Confidential Information

Transcranial Near-Infrared Light in Healthy Subjects: a Cerebral Blood Flow Study with Diffuse Correlation Spectroscopy (NIR-flow).

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1. Study Summary

Protocol #:

Study Purpose: The purpose of this study is:

- To assess the change in cerebral blood flow induced by the Transcranial Continuous and Pulse Near-Infrared Light Therapy in healthy subjects
- To correlate with cognitive performance the change in cerebral blood flow induced by the Transcranial Continuous and Pulse Near-Infrared Light Therapy in healthy subjects
- To correlate with skin pigmentation the change in cerebral blood flow induced by the Transcranial Continuous and Pulse Near-Infrared Light Therapy in healthy subjects
- To correlate with brain electrical activity the change in cerebral blood flow induced by the Transcranial Continuous and Pulse Near-Infrared Light Therapy in healthy subjects
- To assess the safety and tolerability of the Transcranial Continuous and Pulse Near-Infrared Light Therapy in healthy subjects

Trial Design: In essence, this study consists in three sequential sessions of Diffuse Correlation Spectroscopy (DCS), each one week apart, to test three different modes of intervention with Transcranial Light Therapy (TLT). Healthy subjects will receive first a session with continuous light (c-TLT), then a session with sham (s-TLT) and one session with pulse light (p-TLT). Before and after each session, subjects will undergo DCS, which will allow measuring the impact of the intervention on cerebral blood flow. We expect that out of 30 subjects enrolled 10 subjects will be eligible to complete the study. The study will be conducted in single blind.

Study Population: Subjects eligible for study participation will be healthy by Structured Clinical Interview for Diagnostic Statistical Manual-IV (SCID) 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 5 weeks.

Endpoints:

Engagement of cerebral blood flow – The effect of TLT on cognition will be assessed before and after each session (c-TLT, s-TLT and p-TLT) by measuring the Diffuse Correlation Spectroscopy (DCS) signal.

Engagement of cerebral electrical activity – The effect of TLT on brain electrical waves will be assessed before and after each session (c-TLT, s-TLT and p-TLT) by measuring the Electro-Encephalographic (EEG) signal.

Engagement of cognitive circuitry – The effect of TLT on working memory will be assessed immediately after each session by n-back task.

Safety - The safety endpoints will evaluate any reported adverse events within one week from each sessions of TLT.

Entry Criteria:

Inclusion Criteria

1. Subject's age at screening will be between 18 and 70 years old (inclusive).
2. Women of child-bearing potential must use a double-barrier method for birth control (e.g. condoms plus spermicide) if sexually active.
3. Subject Informed Consent obtained in writing in compliance with local regulations prior to enrollment into this study.
4. The subject is willing to participate in this study for at least 5 weeks.

Exclusion Criteria:

1. The subject is pregnant or lactating.
2. The subject is on any psychotropic medication.
3. Psychotherapy is exclusionary unless the subject has had at least 8 weeks of treatment prior to the screening visit.
4. Any current psychiatric disorder (per SCID assessment)
5. Substance or alcohol dependence or abuse in the past 6 months.
6. History of a psychotic disorder or psychotic episode (current psychotic episode per SCID assessment).
7. Bipolar affective disorder (per SCID assessment).
8. Unstable medical or neurological illness, defined as any illness which is not well-controlled with standard-of-care medications (e.g., insulin for diabetes mellitus, HCTZ for hypertension).
9. Suicidal or homicidal ideation as determined by SCID screening.

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 Arteriovenous Malformation (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. Personality traits that rend the subject unsuitable for the study, based on the investigators' clinical judgment.

2. List of Abbreviations

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
CHRT = Concise Health Risk Tracking
CATSD = Center for Anxiety and Traumatic Stress Disorders
EEG = electroencephalogram
FDA = Food and Drug Administration
LED = Light Emitting Diode
HAM-D-17 = Hamilton Depression rating scale
MOCA Montreal Cognitive Assessment
MDD = Major Depressive Disorder
MGH = Massachusetts General Hospital
NEURO-QOL = Quality of Life in Neurological Disorders
TSRQ = TLT Self-Report Questionnaire
OSHA = Occupational Safety and Health Administration
PANAS = Positive and Negative Symptoms Questionnaire
PBQ = Perceptions of Blinding Questionnaire
PI = Principal Investigator at the site
SAE = Serious Adverse Event
SAFTEE-SI = Systemic Assessment for Treatment Emergent Events
SCID = Structured Clinical Interview for Diagnostic Statistical Manual-IV

SDQ = Symptoms of Depression Questionnaire
SFI = Sexual Functioning Inventory
TLT = Transcranial Light Therapy

3. Introduction

Transcranial Light Therapy (TLT) with near-infrared light has recently emerged as a potential new antidepressant treatment in both animal models [1] and human studies [2–4]. TLT consists of delivering invisible near-infrared radiation (NIR), or red light to the scalp of the subject, which penetrates the skull and modulates function of the adjacent cortical areas of the brain. Red and/or near-infrared light appears to increase cellular brain metabolism and neuroplasticity, and to modulate endogenous opioids, while decreasing inflammation and oxidative stress [5–7]. TLT has been shown to penetrate deeply into the cerebral cortex [8–10], to modulate cortical excitability [11] and improve cerebral perfusion [10] and oxygenation [12]. Studies have suggested that it can significantly improve cognition in healthy subjects [13–15], and in subjects with traumatic brain injury [16–18]. The safety of TLT has been studied in a sample of 1,410 acute stroke subjects, with no significant differences in rates of adverse events between TLT and sham exposure [19–21]. Converging evidence from uncontrolled studies suggests both an antidepressant and anxiolytic effect of TLT in subjects suffering from Major Depressive Disorder (MDD) [2–4]. It is critically important to understand the biological targets of TLT and we hypothesize that the improvement in cerebral brain flow is one of the underlying mechanism for the boost in bioenergetic metabolism leading to therapeutic effects, such as enhancement of cognitive functioning.

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) (6). All subjects (n=4) were treated with TLT. Baseline mean Hamilton Depression rating scale (HAM-D-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 subjects with MDD. Both the Laser study (ELATED-1) and the LED study (ELATED-2) were conducted by our group at Massachusetts General Hospital (MGH).

NIR-flow (the present submission) will test for the first time the biological underpinning of TLT, by focusing on the effect of TLT on cerebral blood flow.

The LiteCure® The PhotoBioModulation-1000 (TPBM-1000) device to be used in this study does not require Food and Drug Administration (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 Light Emitting Diode (LED)System

LiteCure® TPBM-1000 is a new device with the flexibility to safely deliver either continuous light (c-TLT), pulsed light (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 (s-TLT). 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 subjects will not experience any heating sensation ensuring the study is single-blind. The device will shut off automatically if skin temperature above 41C were detected.

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 the DCS Device

Separate hybrid FDNIRS-DCS instruments are described in detailed in the relative medical device brochure. FDNIRS-DCS devices are approximately 23" (l) x 18" (w) x 9" (h). Technical specs are provided at:
iss.com/biomedical/instruments/metaox.html. The devices are limited by Federal (or United States) law to investigational use. The devices contain no conductive patient leads and no user serviceable parts. They have a highest rated current of 3 Amp and are designed for safety compliance with UL/IEC 60601-1 3rd Edition as a Class I, Type B device. Prior to investigational use, devices are tested for conformance with ground bond, earth leakage, and enclosure leakage requirements. The devices are not yet under Food and Drug Administration (FDA) investigational device exemption (IDE) regulations, and are considered non-significant risk. During the conduct of human studies the investigators should follow good clinical practice requirements and 21 CFR 812 investigational device exemption regulations.

The FDNIRS portion of the instrument consists of 8 radio- frequency (110 MHz) modulated laser diodes operating at 8 different wavelengths ranging from 670-830 nm and 2 to 4 photomultiplier tube (PMT) detectors for heterodyne detection (similar to the Imagent from ISS, Inc).

The DCS component of the instrument consists of a long-coherence length 852 nm laser, 4 to 8 low dark-count photon counting avalanche photodiodes, and a custom-made correlator board. A short-pass filter in front of the FDNIRS PMTs, along with in-house designed software enables simultaneous FDNIRS-DCS measurements.

Furthermore, to customize this instrument for use in humans, the MGH group has designed optical probes which host fiber optics. Each probe consists of 1 source and up to 8 detector fiber bundles terminated with 90 deg prisms providing multiple source-detector separations (ranging from 0.3 to up to 5 cm).

The optical parameters for the FNIRS- DCS devices can be summarized as follows, irradiance 250-400 mW/cm2; area: 0.0962 cm2 and 10 min exposure (twice 10 min, before and after the session with TPBM-1000); fluence: 150-240 J/cm2. Total energy delivered in 10 min by the FNIRS-DCS devices: 14.43 – 23.08 J (this energy will be delivered twice before and after each TPBM-1000 sessions). The incremental exposure to NIR resulting from the FNIRS-DCS devices are minimal: in fact the NIR light is shed to a tiny area 1/350 of the area irradiated by TPBM-1000 and the energy shed in 10 min by the FNIRS-DCS are inferior to the total energy shed by the TPBM-1000 by more than 2 orders of magnitude.

3.1 Overview of the EEG Device

The StatX24 (Advance Brain Monitoring) is an internally battery powered Type BF device intended for continuous use (16-17 hours). The StatX24 provides an integrated approach for wireless acquisition and recording of electroencephalographic (EEG), electrooculographic (EOG), and electrocardiographic (ECG) signals. The system

utilizes the patented Sensor Headset and patented EEG sensors, which record high quality EEG, obtained with less than five-minutes of set-up time and no scalp abrasion required. The wireless technology allows the user to be un-tethered and move around the home, healthcare facility, or clinical research environment while real time data is collected and displayed.
The StatX24 acquires twenty channels of monopolar EEG recordings with a linked mastoid reference and optional channel for ECG, EOG, or EMG. The 1-10th channels, as well as the 21st channel, are differential channels. The 22nd-24th channels are referential channels. The StatX24 consists of: (1) StatX24 Headset with a Bluetooth (BT) Receiving Unit for bi-directional transmission of digitized physiological signals, (2) a Neoprene Strap, and (3) a Strip with EEG sensors sites in the standard 10-20 format + POz (Fz, Cz, Pz, F3, F4, C3, C4, P3, P4, O1, O2, T5, T3, F7, Fp1, Fp2, F8, T4, and T6 with Linked Mastoids).
The Sensor Headset collects signals from the sensors placed on the patient and performs analog-to-digital conversion, encoding, formatting, and transmitting of all signals. The signals communicate using a 2.4 to 2.48 GHz radio transmitter. StatX24 acquisition utilizes the bi-directional capabilities of the system to initiate scalp-electrode impedance monitoring and monitors the battery capacity in the StatX24 Headset. A BT Receiving Unit is used as the base unit affixed to the PC workstation.

3.2 Overview of Rationale for Diffuse Correlation Spectroscopy Cognitive Tasks and Electroencephalogram

Diffuse Correlation Spectroscopy
From a cellular and molecular perspective, the beneficial effect of TLT (NIR) on brain metabolism is the primary putative mechanism for its therapeutic effects. In experimental and animal models NIR (and red) light delivered noninvasively is absorbed by cytochrome c oxidase, and by stimulating the mitochondrial respiratory chain, leads to increased adenosine triphosphate (ATP) production [22]. In a study on healthy subjects, TLT (NIR) improved cerebral oxygenation, supposedly through a mechanism of neurovascular coupling, which entails an increase in cerebral blood flow coupled with increased oxygen demands [12]. Similarly, a study in MDD subjects found that a single session of t-PBM led to a nearly significant increase in regional cerebral blood flow [2] — both studies relied on functional NIR spectroscopy (fNIRs) to assess, respectively, oxygenation and blood flow. In a case report of moderate, chronic TBI, the improvement in brain perfusion subsequent to TLT was imaged with single positron emission computerized tomography (SPECT) [23].

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.

Electroencephalogram
EEG recorded at the scalp offers the opportunity to access the synchronization of oscillatory frequencies (1Hz to 100Hz) from within and between cortical regions, revealing network changes that reflect abnormal connectivity. Synchronization within and between cortical regions is of interest in the field of TLT. In animal models of depression, TLT was found to regulate the EEG tracing in all wave frequency bands except for theta. Similar effects have been documented in dementia subjects who experienced normalization of delta and alpha waves power during the course of a pilot study with TLT treatment (Berman M & Hamblin M unpublished data).

4. Investigational Plan

4.1 Rationale

The specific aims of this study are:

1. To assess the change in cerebral blood flow induced by the Transcranial Continuous and Pulse Near-Infrared Light Therapy (c-TLT and p-TLT) in healthy subjects
2. To correlate with cognitive performance the change in cerebral blood flow induced by the Transcranial Continuous and Pulse Near-Infrared Light Therapy in healthy subjects
3. To correlate with brain electrical activity the change in cerebral blood flow induced by the Transcranial Continuous and Pulse Near-Infrared Light Therapy in healthy subjects
4. To correlate with skin pigmentation the change in cerebral blood flow induced by the Transcranial Continuous and Pulse Near-Infrared Light Therapy in healthy subjects
5. To assess the safety and tolerability of the Transcranial Continuous and Pulse Near-Infrared Light Therapy in healthy subjects

4.2 Study Design

This study is a single-blind, sham-controlled, study on the use of Transcranial continuous light therapy (c-TLT) and pulse light therapy (p-TLT) as an enhancer of cerebral blood flow in healthy subjects.

We expect that out of 30 subjects enrolled 10 subjects will be eligible to complete a sequence of three sessions of Transcranial Light Treatment (TLT) and sham.

All eligible subjects will receive two TLT sessions and one of sham (s-TLT) within the course of 5 weeks. While the sequence (c-TLT, s-TLT, p-TLT) will be the same for all subjects, the subjects will be blind to the specific mode of function used in each session.

The study site (MGH) will have one Litecure device, which will recognize the subjects' codes generated by the study statistician. Only subjects will be blind to the treatment group assignment. The Sham treatment consists of 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 subject-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). The entire sessions is estimated to last about 1.5 hours. The additional time is needed to have the subject complete a urine toxology and self-report forms, to prepare the subject, to place the necessary protections (e.g. goggles), to inspect the subject's skin, to complete DCS before and after TLT, to set the TLT devices, and to give the subject time to rest 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 Laser Safety Committee. The treatment will follow these specifications: wavelength 830 nm; average irradiance 54.8 mW/cm2; average fluence of 65.8 J/cm2 (consistently with energy density used in the ELATED-2 study at MGH with the device Omnilux New U).

4.3 Study Population

Healthy subjects 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 study.

4.3.1 Population Description

The subject population for this study will consist of healthy subjects between the ages of 18 and 70, of any ethnic background. We expect to enroll 30 subjects in order to have 10 who are eligible and complete the study. 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. 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 site's 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 preliminary 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 NIR-flow study will be identified by subject numbers 101 through 130 (to account for screen failures and drop-out before TLT sessions).

4.5 Sample Size

The NIR-flow study is expected to enroll 30 subjects, with 10 subjects who will be eligible and have 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 Center for Anxiety and Traumatic Stress Disorder (CATSD) in Boston.

The study involves screening, 3 TLT session paired with Diffuse Correlation Spectroscopy (DCS), and one follow-up visit.

Screening Visit (Week 1)
The screening visit is expected to take about 2 hours. The Principal Investigator (PI) at the site or a Sub-investigator will assess subjects against the inclusion and exclusion criteria. If a subject appears to qualify and agrees to participate in the study, then the subject 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.

TLT-DCS Visits – Visits 2-4 (Weeks 2-4)
There is one TLT session per week for 3 weeks. TLT-DCS Visit 2-4 will consist of all subjects receiving TLT, with DCS before and after TLT. Two groups of assessments will be conducted: **1.** Before the TLT session to reflect the symptoms endorsed in the past week: Anxiety Symptoms Questionnaire (ASQ), Symptoms of Depression Questionnaire SDQ, Quality of Life in Neurological Disorders (Neuro-QoL), Sexual Functioning Inventory (SFI), Systemic Assessment for Treatment Emergent Events (SAFTEE), and **2.** After the TLT session to reflect the immediate changes associated with the session: Positive and Negative Symptoms Questionnaire (PANAS), TLT Self-Report Questionnaire (TSRQ), Perceptions of Blinding Questionnaire (PBQ). Cognitive tasks will occur right after the TLT session. A detailed description of the study visits can be found below. EEG recording will immediately precede and follow the DCS-TLT-DCS triad and will in part overlap with the cognitive task.

Follow-Up Visit – Visit 5
All subjects will undergo assessments including ASQ, SDQ, Neuro-QoL, SFI, SAFTEE, PANAS and cognitive tasks at the follow-up visit (Week 5).

4.8 Detailed Study Visits

4.8.1 Screening Visit – Visits 1 (Week 1)

Purpose
The purpose of the Screening Visit will be to determine the eligibility of subjects for participation in the NIR-flow study.

Procedures

The screening visit will occur at the MGH Center for Anxiety and Traumatic Stress Disorders (CATSD) in Boston. At this visit subjects will be consented for the study, will receive a psychiatric evaluation, and will be screened for presence of psychiatric disorders using the Structured Clinical Interview for Diagnostic Statistical Manual-IV (SCID-IV). The SDQ, ASQ and PANAS will be used to assess for subthreshold symptoms. The SFI and NEURO-QoL will be used to assess sexual and cognitive functioning.

Routine laboratory tests (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.

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.

4.8.2 TLT-DCS Visits – Visits 2-4 (Weeks 2-4)

The TLT sessions will occur once weekly for three weeks at the MGH Martinos Center for Biomedical Imaging in Charlestown. Subjects missing one session will be allowed to still receive their TLT session, by shifting the overall study schedule of one week. The study PI will be an active participant in the delivery of the TLT. The PI and other collaborators from MGH CATSD 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 CATSD staff in the use of the device and will provide training certificates at the completion of the training.

The overall study visit is estimated to last about 1.5 hours. The TLT session is estimated to last about 50 min – 5 minutes of EEG before DCS, 10 minutes of DCS before TLT, 20 minutes of TLT, 10 minutes of DCS after TLT and 5 minutes of EEG after DCS. EEG recording will extend to cover at least part of the time devoted to the cognitive tasks. Study sessions will take place in an office dedicated to TLT treatment at MGH Martinos Center for Biomedical Imagining in Charlestown. Only the subject and the staff administering the TLT will be allowed in during the session. The subject will comfortably lie down on an exam bed or sit on a 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. The study staff administering the TPBM device at the TLT-DCS sessions will appropriately remove any reflective objects to fall into or obstruct the path of the light energy produced by this device. Both the subject receiving the TLT and the study staff will wear protective eyewear in the form of goggles. During treatment sessions the office will be kept completely dark and a warning sign will hang 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 CATSD (Boston).

During TLT-DCS Visit 2-4, subjects will receive TLT-c (Visit 2), TLT-s (Visit 3) and TLT-p (Visit 4).

The following tasks will be completed prior to every TLT session:

- Utox
- Concomitant medications form
- ADVERSE EVENTS FORM
- ASQ (self-rated)
- SDQ (self-rated)
- NEURO-QoL (self-rated)
- SFI (self-rated)
- SAFTEE (self-rated)
- PANAS (self-rated)
- EEG- Recording
- DCS – Spectroscopy

The following tasks will be completed after every TLT session:

- DCS – Spectroscopy
- EEG- Recording
- Cognitive tests
- PANAS (self-rated)
- TSRQ (self-rated)
- PBQ (self-rated)

Subjects 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.

4.8.3 Follow-up Visit – Visit 5, (Week 5)

After treatment completion, subjects will continue with one more clinical study visit to measure the long-term pro-cognitive effect of TLT. The follow-up visit will typically occur at the MGH Center for Anxiety and Traumatic Stress Disorders (CATSD) in Boston but could be performed at the Martinos Center for Biomedical Imaging in Charlestown if preferable to the subject.

At Visit 5, the following will be performed:

- Concomitant medications form
- ADVERSE EVENTS FORM
- ASQ (self-rated)
- SDQ (self-rated)
- NEURO-QoL (self-rated)
- SFI (self-rated)
- SAFTEE (self-rated)
- PANAS (self-rated)
- Cognitive tests

4.8.4 Termination

We will encourage subjects to complete the follow-up visit in the study regardless of discontinuation of the TLT sessions.

4.9 Scales & Forms

We will offer clinical interviews and study forms in English. All scales will explore the past 7 days except for SCID (lifetime), PANAS (past 5 min), TSRQ and PBQ (both past session). Below are brief descriptions of the forms that will be utilized in this study.

- **Structured Clinical Interview for DSM-IV (SCID-I/P)** – The SCID-I/P, administered by the clinician, proceeds by modules to diagnose the different psychiatric disorders. Questions here are asked exactly as written, and each is based on the individual criteria from DSM-IV. Answers are generally rated on a scale of 1-3 (1 = doubtful, 2 = probable, 3 = definite), and based on the number of positive answers, a diagnosis is determined. The entire SCID-I/P is administered at screening as per Table 1.
- **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.
- **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.
- **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.

- **Positive and Negative Symptoms Questionnaire (PANAS)** – The 20-item PANAS, comprises two mood scales, one measuring positive affect and the other measuring negative affect. Each item is rated on a 5-point scale ranging from 1 = very slightly or not at all to 5 = extremely to indicate the extent to which the respondent has felt this way in the indicated time frame. The authors have used the scale to measure affect at this moment, today, the past few days, the past week, the past few weeks, the past year, and generally (on average).
- **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 subject-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.
- **Concomitant medications form –** This form will be completed at every TLT study visit, including the screening visit, as a safety-monitoring tool.
- **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.

4.10 Cognitive tests

n-Back Task: The subject is presented with a sequence of stimuli, and the task consists of indicating when the current stimulus matches the one from n steps earlier in the sequence. The load factor n can be adjusted to make the task more or less difficult.

The n-back task captures the active part of working memory. When n equals 2 or more, it is not enough to simply keep a representation of recently presented items in mind; the working memory buffer also needs to be updated continuously to keep track of what the current stimulus must be compared to. To accomplish this task, the subject needs to both maintain and manipulate information in working memory.

4.11 EEG recording

The participant will be fitted with an **EEG sensor headset and ECG leads** to start their resting state data acquisition. For resting state acquisition with eyes open, the participant will be asked to relax, sit still and focus on a cross on the computer for 5 minutes while EEG data is acquired. The participant will be instructed to try to limit blinking and any face movement including clenching their jaw. Several acquisitions and/or longer acquisitions may be necessary if the participant is particularly restless. The participant will then be asked to relax and close their eyes for 5 minutes during the eyes closed resting state acquisition. Multiple and/or longer eyes closed acquisitions may be necessary if the participant has difficulty or is unable to sit still for 5 minutes. Any issues, including inability to remain still for the acquisition, should be noted in the participant's file.

Data Collection Forms

All study subjects will be blind to TLT assignment (single-blind). 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 subjects 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 and independent evaluators (IEs) 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 principal investigator, Dr. Cassano is 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. Data management will include clinician and subject rated assessments (see assessments), screening data, fidelity data, visit adherence data and rater reliability data and safety reporting, including DCS and cognitive data. 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.

Coded data may be sent to collaborators within and outside of Partners for research purposes. Coded sample/data may be shared with for profit companies that are working with Partners researchers, such as Litecure, LLC or Advanced Brain Monitoring, Inc (the manufacturer of the EEG device used this in study). Samples/data will not be sold to anyone for profit. Data may also be shared with other Partners IRB approved protocols supervised by the principle investigator of this study.

4.13 Training

Trained study clinicians 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.

4.14 Blinding of the Study

Since this is a single-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 subjects include detectors sensitive to heating of the skin (which will switch off the device to prevent overheating) and the introduction of a rating scale for subjects 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 $50 per screen (only if deemed eligible) and $100 per TLT-DCS visit, and $50 per follow-up visit; for a total of $400, if all five visits are completed. Compensation will be provided after Weeks 2 ($150), 3 ($100), 4 ($100), and 5 ($50), with the correspondent amount subtracted for each visit missed. Any research participant parking in one of the three Mass General Hospital garages will receive a parking voucher.

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 mood and anxiety scores, concomitant medications, adverse events, and serious adverse events and unexpected device events will be recorded from study entry through completion.

If skin erythema is present, treatment will be suspended. Subjects 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 subthreshold symptoms. If at any study visit the subjects' clinical condition is significantly worsened from baseline, the subject will be referred to appropriate level of care. (see 4.8.4 Termination).

Recording of physiological data with the EEG system poses no known health risk. Participants may experience mild discomfort due to the pressure exerted by the sensor headset; to minimize this risk, caps are adjustable and sensor strips are available in a variety of sizes. Discomfort may also arise from a mild allergic response to extended exposure to the conductive gel used in the EEG sensors; to mitigate this risk, alcohol wipes will be available to remove the gel immediately to reduce any allergic reactions.

The site Principal Investigator (Dr. Cassano) will have monthly conference calls or meetings (which will include study RAs); during these conference calls he will discuss all Adverse Event reports to identify any safety concern, based on such concerns he will be able to decide temporary discontinuation of study enrollment, modifications of the study protocol, or to terminate the study.

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 subject 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 judges to impose a significant hazard, contraindication, side effect, or precaution. Notification by e-mail of all related study forms shall be made to the IRB within 2 days of the occurrence of any SAE. Information will be reviewed and a determination made of whether there was any possible relevance to the study TLT exposure. Additional reporting to local IRBs will be done within 5 business days/7 working days of the date the investigator first becomes aware of the problem.

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 reporting Unanticipated Adverse Events, "None" and "Remote" will be considered as having no association with the device or 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

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.

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

- Concomitant medications form
- ADVERSE EVENTS FORM
- ASQ (self-rated)
- SDQ (self-rated)
- NEURO-QoL (self-rated)
- SFI (self-rated)
- SAFTEE (self-rated)
- PANAS (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 the site is unable to contact a subject or if the subject fails to appear for a visit, three documented phone calls should be made. The site's unsuccessful attempts to contact the subject and the subject's failure to contact the site will result in the subject being withdrawn from the study. The subject will be considered Lost to Follow-up.

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 lingering Depression of Anxiety and Manic Switch: We will minimize this risk by selecting only healthy subjects. We will also discontinue any subject who develops active suicidal ideation during the course of the study; 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 treatment as well. 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 45-60 minutes at baseline and 45 minutes from week 1 to week 5. There is no expected risk to the subjects beyond the ~45-60 minutes of sitting in front of computer for testing and possible experience of startle. Every effort will be made to ensure that the subjects 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 subjects.

4.20.2 Benefits

Study subjects will receive systematic MINI assessment of their DSM comorbidity and subthreshold symptoms. This information will be readily available to their treating clinicians of PCP if the subjects so desire and agree to disclosure. This information might guide long term treatment if subjects are indeed symptomatic. In the short-term, the subject will receive close and systematic monitoring for anxiety and depressive symptoms and formalized cognitive assessments, beyond current standards of care. Easy access to routine physical exams are also a potential benefit.

5 Data Analysis Methods

5.1 General Considerations

The purpose of this study is to assess the change in cerebral blood flow induced by the Transcranial Continuous and Pulse Near-Infrared Light Therapy in healthy subjects

This study is expected to enroll 30 subjects and treat 10 subjects with TLT for a total study duration of 5 weeks.

5.2 Specific Primary Aim

To assess the change in cerebral blood flow induced by the Transcranial Continuous and Pulse Near-Infrared Light Therapy in healthy subjects

Hypothesis 1: We anticipate that p-TLT will trigger a significantly greater increase in CBF as compared to c-TLT, and c-TLT will trigger a significantly greater effect than sham (s-TLT). We expect that we will be also able to estimate the effect size of each type of TLT in terms of increase of CBF.

5.3 Sample Size

With respect to the TLT study, we expect 30 subjects to sign informed consent, including screen failures.

5.4 Subject Demographic and Baseline Characteristics

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

5.5 Efficacy Analyses for Primary Aims

For the primary analysis, delta CBF (measured with DCS-spectroscopy pre- and post-TLT) will be compared in all ten subjects by paired t-test. The primary comparisons will include: a) delta CBF for p-TLT and delta CBF for c-TLT subjects (n=20 within subject comparison) and b) delta CBF for c-TLT and delta CBF for s-TLT (n=20 within subject comparison).

5.6 Safety Analyses

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

5.7 Additional Safety Analyses

Descriptive statistics for safety endpoints 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, pain or discomfort lasting more than 24 hours at the sites of TLT delivery (to prevent risk of subject's having an unknown skin photosensitivity).

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 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

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

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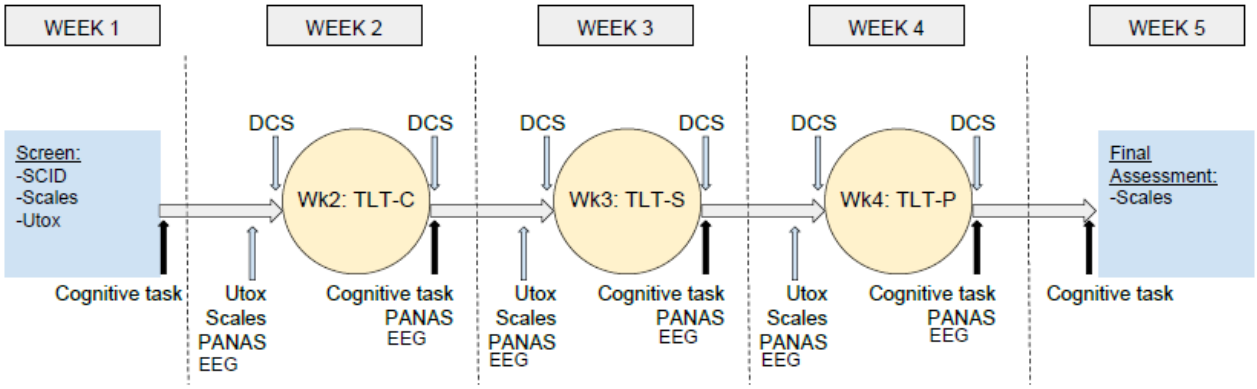
8 List of Protocol Attachments

8.1 Protocol attachment 1 – Time Table of Study Procedures



8.2 Protocol attachment 2 – Schema

Week	**1**	**2**			**3**			**4**			**5**
		Before	TLT	After	Before	TLT	After	Before	TLT	After	
Consent	X		TLT-C			TLT-SHAM			TLT-P		
SCID	X										
Utox	X	X			X			X			
Concomitant Medications	X	X			X			X			X
Adverse Event	X	X			X			X			X
ASQ	X	X			X			X			X
SDQ	X	X			X			X			X
Neuro-QoL	X	X			X			X			X
SFI	X	X			X			X			X
SAFTEE	X	X			X			X			X
PANAS	X	X		X	X		X	X		X	X
TSRQ				X			X			X	
Blinding Scale (PBQ)				X			X			X	
Cognitive Task	X			X			X			X	X
EEG		X		X	X		X	X		X	