A sentence/passage reading. In this portion, participants are shown text and asked to read it out loud. This ranges from holding a single syllable (eg. "ahhhh"), to a diadochokinetic task (repeating syllables like “PA, TA, KA”), to reading a simple sentence (e.g. “the horse trotted around the field at a brisk pace,” or “Okay Google”), to reading a longer passage.

iv. Free response portion. In this portion, participants are given a specific prompt or may select a prompt from a list of options, for example, "how is the weather today?" or "describe your morning." The participants are instructed to freely respond to the prompt for a set period of time ranging from 30 seconds to 3 minutes.

Together, these tasks present a varied set of speech and sound data for each participant.

4.11 Data Collection Forms

All study clinicians and subjects will be blind to randomized treatment assignment.

A number of procedures are in place to assure data integrity and protocol adherence. We will use Research Electronic Data Capture to support direct data entry by patients and study staff. REDCap is a free, secure, HIPAA compliant web-based application hosted by the Partners HealthCare Research Computing, Enterprise Research Infrastructure & Services (ERIS) group. Web-based surveys rely on a study-specific data dictionary defined by members of the research team with planning assistance from Harvard Catalyst/The Harvard Clinical and Translational Science Center EDC Support Staff. These support staff will then oversee the automated export of study data from REDCap to a relational study database in Microsoft Access 2000, allowing for systematic data querying and checking.

Self-report measures will be completed by participants on a computer, directly into REDCap, thus minimizing errors due to data entry. For clinician-administered measures, all clinicians will enter responses directly in REDCap.

To minimize missing data for self-report forms, we will program missed question warnings in REDCap that will alert participants in real-time if they inadvertently skip a question. Participants may then go back and answer any missed questions, or, if they intentionally skipped questions, they may ignore the warning message and continue answer the remaining questions. We will also program real-time range checks in REDCap that generate error messages if a value outside the acceptable range is entered for a given field. To ensure confidentiality, data will be identified in the database only by subject number, visit number, and date of visit. By recording the study data in this manner, the information can be considered 'de-identified' and therefore compliant with the Standards for Privacy of Individually Identifiable Health Information ("Privacy Rule") of the Health Insurance Portability Act of 1996 (HIPAA). Any data that is transmitted electronically will be fully encrypted and password protected. Subjects' names will not be entered into the database; each will be uniquely identified only by an ID number. Consent forms, any hard copy PHI, and any study measures that are completed on paper will be kept and filed in locked office cabinets.

4.12 Data Management

The study biostatisticians (Dr. Anastasia Ivanova) will oversee management of the study database. The principal investigators, Dr. Cassano and Dr. Iosifescu, are ultimately responsible for the quality of the data collection and overall conduct of the study, and directly supervise the study coordinators and data management staff at MGH and NYUSOM, respectively. Data management will include clinician and patient rated assessments (see assessments), screening data, fidelity data, visit adherence data and rater reliability data and safety reporting. Weekly reports from REDCap will monitor subject enrollment, completion, attrition, and individual subject progress as well as the completion of critical assessments. Additional reports will be done as needed to monitor baseline characteristics, protocol adherence, and other issues of interest.

All measures that are completed on paper will be entered by the RA into REDCap, and a two pass verification system will be used to minimize any data entry errors. All records in

REDCap have a form completion status that may be Incomplete (appears as a red circle in Record Status Dashboard), Unverified (yellow circle), or Verified. The RA initially entering data will save each record entered as Unverified (at which point it will appear as a yellow circle in the Record Status Dashboard). A second RA at each site will then go into each unverified record, compare each entered value against the paper source document, make any corrections, and then re-save the record as Verified (at which point it will appear as a green circle in the Record Status Dashboard). The color coding system built into REDCap readily allows for identification of unverified records.

4.13 Training

Trained study clinicians blind to randomized treatment assignment will administer diagnostic assessments and rating scales. All raters will be experienced clinicians who will have undergone specific training to criteria in the use of the study measures. Any new raters will undergo training. Periodically, all study clinicians will co-rate an audiotape during a staff meeting. These duplicate ratings will be used both to calculate kappa coefficients and for supervision. Differences between raters will be discussed during supervision to identify reasons for disagreement and improve inter-rater reliability. These procedures will help us ensure that study clinicians refine their diagnostic skills and will also establish common guidelines for ongoing use in diagnostic decision-making. Inter-rater agreement will be assessed via evaluation of recordings of diagnostic interviews. Kappa coefficients will be calculated every 12 months. If reliability falls below criteria (ICC $\geq$ 0.8 for QIDS-C), study clinicians will be retrained.

4.14 Randomization Procedure and Blinding of the Study

The study statistician will provide the two study sites (MGH and NYUSOM) randomization codes (block randomization) which will be pre-loaded in the Litecure devices (TPBM-1000). The randomization codes will differ from the study ID assigned at the time of screening. The randomization codes will be two digit only to prevent any confusion. Study ID and randomization code will be matched at the time of the randomization in the subject binder and in a master list of study subjects at each site. In order to administer the treatment the study investigator will enter twice the randomization code in the Litecure instrument. In the event that a medical emergency will necessitate unblinding of a subject's treatment modality, the site's Principal Investigator (or Subinvestigator in the event that the PI is not readily accessible at the time of the medical emergency) will be able to retrieve the unblinding information from on-site sealed envelopes with codes paired to corresponding treatment.

Since this is a double-blind, sham-controlled study, measures have been taken to assure that the integrity of the blinding of the treatment modalities remains intact. The two means of assuring that the treatment modalities will not be disclosed to either subjects or site research personnel include automated LED control sensor based on the temperature of the skin. Also, he introduction of a rating scale for subjects and raters to assess their best guess on treatment assignment.

The performance/output of all visible and audible indicators is identical for all three treatment modalities, (i.e. other than the emission of invisible light radiation, the behavior of the system is identical for all).

4.15 Compensation

Subjects will be compensated $12.50 per treatment visit, for a total of $300 if all twenty four treatment visits are completed. Compensation will be provided after Weeks 2 ($50), 4 ($50), 6 ($50), and 8 ($50), 10 ($50) and 12 ($50), and will be pro-rated for missed treatment visits.

4.16 Accountability/Investigational Product Control

U.S. federal law and ICH Guideline E6 § 5.14 requires that all investigational medical devices be strictly controlled. All study devices must be kept in a secured area at the clinical sites in compliance with all applicable FDA (U.S. sites) regulations.

The Principal Investigators or designated study site personnel who verify the receipt of the devices/device accessories must complete the Device/Accessories Acknowledgment Form and fax a copy to Litecure Inc. Device Accountability and Acknowledgment Logs will be maintained at each study site. These logs will list all equipment received, the receiving date, the serial number of each device. Study site personnel will initial the log each time the device is used. TPBM-1000 use will also be recorded on the appropriate CRF.

Malfunctioning devices and device accessories, including all components, will be returned to Litecure Inc. for investigation, at Litecure expense.

4.17 Clinical Adverse Events

4.17.1 Overview and Definitions

All adverse events will be recorded from the time of Informed Consent through study completion, or termination. The Adverse Event CRF must be completed and submitted to the IRB, FDA, and Litecure, as required. Regulations for adverse event handling and reporting contained in the FDA and ICH Guidelines will be adhered to.

Consideration of Adverse Events will hereafter consist of Adverse Events, Serious Adverse Events, and Adverse Device Effects, including Anticipated Adverse Device Effects and Unanticipated Adverse Device Effects.

- Adverse Event is defined as any untoward/undesirable clinical occurrence in a clinical investigation of a subject using a device and/or product and which does not necessarily have a causal relationship with this treatment. An Adverse Event can therefore be any unfavorable and/or unintended sign, symptom, or disease temporally associated with the use of a device product, whether or not considered

related to the device product. Only abnormal laboratory values that are deemed clinically significant by the investigator will be classified as adverse events.

- Serious Adverse Event is defined as any untoward/undesirable adverse experience that results in any of the following outcomes: 1) death; 2) a life-threatening adverse experience; 3) inpatient hospitalization or prolongation of existing hospitalization; 4) a permanent/persistent or significant disability/incapacity or a congenital anomaly/birth defect; 5) important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
- Anticipated Adverse Device Effect is defined as any adverse effect related to the device or procedure, which is identified in the protocol.
- Unanticipated Adverse Device Effects is defined as any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

4.17.2 Safety Monitoring

The study subjects will undergo frequent clinical evaluations including depressive scores, concomitant medications, adverse events, and serious adverse events and unexpected device events will be recorded from study entry through completion. Additionally, doctors must monitor a subject's safety by asking the subjects frequently about the subject's own comfort during treatment application.

If skin erythema is present, treatment will be suspended. Patients will be instructed to contact the study site principal investigator or a member of his staff at any time between visits concerning adverse events or worsening of symptoms. If at any study visit the subjects' clinical condition is significantly worsened from baseline (operationalized as the clinical global impression severity score, CGI-S, of 6 or higher) or if a subject becomes actively suicidal with intent and/or plan, based on the C-SSRS scale and/or the clinical interview, the subject will be offered to start an antidepressant medication. If the subject were deemed at imminent danger as a result of suicidality, s/he would be discontinued from the study and referred to appropriate clinical treatment (see 4.8.4 Termination).

The two site Principal Investigators (Drs. Cassano and Iosifescu) will have bi-weekly conference calls (which will include study RAs); during these conference calls they will discuss all Adverse Event reports to identify any safety concern, based on such concerns they will be able to decide temporary discontinuation of study enrollment, modifications of the study protocol, or to terminate the study.

Additionally, A Data and Safety Monitoring Board (DSMB), consisting of at least 2 clinicians and one biostatstician not directly involved in the study, will review SAEs annually. Research assistants responsible for data collection and storage will be aware of and comply with all regulatory requirements related to adverse events. In the event that a patient becomes ill or is injured as a direct result of study participation, medical care will be made available. All adverse events (and device events) will be followed to resolution and reported to the MGH IRB as serious in the event that **1.** they are unanticipated and possibly related to the study (same reporting as SAE) or **2.** if they meet any one of the following criteria: Any fatal event, immediately life-threatening event, permanently or substantially disabling event, event requiring or prolonging inpatient hospitalization, or any congenital anomaly. This also includes any event that a study investigator judges to impose a significant hazard, contraindication, side effect, or precaution.

Expedited review will occur for all events meeting the FDA definition of Serious Adverse Events (SAEs) – i.e., any fatal event, immediately life-threatening event, permanently or substantially disabling event, event requiring or prolonging inpatient hospitalization, or any congenital anomaly. This also includes any event that a study investigator or the DSMB judges to impose a significant hazard, contraindication, side effect, or precaution. For purposes of this study, all SAEs will be required to be reported to the DSMB, regardless of any judgment of their relatedness to the study device. All relevant information will be reported to the DSMB for each SAE including information about the event and its outcome, dosing history of all study TLT applications, concomitant medications, the subject's medical history and current conditions, and all relevant laboratory data. Per Partners IRB protocol, the principal investigator at each site will report all serious unanticipated adverse events to Insight/eIRB within 5 working days/7 calendar days of the date the investigator first becomes aware of the problem. If at any time during the course of the study, the DSMB judges that the risk to subjects outweighs the potential benefits, the DSMB shall have the discretion and responsibility to recommend that the study be terminated.

4.17.3 Reporting Procedures for All Adverse Events

After review with the subject by the study site personnel, all Adverse Events occurring during the study, whether or not attributed to the TPBM-1000 device or TLT procedure, observed by the Investigator or reported by the subject, will be documented in the subject's source document and on the appropriate CRF pages. The following attributes must be assigned:

1. Description of event
2. Date of onset
3. Date of resolution (if applicable)
4. Seriousness
5. Relationship to the study device and/or procedure(s)
6. Intensity

7. Action(s) taken
8. Outcome(s)

Intensity is defined as a measure of the severity of a reaction, effect or experience. The measurement(s) are described as mild, moderate or severe. The event itself, however, may be of relative minor medical significance.

The intensity of Adverse Events is assessed as mild, moderate or severe according to the following index scale:

Mild
The Adverse Event is transient, requires no treatment, and does not interfere with the subject's daily activity.
Moderate
The Adverse Event introduces a low level of inconvenience or concern to the subject and may interfere with daily activities, but is usually ameliorated by simple therapeutic measures.
Severe
The Adverse Event interrupts the subject's usual daily activity and requires systematic therapy or other treatment.

If the Adverse Event is of such intensity in the Investigator's judgment that it warrants withdrawal from the study, the subject should be withdrawn from treatment. The subject should be given appropriate care under medical supervision until symptoms resolve.

The relationship of an Adverse Event to the study device or procedure will be graded as follows:

None
The Adverse Event is not associated with the study device use.
Remote
The temporal association is such that the study device is not likely to have had an association with the observed Adverse Event.
Possible
This causal relationship is assigned when the Adverse Event:
a) Follows a reasonable temporal sequence from device use, but
b) Could have been produced by the subject's clinical state or other modes of therapy administered to the subject.

Probable
This causal relationship is assigned when the Adverse Event:
a) Follows a reasonable temporal sequence from device use;
b) Abates upon discontinuation of the treatment;
c) Cannot be reasonably explained by known characteristics of the subject's clinical state.

Definite
This causal relationship is assigned when the Adverse Event:

a) Follows a reasonable temporal sequence from device use;
b) Abates upon discontinuation of the treatment; and
c) Is confirmed by the reappearance of the Adverse Event on repeat exposure.

*For purposes of immediate reporting of Unanticipated Adverse Events, investigator's judgment will be considered for determining that the adverse event is "more than 50% likely" related to use of the device or to the treatment procedure.

4.17.4 Serious Adverse Events

All Serious Adverse Events must be reported to the overseeing Institutional Review Board (IRB), FDA, and Litecure Inc as required.

If the Investigators are notified by Litecure Inc or its designee of any Serious Adverse Events that are considered to be Unanticipated Adverse Device Effects, the Investigators must notify his/her own IRB/EC as required.

4.17.5 Deaths

Deaths which must be reported to Litecure Inc include all deaths while participating in the study.

4.17.6 Withdrawals for Adverse Events

All Adverse Events which result in the subject's withdrawal from the study must be reported immediately by telephone to Litecure Inc.

The Investigator may be asked to provide detailed follow-up information. The Investigator will determine the reportability of the event on a case-by-case basis, and will report to the appropriate regulatory authorities evaluating the study device as necessary.

4.18 Measures to Assure Subject's Safety

The Investigator will be responsible for monitoring the safety of subjects who enter this study and for alerting Litecure Inc of any study-related event that seems unusual and/or unanticipated for his/her site.

The Investigator will be responsible for the appropriate medical care of the subjects during the study in connection with protocol procedures for his/her site.

The Investigator will remain responsible for providing any appropriate health care options after a subject's completion or discontinuation from the study due to adverse events.

4.19 Subject Disposition Criteria

4.19.1 Withdrawal from the Study

Each subject and the Investigator reserve the right at any time to terminate a subject's participation in the clinical investigation.

Possible reasons for withdrawal or removal from the study may include:

1. The subject voluntarily withdraws consent.
2. The subject was not eligible based on the study inclusion and exclusion criteria.
3. The subject develops an Adverse Event that would not allow continuation in the study.
4. The subject has an Adverse Event which in the opinion of the Investigator warrants withdrawal from the study. Litecure Inc must be notified within two business days.
5. A decision is made by the subject and/or Investigator that the subject should be withdrawn from the study.
6. Subject death
7. Positive pregnancy test during the eligibility procedures

When a subject withdraws or is removed from the study, the following will be performed, if feasible, at the study termination (exit) visit:

- HAM-D-17
- QIDS-C
- CGI
- C-SSRS
- ADVERSE EVENTS FORM
- Concomitant medications form
- ASQ (self-rated)
- SAFTEE-SI (self-rated)
- SDQ (self-rated)
- NEURO-QOL (self-rated)
- SFI (self-rated),
- Cognitive tests

For all subjects who withdraw from the study prematurely, the date, and reason for withdrawal will be documented.

4.19.2 Lost to Follow-up

If a site is unable to contact a subject or if the subject fails to appear for a visit, three documented phone calls should be made, followed by a certified letter (or its equivalent). The certified letter should detail the need for the subject to appear for a visit, the site's

unsuccessful attempts to contact the subject, and that failure to contact the site will result in the subject being withdrawn from the study.

If the certified letter is returned to the site as undeliverable or the letter is delivered but the subject does not contact the site and no other contact is made with the subject or the subject's caregiver, then the subject will be considered Lost to Follow-up and discontinued from the study. All attempts to contact the subject will be documented.

4.20 Risk and Benefits Overview

4.20.1 Potential Adverse Events

Risks to the subject may include but are not limited to the following:
The TPBM-1000 emits light with a longer wavelength than the human eye can see. The TPBM-1000 output is less than the maximum permissible exposure (MPE). The staff will be provided training on basic safety procedures relative to the use of the device. The staff administering the TLT will be careful not to operate the TPBM-1000 device unless it is in direct contact with the subject's skin. Both the subject receiving the TLT and the study clinician present in the TLT delivery room will wear protective eyewear in the form of goggles or eye pads. The goggles provided with the TPBM-1000 are in direct contact with the area surrounding the subject's eye. Since they are reusable they should not be shared between subjects. The eye pads are disposable and should be discarded after use. The eye pads use an adhesive to adhere to the subject's eyelids so there is potential for an allergic reaction.

Failure of the TPBM-1000, resulting in the cessation of investigative therapy can cause:

1. No adverse event to our knowledge
2. Unforeseeable adverse events

Delivery of the infrared energy to an inappropriate site, such as directly over the open eye, is not recommended and could pose a risk to the subject.

Application of the TPBM-1000 may result in mild thermal sensation of warmth during the use. The temperature of the skin is well below the level for thermal damage.

Based on human clinical trial experience to date, each adverse event listed below has been reported with TLT:

1. Application Site Erythema
2. Application Site Pain
3. Application Site Discomfort
4. Application Site Warmth
5. Application Site Reaction
6. Headache

Additional potential side effects of TLT, documented at MGH in prior trials, include:

- Seeing vivid colors, having abnormal taste
- Feeling "out-of-body" experiences
- Insomnia, restless sleep, erratic sleep, early morning awakenings
- Vivid dreams
- Irritability
- Word finding difficulties
- Abdominal bloating

Other potential risks are described below:

Risk of Worsening of Depression, Suicidality & Manic Switch: Worsening of depression and increased suicidality are possible complications of antidepressant treatments prescribed to subjects with MDD. We will minimize this risk by selecting only subjects who do not present active suicidal ideation at screening. We will also discontinue any subject who develops active suicidal ideation during the course of the study (C-SSRS) and is deemed at imminent danger; we will then arrange for appropriate levels of care and standard antidepressant treatment (see 4.8.4 Termination). Manic switches are possible adverse events and will be closely monitored during antidepressant treatment as well. Our tight schedule of clinical study visits will allow early recognition of treatment-emergent suicidal ideation or prodromal hypomanic signs. Subjects who develop mania or hypomania will be discontinued and provided appropriate level of care.

Answering detailed questionnaires may create a mild degree of inconvenience for the subjects.

Length of testing for cognitive circuitry:

The total length of this task takes a total of 40 minutes at baseline and 40 minutes at week 3, 6, 9 and 12. There is no expected risk to the patients beyond the 40 minutes of sitting in front of computer for testing and possible experience of startle. Every effort will be made to ensure that the patients are comfortable and safe during this period, and the study can be stopped at any time. Beyond these issues, there is minimal or no risk to the patients.

Risk of Bruising

Venipuncture might result in accidental bruising at the sight of blood drawing on the forearms.

4.20.2 Benefits

Study subjects will receive systematic MINI assessment of their DSM comorbidity. This information will be readily available to their treating clinicians if the subjects so desire and agree to disclosure. This information might guide long term treatment. In the short-term, the subject will receive close and systematic monitoring for depression and formalized cognitive assessments, beyond current standards of care. Easy access to

routine physical exams are also a potential benefit. The subjects will have access to a different modality of treatment if counseling and/ or medications were not sufficient or not acceptable to them. The treatment itself with Transcranial continuous or pulse Light Therapy (TLT) may potentially provide relief of depressive symptoms at a level equal to antidepressant medication.

5 Data Analysis Methods

5.1 General Considerations

The purpose of this study is to assess the antidepressant effect of TLT in major depressive disorder.

This feasibility study is expected to randomized 50 subjects total across two sites, 1/3-of whom will receive 12 weeks of TLT and 2/3 of whom will receive 6 weeks of sham followed by either more sham (50%) or TLT (50%) for 6 more weeks.

5.2 Specific Aims

A. To assess the antidepressant effect of the Transcranial continuous or pulse Light Therapy in depressed subjects.
Hypothesis 1: We anticipate that TLT will decrease QIDS-C and HAM-D 17 total scores in study subjects significantly more than the Sham treatment. We expect that we will be also able to estimate the effect size of the antidepressant action of Transcranial continuous and pulse Light Therapy, separately.

B. To assess the safety and tolerability of the Transcranial continuous / pulse Therapy in depressed subjects
Hypothesis 2: We predict that the Transcranial continuous and pulse Light Therapy will be safe and well-tolerated by depressed patients, as assessed by the following rating scales: SAFTEE-SI and ADVERSE EVENTS FORM. We anticipate differences in between TLT and Sham treatment as concerns side-effects.

Secondary Aims include the assessment of neuro-inflammation and cognitive circuitry. We predict that TLT decreases plasma levels of IL6, IL8, TNF-α, IL-1β more than sham. TLT will also improve attention, inhibition and executive functions more than sham.

5.3 Sample Size

With respect to the TLT study, we expect 100 subjects to sign informed consent, including screen failures. Assuming that 1/2 will screen fail this will lead to randomization of 50 subjects. In PART-1 (CW), we will have a n=24 with a 1st randomization 2:1 (sham:NIR), which will result in 16 subjects receiving sham and 8 subjects receiving NIR. We then assume 70% non-response to sham which leads to 11 sham subjects who are non-responders and who will undergo the 2nd randomization (week 6). Assuming a dropout rate of 24% before week 6, we anticipate that 9 subjects from the sham group will be eligible for the 2nd randomization 1:1 (sham:NIR). This 2nd randomization (week 6) will result in approximately 5 subjects receiving sham and 4 subjects receiving NIR. According to the Sequence Parallel Comparison (SPD) design the data from the 1st randomization (week 0 to 6 of the trial) and the data from the 2nd randomization (week 6 to 12 of the trial) can be pooled resulting in a total of 16+5=21 available sham subjects and 8+4=12 available NIR subjects. With this sample we have estimated >80% power to detect a significant difference ($p \leq 0.05$) in depression outcome based

on the antidepressant effect size detected in our prior MGH study (ELATED-2). In PART-2 (PW) we will have an even larger power to detect a significant difference since our sample of subjects at the 1st randomization will be larger (n=26).

5.4 Subject Demographic and Baseline Characteristics

Subject demographic and baseline characteristics will be summarized for each treatment. Descriptive summaries will include the number of subjects, mean, standard deviation, median, minimum and maximum for continuous parameters, frequencies and percentages for categorical parameters.

5.6 Efficacy Analyses for Primary Aims

The study (both PART-1 continuous and PART-2 pulse) will utilize a 2-phase sequential parallel design with 6 weeks treatment duration in each phase. Eligible patients will be randomized to two groups with a 1:2 ratio, to ensure adequate sample in phase 2 (week 6-12). Patients in the first group (TLT) will receive TLT (contionus in PART-1 and pulse in PART-2) at both phases (week 0-12). Patients in the second group (Sham) will receive sham at phase-1 and will undergo a 1:1 ratio randomization to either TLT or sham in phase-2 (week 6-12), respectively. Patient retention rates during phase-1 of the trial are assumed to be 76%, based on our earlier study ELATED-2. The treatment differences from phase-1 and phase-2 will be combined with equal weights to estimate the overall treatment effect.

For the primary efficacy analysis, all patients in phase 1 will be compared, together with sham non-responders from phase 2 (sham non-responders are defined as those patients who failed to achieve a 50% decrease in their QIDS-C score at visit 13).

A. Antidepressant effect of the TLT: To test the significance of the antidepressant effect of TLT (in both PART-1 and PART-2) we will use a T-test for mean differences of two related measures and will primarily look at QIDS-C and HAM-D 17 total score mean change on TLT and compare it with the QIDS-C and HAM-D 17 total score mean change on Sham, the significance will be set at a p-value ≤ 0.05. The time-points to estimate the mean differences are baseline and week 6 for all subjects and for subjects non-responders to sham who are re-randomized also week 6 and week 12. Rates of responders according to the CGI-Improvement (score 1 or 2) will be estimated at week 6 and 12 of the study (no statistics planned). Both completer analysis and intent-to-treat analysis will be performed to analyze the severity of depression at endpoint. Patient attrition and dropouts

will be analyzed. Potential differences between treatment groups in dropouts will be examined with the Fisher's Exact test.

B. Tolerability of the TLT: The frequency and severity of the reported side-effects and adverse events with the TLT will be compared in the TLT vs. Sham group (Fisher's Exact Test), following the SPCD design. The corrective measures adopted by the clinicians will also be reported, as well as the clinical judgment on the likelihood of the association of the side effect/ adverse event with the TLT.

C. Cognitive Tasks Analyses:

The secondary efficacy variable is the effect of TLT on cognitive performance as measured by selected cognitive tasks. All analyses will be conducted in accordance with SPCD design.

5.7 Safety Analyses

The number and percent of Adverse Events through study completion will be presented for each treatment group by adverse event type.

5.8 Additional Safety Analyses

Descriptive statistics for safety endpoints for each treatment group will include number of subjects, mean, standard deviation, median minimum and maximum for continuous variables, and frequencies and percentages for categorical variables.

6 Ethical Review & Regulatory Considerations

6.1 Ethical Review

Prior to the start of the study the Investigator will obtain IRB approval of the protocol and the Informed Consent Form. Additional documentation may be required pending applicable local requirements. At least the following documentation must be obtained:

1. The IRB/EC approval of the protocol.
2. The IRB/EC approval of the Informed Consent Form.
3. The IRB/EC annual (or any other frequency when applicable – i.e. quarterly, semiannually according to the local IRB/EC standard operating procedure) renewed approval of the protocol.
4. The IRB/EC approval of any revisions to the Informed Consent Form or amendments to the protocol.

6.2 Regulatory Considerations

This study will be conducted in accordance with the Good Clinical Practice (GCP) guidelines and other applicable regulatory requirements including but not limited to:

- The Food and Drug Administration (FDA) Regulations on Investigational Device Exemption (21 CFR 812),
- The FDA Regulations on research with human beings (21 CFR 50, 54 and 56),
- The Health and Human Services (DHHS) Regulations on research with human beings (45 CFR 46 Subparts A, B, C, and D) and
- The International Conference on Harmonization (ICH) "Guidance for Industry-E6 Good Clinical Practice: Consolidated Guideline."

The Study will be conducted in the US in accordance with the Privacy Rule (45 CFR Parts 160 and 164) of the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

6.3 Monitoring Procedures

Monitoring will be conducted by the PI who will periodically review lab results and clinical information. The PI will be coordinating the different phases of the protocol implementation such as TLT delivery and follow up. The PI will be frequently in contact with the other staff members responsible for TLT delivery and with Litecure Inc, which will lend the TLT device. We have minimized risks potentially associated with the use of Transcranial Light Therapy (TLT) by requiring two methods of contraception (although TLT has not been associated with teratogenic effect). In addition, we are providing the subjects with safety goggles or eye pads during the TLT delivery. We have also included treatment discontinuation rules based on the development of skin erythema, or discomfort

lasting more than 24 hours at the sites of TLT delivery (to prevent risk of subject's having an unknown skin photosensitivity).

Treatment will end for participants who experience temporary pain or discomfort during a treatment session. A research assistant or the study doctor will follow-up within a 24-hour period, after the discontinued treatment, with the patient who experienced discomfort. The subject may continue with the study if the acute pain stopped. If the subject experiences discomfort after 24-hours the aforementioned treatment discontinuation process may begin (e.g., Section 4.19.1, p. 29).

6.3.1 Training of study site personnel

Training will be initiated prior to the protocol being implemented. Training will consist of both lecture and practicum. Application of TLT procedure will be performed only by PI or his/her designee trained by the Sponsor (or its designee) to perform the procedure.

6.4 Informed Consent

The Principal Investigator will be responsible for developing the Informed Consent Form The Informed Consent Form will be prepared in accordance with FDA 21 CFR Part 50 for all US sites. The Informed Consent Form will be used to explain in simple terms, before the subject is entered into the study, the possible risks and benefits to the subject. The Informed Consent Form will contain a statement that the consent is freely given, that the subject is aware of the risks and benefits of entering the study, and that the subject is free to withdraw from the study at any time.

Prior to a subject's participation in the study, the written Informed Consent Form will be signed and personally dated by the subject or by an individual authorized to sign on behalf of the subject per compliance with local regulations.

If a subject is unable to read or if a legal representative is unable to read, an impartial witness will be present during the entire Informed Consent Form discussion. After the written Informed Consent Form and any other written information to be provided to the subject is read and explained to the subject or legal representative, and oral consent to the subject's participation in the study has been given by the subject, the witness will sign and date the Consent Form.

A witness in the consent process is considered to be an individual that is impartial and independent of the study.

Note: ***The Investigator must document acquisition of the written Informed Consent Form in the subject's medical records, and the subject or legal representative must be given a copy of the Informed Consent Form document prior to enrollment into the study.***

6.5 Protocol Adherence

The protocol must be read and followed by all participating study personnel.

6.6 Data Collection

Data will be captured on Case Report Forms - RedCAP. For each subject, a study binder will be kept locked in the MGH and NYUSOM site.

6.7 Record Retention

The Investigator must maintain a file of all documents and records relating to the conduct of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, or as specified below.

Study records are subject to inspection by the FDA and other regulatory agencies.

6.8 ClinicalTrials.gov

ELATED-3 study will be registered with and posted on ClinicalTrials.gov (www.clinicaltrials.gov).

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PROTOCOL ATTACHMENT 1 – TIME TABLE OF STUDY PROCEDURES

Table of Assessments

	Screening Visit	Treatment Visits																								Follow-Up Visits
Week	0	1		2		3		4		5		6		7		8		9		10		11		12		13
Visit	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25
PROCEDURES																										
Consent	Δ																									
MINI	Δ																									
ATRQ	Δ																									
HAM-D 17	Δ	Δ						Δ						Δ						Δ						Δ
QIDS-C	Δ	Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ
CGI	Δ	Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ
MoCA	Δ																									
C-SSRS	Δ	Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ
CADSS		Δ												Δ												Δ
TLT/Sham Treatment		Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	
PBQ-CR											Δ												Δ			
Physical Exam	Δ																									
Adverse Events Form		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ
Concomitant Medications	Δ	Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ		Δ
Progress Note		Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ
Vital Signs	▌													▌												▌
Laboratory	▌																									
Cytokine Blood Draws	▌													▌												▌
MSIT		▌						▌						▌						▌						▌
Voice Pattern		▌						▌						▌						▌						▌
MST		▌												▌												▌
SDQ	▼	▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼
ASQ	▼	▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼
SAFTEE-SI		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼
NEURO-QOL		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼
MGH SFI		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼		▼
PBQ-SR											▼												▼			
TSRQ											▼												▼			
PEG		▼												▼												▼

Δ Clinician Rated ▌ Conducted by Research Coordinator ▼ Subject Rated

The CGI improvement scale is unmeasured on Visit 0 (screening visit).