CONFIDENTIAL INFORMATION

The information contained in this protocol is confidential and is intended for use by clinical investigators. It should not be copied or distributed to persons not involved in the clinical investigation of the LiteCure laser device, unless such persons are bound by a confidentiality agreement with Pthera LLC.

Near Infrared Transcranial Laser Therapy in Subjects with Major Depressive Disorder: A study of Dosing with Laser.

Study Sponsor:

Pthera LLC

18 She Way, Suite 107.

Newark, Delaware 19713

Protocol number:

NITLT01

Table of Contents

SIGNATURE PAGE

Protocol Number: NITLT01

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INVESTIGATOR'S AGREEMENT

By signing below I agree that:

I have read this protocol. I approve this document and agree that it contains all the details necessary to carry out the study as described. I will conduct this study in accordance with the design and the specific provision of this protocol and will make every reasonable effort to complete the study within the designated time. I will provide copies of this protocol and access to all information provided by Peruvian Clinical Research S.A.C. (Contract Research Organization responsible for the conduct of the study) to study personnel under my supervision. I will review this material with them to ensure that they are fully informed about the study product and study procedures.

I will let you know that this information is confidential and proprietary to Pthera LLC, sponsor of the study and may not be disclosed to any third party. I understand that the study may be terminated or enrollment may be suspended at any time by Pthera LLC, with or without cause, or by me if necessary to protect what is in the best interest of the study participants.

I agree to conduct this study in full compliance with INS Regulations, DIGEMID, Institutional Review Board/Ethics Committee Regulations and the International Conference on Harmonization of Good Clinical Practice Guidelines.

Investigator's Name

Investigator's Signature

Date:

SUMMARY TABLE OF CHANGES TO PROTOCOL AMENDMENT version 5.1 dated December 17, 2023

JUSTIFICATION: Amendment 5.1 is proposed due to typographical errors observed in official communication No. 1046-2023-DIIS/INS dated October 31, 2023.

N°	It said	It should say	Justification of the change	References
1	In section 1. Study Summary: (...) All NIR-TLT sessions will be ~~20 minutes~~, regardless of group assignment (...). In section 3.2 Lite Cure Laser System Overview: (...) the treatment time will be the same in all modes: ~~20 minutes~~. In Section 4.4 Study Design: (...) The entire session is estimated to last 30 minutes (...) In section 4.8.2 Treatment Visits 1 to 18- Visits 1-6: (...) Treatment visits are estimated to last approximately ~~20 minutes~~ (...)	In section 1. Summary of the Study: All NIR-TLT sessions will be 429 seconds (7.15 minutes), regardless of group assignment. In section 3.2 Lite Cure Laser System Overview: (...) the treatment time will be the same in all modes: 429 seconds (7.15 minutes). In section 4.4 Study Design: (...) The entire session is estimated to last (which includes the 429-second treatment and all procedures described in the "Study Visits" section) 30 minutes (...) In section 4.8.2 Treatment Visits 1 to 18 - Visits 1-6: (...) It is estimated that treatment visits will last approximately 429 seconds (7.15 minutes) (...)	It is being standardized to 429 seconds (7.15 minutes) in all sections to standardize the amendment.	N.A.

2	In section 5.1 General Considerations: (...) ~~120~~ patients were included, randomly distributed in five groups. (...) (...) The purpose of this study is to compare the antidepressant effect of ~~five~~ different doses of NIR-TLT (...) In section 5.2 Objectives of the study: (...) To compare the effect(s) of each of the ~~five~~ doses of NIR-TLT (...)	In section 5.1 General Considerations: (...) <u>112</u> patients were included, randomly distributed in <u>four</u> groups. (...) (...) The purpose of this study is to compare the antidepressant effect of <u>four</u> different doses of NIR-TLT (...) In section 5.2 Study objectives: (...) To compare the effect(s) of each of the <u>four</u> doses of NIR-TLT (...)	The arm that was to receive the NIR-TLT dose is eliminated: Mode 4 (UPW Group). The number of patients for the study is corrected.	N.A.
3	In the paragraphs of the document referring to the name of the sponsor, it has been changed. Formerly as: Lite Cure LLC	In the paragraphs of the document referring to the name of the sponsor, it has been changed. Now it should say: Pthera LLC	Due to the change in the sponsor of the study, the change was made to standardize the information in the document.	N.A.
N°	**OTHER CHANGES**			
1	NOT APPLICABLE			

1. Summary of the Study

Objectives of the Study:

The objectives of this study are:

Primary:

- A. To evaluate and compare the effect(s) of each of the four doses of NIR-TLT on depressive symptomatology: antidepressant effect, in patients with major depressive disorder (MDD).

Secondary:

- B. Compare safety and tolerability at each dose of NIR-TLT.
- C. Compare the effect(s) of each of the four doses of NIR- TLT on brain electrophysiology.

Exploratory:

- D. To describe the findings visualized in PET, related to the brain metabolism of the participants, before and after receiving the intervention under study.

Study Design:

Multi-center, randomized, sham, double-blind.

This trial will randomize 112 subjects into 4 equal groups: bilateral continuous wave (BCW), bilateral pulsed wave (BPW 1-2) and SHAM. All subjects, investigators (except the study statistician) and outcome assessors will be blinded to group assignments. The device operator will be aware of group assignments.

All subjects will receive 3 NIR-TLT sessions per week for 6 weeks. A follow-up evaluation will be performed 2 weeks after the end of the study. The treatment will follow these specifications: wavelength 830 nm; average irradiance 54.8 mW / cm2; average fluence of 65.8 J / cm2 (according to the parameters used in the ELATED-2 study at MGH with the Omnilux New U device), being the total fluence delivered: 3.6 kJ (bilateral) or 1.8 kJ (unilateral), either on the subject's forehead, at or near the electroencephalography (EEG) sites F3 and F4.

All NIR-TLT sessions will be 429 seconds (7.15 minutes), regardless of group assignment.

Study population:

Patients eligible to participate in the study will be diagnosed with MDD by DSM criteria (MINI). Subjects will be between 18 and 75 years of age (not yet 76 years of age) on the date of the examination.

Duration of participation:

Research subjects will remain in the study for 8 weeks.
(6-week treatment period, plus 2-week follow-up). The total duration of the study will be 24 months.

Criteria for valuation:

- **Main:** Improvement of depressive symptoms from each of the four doses of NIR-TLT. Assessed using HAMD-17, at 3 time points (visit 1, visit 9 and visit 18).

- **Secondary:** Safety and tolerability. Assessed by SAFTEE- SI (self-assessed), and collection of serious adverse events throughout the study. Safety data will be used to assess the tolerability of each of the NIR-TLT doses.
  Changes in brain electrophysiology (electrical activity): Commercially available EEG devices will be used to collect data on brain electrical activity. At 3 time points (visit 1, visit 9 and visit 18).

- Changes in measurements of MDD symptom severity assessment scales:

  - Doctor Administered Scales: QIDS-C and CGI-S
  - Patient-reported scales: ASQ and SDQ

Entry Criteria:

Inclusion Criteria

1. The age of the subjects in the examination will be between 18 and 75 years (inclusive).
2. Diagnosis of major depressive disorder (MINI).
3. QIDS-C≥12 in the selection
4. CGI-S ≥4 or greater, i.e., "moderately depressed".
5. Women of childbearing age should use a double-barrier method of birth control (e.g., condoms plus spermicides) if they are sexually active.
6. Subject's informed consent obtained in writing in accordance with local regulations prior to enrollment in this study.
7. The subject is willing to participate in this study for at least 12 weeks.
8. Subjects should receive stable doses of antidepressants (if taking any) afor at least six weeks prior to enrollment.

Exclusion Criteria:

1. Decrease in SDQ self-reporting from screening to initiation. >=30%, calculated as [(SDQselection-88) - (SDQinitial-88) / (SDQselection-88)] >=30/100. A score of 88 is "normal" in SDQ.
2. Subject is pregnant or breastfeeding.

3. Subject failed more than 2 adequate FDA-approved antidepressant treatments during the current episode according to ATRQ criteria (less than 50% decrease in depressive symptomatology).
4. Structured psychotherapy focused on treating the subject's depression (i.e., CBT or IPT) is allowed if initiated at least 8 weeks prior to the screening visit.
5. Substance dependence or abuse in the last 3 months.
6. History of a psychotic disorder or psychotic episode (current psychotic episode by MINI assessment).
7. Bipolar affective disorder (according to MINI evaluation).
8. Unstable medical illness, defined as any medical illness that is not well controlled with standard of care medications (e.g., insulin for diabetes mellitus, HCTZ for hypertension).
9. Active suicidal or homicidal ideation (both intent and plan are present), as determined by C-SSRS 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 in close proximity to any of the procedure sites.
11. Subject has an implant of any type in the head (e.g., stent, clipped aneurysm, embolized AVM, implantable shunt - Hakim valve).
12. Any use of light-activated medications (photodynamic therapy) within 14 days prior to study enrollment (in the U.S .Visudin (verteporfin) - for age-related macular degeneration; Aminolevulinic acid - for actinic keratoses; Photofrin (sodium porfimer) - for esophageal cancer, non-small cell lung cancer; Levulan Kerastick (aminolevulinic acid HCI) - for actinic keratoses; 5-aminolevulinic acid (ALA) - for nonmelanoma skin cancer)
13. Recent history of stroke (90 days).
14. Subject had a failed intervention based on an FDA-approved device for the treatment of depression, during the current episode (e.g., less than 50% decrease in depressive symptomatology with TMS, ECT, or VNS).
15. History of dementia, traumatic brain injury (TBI) or other organic neurological disorder.

2. List of Abbreviations

ANCOVA = Analysis of Covariance
ANOVA = Analysis of Variance
ASQ = Anxiety Symptom Questionnaire
ATP = Adenosine Triphosphate
BOLD fMRI = blood-oxygen level dependent functional magnetic resonance imaging
C-SSRS = Columbia Suicide Severity Rating Scale
CFR = Code of Federal Regulations
CGI-I = Clinical Global Impressions: Improvement
CGI-S = Clinical Global Impressions: Severity
CRF= Case Report Form
CW = Continuous Wave
DNA = Deoxyribonucleic acid
DSMB = Data and Safety Monitoring Board
ECT= Electroconvulsive Therapy
ETC= Electron Transport Chain
FDA= Food and Drug Administration
HAM-D-17 = Hamilton Depression Rating Scale
HCTZ = Hydrochlorothiazide
ISO = International Standards Organization
MDD = Major depressive disorder
MINI= Mini-International Neuropsychiatric Interview
NEST 1 = NTS® Effectiveness and Safety Trial - 1
NEST 2 = NTS® Effectiveness and Safety Trial - 2
NIMH = National Institute of Mental Health
NIR-TLT = Near-infrared Transcranial Laser Therapy
OSHA = Occupational Safety & Health Administration
PBM = Photobiomodulation
PET *scan* / PET: Positron Emission Tomography
PI = Principal Investigator at the center
QIDS-CR = Quick Inventory of Depressive Symptomatology: Doctor-rated scale
RA = Research associate
SAE = Serious Adverse Events
SAFTEE-SI = Systematic Assessment for Treatment Emergent Effects
SCID = Structured Clinical Interview for DSM disorders
SQL = Structured Query Language
SSRI = Selective serotonin reuptake inhibitor
t-PBM = Transcranial photobiomodulation
TLT = Transcranial Laser Therapy
TMS = Transcranial Magnetic Stimulation
VNS= Vagus Nerve Stimulation

3. Introduction

Major depressive disorder (MDD) is a prevalent and disabling condition: 16.2% of the U.S. population has MDD at some point in life (Kessler et al., 2003). In the United States, depression is the third leading cause of years lost to disability or death, after cardiovascular disease and lung and breast cancer (Michaud et al., 2006). Despite the availability of effective treatments such as antidepressants and psychotherapy, MDD often remains untreated, with only one-fifth of cases receiving adequate treatment (Kessler et al., 2003). In addition, diagnosis and treatment rates for depression among Blacks and Hispanics are less than half the rates observed for Whites (Sclar et al., 2008). Hispanics and Blacks have far more concerns regarding the effects of medications on quality of life than Whites (Huang et al., 2009). Among Hispanics, the stigma associated with receiving antidepressants is a major concern (Interian et al., 2007). Delayed treatment for depression not only prolongs associated disability, but may result in lower rates of response to antidepressants (de Diego-Adelino et al., 2009).

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

Due to the limitations of drug treatment and talk therapy, new treatment approaches for depression are needed, especially in primary care and in the community, where several minorities are underserved. We propose a new treatment approach for depression based on the use of transcranial laser therapy (TLT). The treatment consists of bilaterally exposing the forebrain to TLT, which can enhance ATP production in depressed subjects.

Major depressive disorder has been associated with deficits in brain bioenergetic metabolism. In an experimental model of depression, chronic stress inhibited the mitochondrial respiratory chain (the cellular site for energy production) (Rezin et al., 2008). Depressed subjects also have significantly lower production of ATP (an energy vector) in their muscle tissue and a higher incidence of deletions in their mitochondrial DNA (Gardner et al., 2003). Magnetic resonance spectroscopy data in subjects with MDD showed that the response to augmentation of a selective serotonin reuptake inhibitor (SSRI) with triiodothyronine (a thyroid hormone) is associated with restoration of ATP levels in the brain (Iosifescu et al., 2008).

Near infrared light is a non-ionizing electromagnetic wave, is invisible, penetrates the skin and skull into brain tissue, is non-invasive (Zhang et al., 2000), dissipates minimally as thermal energy and is absorbed mainly by specific chromophores (Mochizuki-Oda et al., 2002). The benefits of NIR-TLT are wavelength specific. A mitochondrial enzyme, cytochrome c oxidase, is the primary chromophore for NIR-TLT with a wavelength of about 830 nm (Eells et al., 2003). The energy absorbed by cytochrome c oxidase leads to increased production of adenosine triphosphate (ATP) through the respiratory chain. Ultimately, the increase in ATP leads to an increase in the cell's energy metabolism, and presumably also activates a signaling cascade that promotes cell plasticity and cytoprotection. (Eells et al., 2003).

These properties of NIR-TLT have led to new therapeutic applications in neurology. In subjects with acute ischemic stroke, acute treatment with NIR-TLT led to a significantly better outcome compared to sham treatment (Lampl et al., 2007). These results were confirmed in a different cohort of stroke patients with mild to moderate disease severity (Zivin et al., 2009). Both studies in stroke subjects showed no significant difference in the rate of adverse events or serious adverse events between NIR-TLT and sham-treated subjects (Lampl et al., 2007; Zivin et al., 2009). NIR-TLT has also been used as a treatment for alopecia (Leavitt et al., 2009) and in animal models for methanol-induced retinal toxicity (Eells et al., 2003). Near-infrared light is already widely used for noninvasive 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 NIR-TLT. The greatest risk of NIR-TLT when using a laser as a light source is associated with accidental exposure of the retina, when beams are projected through the lens, which increases the risk of macular injury (Kim et al., 2007). In our study, we will have multiple safeguards against this risk [See Section 4.9, Completion].

Preliminary uncontrolled studies in 10 and 4 depressed subjects, respectively, have shown NIR-TLT to be 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 NIR-TLT. Because NIR-TLT is non-ionizing radiation, multiple sessions, likely necessary for the treatment of depression, are expected to be safe.

The advantage of the NIR-TLT treatment approach over pharmacotherapy is that adherence can be easily monitored (since the treatment is administered in the clinic) and the patient is not required to ingest any substances. It is possible that exposure to NIR-TLT is more acceptable than medication use among some minorities. Compared to talk therapy, NIR-TLT has the advantage of not requiring providers with specific cultural expertise or proficiency in a second language. Our study will help determine whether NIR-TLT has an antidepressant effect and is acceptable in minority populations, thus justifying further study and investment.

3.1 Summary of Prior Clinical Experience

LiteCure conducted two studies on the safety and efficacy of NIR-TLT applied to the forehead (prefrontal cortex) twice a week in subjects with MDD. The device used for ELATED-2 is a predicate device for EXPi-laser.

ELATED-1 was a 3-week proof-of-concept study of TLT (EXPi - class IV laser, wavelength 808 nm; irradiance 700 mW / cm2; fluence 84 J / cm2; total energy 2.4 kJ per session for 6 sessions) All subjects (n = 4) were treated with TLT. Mean baseline HAMD-17 scores for depression decreased from 19.8 ± 4.4 (SD) to 13 ± 5.35 (SD) after treatment (p = 0.004) (Cassano et al. 2015). (Cassano et al. 2015).

ELATED-2 was an 8-week double-blind, randomized, controlled study of TLT (Omnilux New U - LED, 823 nm; 36.2 mW / cm2; up to 65.2 J / cm2; vs. sham for 16 sessions). At completion, the mean HAMD-17 for depression decreased 11.7 ± 7.5 points (TLT, n = 9) vs. 5.3 ± 7.0 points (simulation, n = 9) (p = 0.04); the differences were even more pronounced in the completer sample as HAMD-17 decreased 15.7 ± 4.4 (TLT, n = 6) points vs. 6.1 ± 7.9 points (simulation, n = 7) (p = 0.01).(Cassano et al., 2018).

3.2 Overview of LiteCure Laser System

The EXPi device is a research device based on LiteCure's EXPi Deep Tissue Laser Therapy™ system. For this research study, the EXPi system's beam delivery, Empower ™, is modified to deliver NIR laser energy non-invasively to the brains of subjects diagnosed with neuropsychiatric disorders. Other than the cap, the EXPi device is the same device as LiteCure's EXPi system: model LTS-2500, which is marketed under FDA 510 (K) # K173067. The device is manufactured and supplied by LiteCure LLC, 101 Lukens Drive, Suite A, New Castle, DE 19702.

The EXPi device is a Class II medical device according to 21 CFR 890.5500 and 878.4810 and is manufactured according to 21 CFR 820. It uses laser diode sources that emit in the near infrared (808 +/- 10 nm) with a maximum continuous output of ≤ 30 watts. The device consists of two main assemblies: a console and an optical delivery system. Laser power is generated and controlled within the console assembly and delivered to the optical delivery system: custom helmet (cap), via a flexible double-clad optical fiber. The cap is configured to produce a 4 cm diameter (12 cm2) beam of light at each of two sites on the subject's forehead: adjustable at/near EEG locations F3 and F4.

The console is programmed to produce laser energy in one of 4 modes:

1. Mode 1 (BCW group), NIR-TLT dose:
   i. Treatment site(s): EEG F3 and F4
   ii. Time format:
   continuous wave
   iii. Average radiance: 350 mW / cm2
   iv. Exposure time: 429 sec.
   v. Total fluidity delivered: 3.6 kJ (1.8 kJ per treatment location)

2. Mode 2 (BPW-1 group), NIR-TLT dose:
   i. Treatment site(s): EEG F3 and F4
   ii. Time format: wave
   pulsed, 10 Hz; 50% duty cycle
   iii. Average radiance: 350 mW / cm2
   iv. Exposure time: 429 sec.
   v. Total fluidity delivered: 3.6 kJ (1.8 kJ per treatment location)

3. Mode 3 (BPW-2 group), NIR-TLT dose:
   i. Treatment site(s): EEG F3 and F4
   ii. Time format: pulsed wave, 40-50 Hz; 50% duty cycle
   iii. Average radiance: 350 mW / cm2

iv. Exposure time: 429 sec.
v. Total fluidity delivered: 3.6 kJ (1.8 kJ per treatment location)

4. Mode 4 (SHAM group), NIR-TLT dose:
i. Treatment site(s): none
ii. Time format: none
iii. Average radiance: 0 mW / cm2
iv. Exposure time: 429 sec.
v. Total fluidity delivered: 0 kJ

See section 4.3 - Dosing rationale

The console display does not provide information on the dose irradiance, showing only the mode number, e.g. Mode 1 and, although the laser exposure varies with the treatment mode, to maintain blinding, the treatment time will be the same in all modes: 429 seconds (7.15 minutes). In addition, the behavior of the device, the performance/output of all visible and audible indicators, including the graphical user interface, is identical in all modes, differing only with respect to treatment irradiance. Since the laser radiation emitted during the NIR mode is invisible to the naked eye, the modes cannot be distinguished from each other, which keeps the operator and subject blinded to the group tasks.

Dose administration for the present trial will follow the specifications: wavelength 830 nm; mean irradiance 54.8 mW / cm2; mean fluence 65.8 J / cm2 (according to the parameters used in the ELATED-2 study in MGH with the Omnilux New U device), which demonstrated in terms of improvement of depressive symptomatology evaluated with HAMD17 in patients with major depression. Therefore, by virtue of the ELATED2 findings, doses were considered for this intervention in patients with MDD.

3.3 Overview of Positron Emission Tomography (PET scan)

The main metabolite used by the brain to obtain ATP in mitochondrial oxidative processes is glucose, although this organ constitutes 2% of the body weight, it is known to use 20-25% of the total circulating glucose. We consider that, therefore, it becomes the ideal vehicle to determine metabolic rates in the different regions of the brain.

Positron emission tomography (PET) is a nuclear medicine study that provides maps indicating metabolic activity, especially glucose metabolism in different tissues and lesions (Ropper et al. 2008), using a radiopharmaceutical that is the union of a drug or physiological substance with known pharmacokinetics and pharmacodynamics with a positron-emitting radioactive atom. On the one hand, the drug has a known biodistribution and, on the other hand, the positron-emitting atom indicates the location of this drug after tracking in a PET camera. Because the PET system lacks an anatomical reference to determine the exact location of these abnormalities, PET is currently fused to a computed tomography (CT) system (Roldán-Valadez et al. 2008). The most frequently used radiopharmaceutical is fluorodeoxyglucose, which is 18F-labeled glucose, known by the acronym 18FDG.

The use of positron emission tomography/computed tomography with 18F-fluorodeoxyglucose

(PET/CT with 18F-FDG) as a means of determining brain metabolism is proposed, not only globally but regionally. This tool has already been used in different neuropsychiatric conditions, such as: cognitive impairment, dementia and Parkinson's disease, as well as in patients with affective disorders, such as major depressive disorders (Lyra et al. 2020; Sacher et al. 2012). There is evidence that depression is associated with deficits in brain metabolism.

There is a growing consensus that there are a number of brain areas that are involved in depression. PET shows increased glucose metabolism in the anterior cingulate area, specifically in the right pregenual and subgenual regions (Sacher et al. 2012).

The anterior cingulate cortex has a crucial role in initiation, motivation and goal-directed behavior, thus playing a key role in depressive disorders (Alexopoulos et al. 2008), changes in these areas, as determined by PET CT is even useful to identify eventual responders after starting a treatment (De Crescenzo et al. 2016).

4. Research Plan

4.1 Study Aims

Primary Aim:

A. To evaluate and compare the effect(s) of each of the four doses of NIR-TLT on depressive symptomatology: antidepressant effect, in patients with major depressive disorder (MDD).

Hypothesis 1: Total HAM-D 17 scale scores will vary in a NIR-TLT dose-dependent manner, with BPW dose being more effective.

We also expect to be able to estimate the effect size of the antidepressant action as a function of the irradiance applied to the NIR-TLT skin, with the BPW dose being more effective.

Secondary Aims:

B. Compare safety and tolerability at each dose of NIR-TLT.

Hypothesis 2: The doses of NIR-TLT tested in this study will be safe and well tolerated by patients with MDD, as assessed by SAFTEE-SI and reports of serious adverse events. We do not anticipate dose-dependent differences in reported side effects.

C. To compare the effect(s) of each of the four doses of NIR-TLT on brain electrophysiology.

Exploratory Aim:

D. To describe the findings visualized in PET, related to the brain metabolism of the participants, before and at the end of the intervention under study.

4.2 Rationale for the use of the EXPi device:

Transcranial photobiomodulation (t-PBM) with near infrared (NIR) laser therapy has emerged as a possible antidepressant treatment in both animal models (Mohammed et al.2016; Salehpour 2016-2017; Xu et al.2017;Wu et al.2012), and in human studies (Cassano 2015- 2017; Caldieraro et al.2018) t-PBM. NIR appears to increase brain metabolism (by activating the cytochrome C oxidase in the mitochondria), to increase neuroplasticity and to modulate the

endogenous opioids, while decreasing inflammation and oxidative stress. (Chung et. al. 2012; Yu et. al.1997; Oron et al. 2007, Mochizuki et al. 2002) t-PBM penetrates deep into the cerebral cortex, modulates cortical excitability, and improves cerebral perfusion and oxygenation. The safety of t-PBM has been studied in a sample of 1410 acute stroke patients, with no significant difference in adverse event rates between t-PBM and sham exposure. (Hackee tal.2014; Lamp et al.2007, Zivin et al.2009) Uncontrolled studies suggest an antidepressant effect of t-PBM in subjects suffering from MDD (Cassano 2015-2017; Caldieraro et al.2018).

Different devices have been used in the study of the effects of near infrared radiation (NIR) treatment in humans. We decided to use a Litecure device specifically adapted for this study to allow NIR-TLT testing of different doses in a controlled manner.

4.3 Dosing Rationale

The laser dose to be used in the present clinical trial is derived from previous clinical experience with the use of other versions of the medical device in studies conducted in patients with acute stroke, major depressive disorder and Alzheimer's disease in whom lasers have been administered with this device, which has provided guidance on safe and potentially effective doses in the study population. A summary of this information is presented in Table 1.

Laser devices have a potential risk of retinal injury when used incorrectly; this is mitigated by the use of protective eye equipment "goggles" and proper procedures to ensure eye safety (Barkana & Belkin, 2000).

No serious adverse events were found in our literature review (Caldieraro & Cassano, 2019). Two open-label studies using 1 and 6 sessions of t-PBM reported no treatment emergent side effects (Cassano et al., 2015; Schiffer et al., 2009).

The safety of a t-PBM session was evaluated in three large RCTs with a combined sample of 1410 stroke subjects (Hacke et al., 2014; Huisa et al., 2013; Lampl et al., 2007). No significant differences in the rate of adverse effects were observed between the group receiving NIR laser (808 nm; 5W, 700 mW / cm2) or sham.

A clinical trial with 16 sessions of t-PBM reported a higher number of mild side effects in the active treatment group, the most frequent being insomnia, "seeing bright colors," "an ashtray taste," and irritable mood (Cassano et al., 2018, p. 2). Other potential risks are related to improper administration and will be mitigated by safety procedures, such as the use of protective eyewear.

The risk of thermal injury from PBM administered with the parameters used in the studies we reviewed is considered minimal and limited to the skin. In ten people treated by TBI with 10-15 W lasers, a much higher power than that used in t-PBM, skin temperature increased to no more than 30°C with rapid cooling after removal of the NIR light. Clinically, patients reported slight skin heating, but no discomfort (Morries et al., 2015).

	PUBLISHED					NOT PUBLISHED			PRESENT
	NEST	NEST-2	NEST-3	ELATED	ELATED-2	ELATED-3	TRIADE	TRAP-AD	"ELATED-4"
TITLE	Infrared laser therapy for ischemic stroke: a new treatment strategy: results of the NeuroThera Effectiveness and Safety Trial-1 (NEST-1)	Effectiveness and Safety Trial of a New Ischemic Stroke Treatment Within 24 Hours From Stroke Onset (NEST-2)	Efficacy and Safety Trial of Transcranial Laser Therapy Within 24 Hours From Stroke Onset (NEST-3)	Transcranial Laser Therapy for Major Depressive Disorder	Transcranial Photobiomodulation for the Treatment of Major Depressive Disorder. The ELATED-2 Pilot Trial	Transcranial Laser Therapy, Continuous and Pulsed Light, for Major Depressive Disorder (ELATED-3)	Transcranial Near Infrared Radiation and Cerebral Blood Flow in Depression	Transcranial Photobiomodulation for Alzheimer's Disease (TRAP-AD)	Near-infrared Transcranial Laser Therapy in Subjects with Major Depressive Disorder: A study of Dosing with Laser
LOCATION	GLOBAL	GLOBAL	GLOBAL	MGH/Hardvard	MGH/Hardvard	MGH/Hardvard	MGH/Hardvard NYU NKI	MGH/Hardvard NYU NKI	LIMA
INDICATIÓN	ACUTE STROKE	ACUTE STROKE	ACUTE STROKE	MAJOR DEPRESSIVE EPISODE	MAJOR DEPRESSIVE EPISODE	MAJOR DEPRESSIVE EPISODE	MAJOR DEPRESSIVE EPISODE	ALZHEIMER'S DISEASE	MAJOR DEPRESSIVE EPISODE
Effectiveness	YES	NO	NO	YES	YES	NO	ONGOING	ONGOING	ONGOING
SECURITY	YES (NO SAE)	YES (NO SAE)	YES (NO SAE)	YES (NO SAE)	YES (NO SAE)	YES (NO SAE)	ONGOING	ONGOING	ONGOING
IRRADIANCE (mW/cm^2)	700	700	700	700	~50	~50	Multiple groups Max 770	Multiple groups Max 300	Multiple groups Max 350
EXPOSURE TIME (min)	2	2	2	2	25	25	~6	~11	7
NUMBER OF SITES	20	20	20	4	2	2	2	2	2

Table 1 - Summary of doses and efficacy and safety results in previous studies. Source: Lampl et al. (2007), Huisa et al. (2013), Zivin et al. (2014), Cassano (2017), Cassano et al. (2018), Cassano (2016), NIMH (2021), NYU (2021).

4.4 Study Design

This study is a multicenter, randomized, sham-controlled, double-blind study on the use of transcranial continuous light therapy as a treatment for depressive symptoms in patients with major depressive disorder (MDD). Laser light therapy is tested in a strict parallel design.

This study will include 112 subjects randomly assigned to each of the four NIR-TLT treatment groups (28 subjects/group).

All subjects will receive three NIR-TLT treatments per week for 6 weeks and will complete a follow-up evaluation two weeks after treatment completion. All subjects and investigators (except the study statistician) will be blinded to treatment sequence assignment.

Energy will be administered at a wavelength of 808 nm, bilaterally to two frontal areas on the left and right side of the forehead, at or near electroencephalography sites F3 and F4. To maintain blinding, the treatment duration will be 429 seconds (7.15 minutes) for all groups. The treatment exposure (time during which the laser is active for the duration of the treatment) will be specific for each group, controlled by the device to normalize the treatment fluence of each active session per treatment location. Treatment will follow these specifications: wavelength 830 nm; average irradiance 350 mW / cm2; average fluence of 65.8 J / cm2 (according to the parameters used in the ELATED-2 study at MGH with the Omnilux New U device) being the total fluence delivered: 3.6 kJ (bilateral) or 1.8 kJ (unilateral), either on the subject's forehead, at or near electroencephalography (EEG) sites F3 and F4.

The entire session (which includes the 429-second treatment and all procedures described in the "Study Visits" section) is estimated to take 30 minutes or less (Schiffer et al., 2009) (Lampl et al., 2007, Zivin et al., 2009). Additional time is needed to prepare the subject, fit the necessary protections (e.g., goggles), inspect the subject's skin, set up the NIR-TLT devices, and give the subject time to rest after irradiation. NIR-TLT treatment will only be administered by trained personnel who are on study staff. All personnel administering the treatment must pass laser safety training created by the American Institute for Medical Laser Applications and approved by the relevant Committees. The duration of irradiation will be decreased if clinically indicated, based on tolerability as needed.

4.4.1 Randomization procedure

At screening, participants will be assigned an identification number on a first-come, first-served basis. When it is confirmed that the participant is eligible for the clinical trial, he/she will be randomized.

The assignment of each participant to a particular treatment arm (experimental and control group will be performed in a 1:1:1:1 ratio) will be done by using an electronic system, which is centrally administered.

For each participant at each visit, the investigator or center coordinator will communicate with the central statistician and, through a user and password assigned for the center, will enter the web system where he/she will be able to request and obtain the corresponding treatment number assigned to him/her in the study, and then proceed with the laser application. The participants and the study investigator will be blinded to the treatment assignment. The screen of the medical device does not show information of the dose applied to each participant, only the group to which he/she belongs will be referenced, keeping the dosing details confidential.

At central level, the unblinded statistician (responsible for administering this web system) will know which treatment arm corresponds to each participant from each research center.

4.4.2 Blinding procedure

This study is a clinical trial where blinding of both the investigator and the study participant will be assured. Only the treatment identifiers, the central statistician of the study, will know the treatment identifiers.

The sponsor is responsible for providing the medical device under study, programming and maintenance of the device.

The investigator at each center will be responsible for providing the corresponding dose according to the intervention under study (treatment assigned by the statistician through the web system) to each study participant.

Simulation blinding will be effective because the radiation is not visible and because no dose from the NIR-TLT will produce any perceptible difference from the simulation patient, e.g., skin heating.

4.4.3 Contingency plan for opening of the blind

Only in the event of an emergency or serious adverse event or other particular event that alters the risk-benefit profile of the intervention will the principal investigator determine whether unblinding of a participant's study intervention assignment is warranted. Participant safety should always be the first consideration in making such a determination.

If the investigator decides that unblinding is warranted, every effort should be made to communicate this decision by telephone or mail to the sponsor or medical monitor before unblinding a participant's treatment assignment, unless this would delay the management of unblinding of the participant, as approval from either is necessary to communicate with the unblinded statistician responsible for the administration of the randomization web system, who has access to the arm assignment lists for each participant, to forward this information to the requesting site.

The date and reason for breaking the blind should be recorded in the source documents and in the FRCe by the investigator at the research site.

If the treatment assignment blind is opened, this information should only be shared with the other investigating doctors at the center on a need-to-know basis (especially when the cause is a safety event consistent with a serious adverse reaction). Treatment assignment information should not be shared with others, unless decided by of thepat rocinator or mandated by the regulatory authority.

4.5 Study Population

4.5.1 Population Description

The subject population for this study will consist of 112 randomized subjects between the ages of 18 and 75 years, of any ethnicity and diagnosed with MDD. The subject will meet all inclusion criteria, will have none of the exclusion criteria, and will provide written informed consent to participate in this clinical trial. Any subject who signs an informed consent will be considered enrolled in the study, although they will not be able to participate if they are not eligible for the study. In the event that a subject's ability to understand and communicate is compromised (as assessed by the investigator), local regulations regarding informed consent signatures must be followed.

Subjects will be recruited, through selections, advertisements and publications. Study participants will also be recruited through advertisements on the Internet, study center websites, etc. According to the strategies proposed by each researcher.

Participants who contact the respective study site staff in response to any of the announcements will complete the telephone screening to determine tentative eligibility for the study.

4.5.2 Use in Female Subjects of Childbearing Potential

Women of childbearing age must consent (without any element of coercion) to use a double barrier method of birth control (e.g., condoms with spermicide) if they are sexually active. A pregnancy test will be performed at the screening visit. The sponsor will provide free access to any contraceptive method of the participant's choice.

4.5.3 Criteria for Enrollment - see inclusion and exclusion criteria section.

4.6 Subject Numbering

Subjects enrolled in the ELATED-4 study will be identified by subject numbers 01 through 150 (to account for selection failures and drop-out prior to randomization).

4.7 Sample Size

The study is expected to include 112 subjects with analyzable data (at least 3 sessions - 1 week, after randomization).

4.8 Subject Confidentiality

Any information and data provided to Pthera LLC or its designees in reference to any subject's participation in this research shall be considered confidential. The investigator shall ensure that the anonymity of all subjects is maintained in all documentation submitted to Pthera LLC by completely removing each subject's name and/or other identifying information. Identifying information will be replaced with the study number and subject's initials. The research site should not provide Pthera LLC with information such as subject phone numbers, home address, personal identification numbers such as passport numbers, etc. Site research personnel should be careful when communicating with Pthera LLC representatives by telephone or electronic correspondence not to provide information that could reveal a subject's identity.

Documents associated with the study that are not intended to be sent to Pthera LLC (e.g., signed informed consent forms) must be held in strict confidence by the investigator. Only study site personnel, authorized Pthera LLC personnel, and regulatory authority inspectors will have access to these confidential files.

4.9 Study Visits

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

4.9.1 Selection Visit - Week 0

Purpose
The purpose of the screening visit will be to determine the eligibility of patients to participate in the study.

Procedures
Study personnel will consent subjects to the study, perform a psychiatric evaluation, and screen for the presence of MDD using the MINI, QIDS-C, and CGI-S scales.

Laboratory tests (including urine collection for urine biochemical analysis and urine toxin analysis (also called Urine Drug Screen), urine pregnancy test, and a complete physical examination including vital signs and weight will be performed.

Approximately 15 cc ± 5 cc (15 ml) of blood will be drawn for laboratory safety testing. To confirm that a patient is healthy enough to participate, a complete blood work-up with differentials, thyroid stimulating hormone test, blood biochemistry (glucose, calcium, sodium, potassium, carbon dioxide, chlorine, albumin, total protein, alkaline phosphatase, transaminases, total and fractionated bilirubins, urea, creatinine) and a high sensitivity C-reactive protein test will be performed. A PET scan will be performed on patients within the study subgroup to meet the exploratory objective.

Once all tests and procedures are available for review, study personnel will determine subject eligibility based on all inclusion and exclusion criteria.

Subjects who report having taken antidepressants at the screening examination may choose to continue medication during the study or taper and discontinue therapy prior to the baseline visit (under the supervision of a treating doctor). However, subjects who have discontinued more than two antidepressants in the current episode of depression will be excluded.

4.9.2 Treatment Visits 1 to 18 -Visits 1-6

Regular treatment visits (1 to 18) occur on non-consecutive days, 3 times per week for 6 weeks.

Purpose: The purpose of the treatment visits is to administer NIR-TLT and collect data to assess the effect of each of the NIR-TLT dose effects on MDD symptomatology (antidepressant effect) and tolerability.

Procedures: Trained study personnel will deliver NIR-TLT to study participants. Treatment visits are estimated to last approximately 429 seconds (7.15 minutes) and will take place in a dedicated NIR-TLT (laser safety) office. Only the subject and the staff administering NIR-TLT will be present during the session. The subject will lie comfortably on an examination bed or sit in a recliner, a cervical pillow (U-shaped) may be used for comfort and to prevent the helmet connector cables from being stepped on, bent or damaged. The NIR-TLT application sites (left and right forehead) will be inspected for skin lesions (e.g., lacerations or signs of inflammation) that might contraindicate treatment. During treatment sessions, a warning sign will be hung on the office door. Ideally the place where treatment is administered should be at a temperature of 25°C +/- 2°C.

Prior to treatment with the EXPi device, the subject's forehead is cleaned with 70% isopropyl alcohol, the cap is placed on the subject's head and secured, and the treatment beam locations are adjusted to match the F3 and F4 EEG sites (or very close if the site is covered by hair), the eyebrows may be used as a reference for placement of the lower edge of the helmet. The operator and subject are provided with appropriate laser safety glasses (optical density rating> 5.0 at 808 nm). Disposable caps may be used for hair control if necessary.
Trained personnel administering NIR-TLT will make every effort to never shine light into or near the subject's eyes. Patients and staff will wear safety glasses selected for the biophysical properties of NIR-TLT in accordance with Occupational Safety and Health Administration (OSHA) guidelines. The patient may sleep during the time of therapy administration.

The NIR-TLT delivery is expected to last no longer than 7.15 minutes (429 seconds) in total (simultaneous application to the left and right forehead). The subject will be allowed to rest for 5 minutes after NIR-TLT delivery. The skin at the application sites will be inspected again after laser application. A log will be kept with the dates of delivery of the NIR-TLT.

treatment per study subject. The use of cell phones, tablets, computers or electronic devices will not be allowed during laser administration.

During treatment visits and prior to NIR-TLT, study personnel will collect tolerability (safety) and efficacy data:

1. Safety data: SAFTEE-SI (self-assessed), C-SSRS, adverse events and concomitant medication form.
2. Efficacy data - HAMD-17.

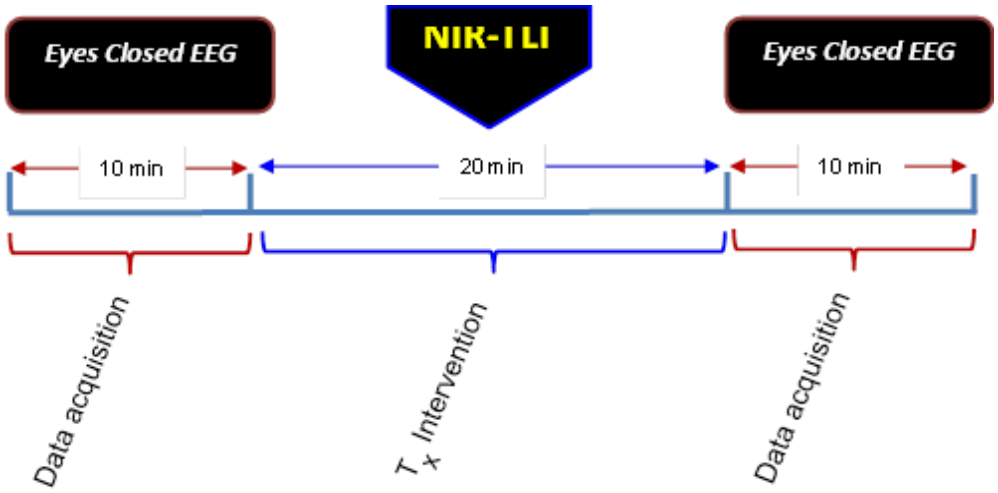
In addition to the evaluation described above, during visits 1, 9 and 18:

Prior to the NIR-TLT study, the staff will collect primary efficacy data from metric measurements and brain electrophysiology data:

1. Efficiency data:

   a. Doctor-administered scales (1): HAMD-17, QIDS-C and CGI-S.
   b. Patient-Reported Scales (1): ASQ and SDQ

2. Brain Electrophysiology Data - Study personnel will conduct a first 10-minute EEG recording session, treat the patient according to their NIR-TLT group randomization, and then conduct a second 10-minute EEG recording session as soon as possible after the end of treatment, but within 15 minutes.



3. Exploratory PET scan data: A PET scan will be performed on the participants selected to be part of the research subgroup, before the first treatment visit and within 24 hours of the end of the last intervention (visit 18).

4.9.3 Follow-up visit (Post Treatments) - Week 8

The next visit will occur two weeks after the last cerebral blood flow impact visit.

Purpose: The purpose of the follow-up visit is to evaluate the long-term antidepressant effect of NIR-TLT.

Procedures: During the follow-up study, staff will collect tolerability (safety) and efficacy data:

1. Safety data: SAFTEE-SI (self-assessed), adverse events and concomitant medication form.
2. Efficiency data:

    a. Doctor-administered scales (1): HAMD-17, QIDS-C and CGI.
    b. Patient-reported scales (1): SDQ and ASQ.

4.9.4 Completion

If, during the study, a subject's CGI-S increases more than 2 points or rises above a score of 5, or if they become actively suicidal according to the C-SSRS scale, the subject will be referred to their treating doctor and advised to start or switch to another FDA-approved antidepressant medication. Such subjects may or may not be terminated from the study, depending on the severity of symptoms, at the clinical judgment of the principal investigator. Subjects who are considered actively suicidal and in imminent danger will be terminated from the study and referred to the appropriate local MGH or NKI emergency room for evaluation and subsequent hospitalization, or, if the subject is appropriate for outpatient monitoring, will be referred to their treating doctor for frequent appointments and follow-up. Each subject will have the investigator's contact information, as well as instructions on how to call emergency services, if necessary. We will encourage subjects to continue enrollment in the study through follow-up visits, regardless of discontinuation of NIR-TLT.

4.10 Scales & Forms

We will offer clinical interviews and study forms in Spanish. All scales will explore the last 7 days, except M.I.N.I (lifetime), TSRQ (study duration).

- **(M.I.N.I.)** - The M.I.N.I. is a brief 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 in the Division of Clinical and Treatment Research at the National Institute of Mental Health (NIMH), 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). Validated in Spanish (Ferrando L. et. al, 1998).
- **Systematic Assessment for the Treatment of 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 we plan to use is the same

used by the NIMH-sponsored CO-MED multicenter trial, and systematically examines all potential treatment-emergent side effects and discusses specific adverse symptoms, including suicidal thoughts and behaviors, and self-injurious behavior.

- **Hamilton Depression Rating Scale - 17 items (HAM-D-17;** Hamilton, 1960**)**
  - The doctor completes this instrument based on his or her assessment of the patient's depressive symptoms, using a structured interview and defined anchor items. The goal of the HAM-D is 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 in the past 7 days. There are several different versions of the HAM-D; they differ only in the number of questions included. The standard form generally used in research studies is the 17-item Hamilton D (HAM-D-17). Responses to the questions are scored on a scale of 0-4 or 0-2, with higher scores indicating more severe pathology. Scores on the HAM-D-17 generally 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 or more. Validated in Spanish by Ramos-Brieva (1986).
- **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. Validated in Spanish by Planas et al. (2014).
- **Clinical Global Impressions: Severity and Improvement (CGI-S, CGI-I) -** The clinician scores these two instruments from 1 to 7 based on the assessment of the subject's overall clinical status. Measured based on history and scores on other instruments: (a) depressive severity (CGI-S) and (b) clinical improvement (CGI-I). Validated in Spanish by García-Portilla (2011).
- **Anxiety Symptom Questionnaire (ASQ) -** This is a 17-item self-report questionnaire, used in Spanish, that measures the frequency and intensity of 17 anxiety symptoms, including nervousness, worry, irritability, trouble relaxing, insomnia, lack of energy, difficulty concentrating, somatic symptoms, and impaired functioning due to anxiety.
- **Symptoms of Depression Questionnaire (SDQ)** - This is a comprehensive measure of depression that includes assessment of symptoms on the anxiety-depression spectrum. It assesses irritability, anger outbursts, and anxiety symptoms along with symptoms commonly considered to be symptoms of depression. Factor structure analysis of the SDQ identified 5 subscales, including one on the anxiety-depression spectrum, with adequate internal consistency and concurrent validity in Spanish (Pedrelli et al., 2014).
- **Columbia Suicide Severity Rating Scale (C-SSRS)** For suicidal ideation and behavior: an FDA-endorsed instrument for clinical trials. This instrument systematically tracks suicidal ideation and behavior (e.g., suicide attempts, desire to die, suicidal thoughts, plan and intent) (Posner et al. 2007) Validated in Spanish by Al-Halabí et al.(2016).
- **Adverse Event Form-** The adverse event form captures any adverse events (serious or otherwise) specifically related to the application of TLT. This form will help determine if the side effects of TLT are too great for a participant to continue in the study.
- **Concomitant Medication Form -** This form will be completed at each TLT study visit, including the screening visit, as a safety monitoring tool.

4.11 Data Collection Forms

Study investigators, subjects, and outcome assessors will be blinded to the random assignment of treatment

Several procedures are in place to ensure data integrity and protocol compliance. We will use HIPAA-compliant electronic research data capture to support direct data entry by patients and study personnel. Web-based surveys will be based on a study-specific data dictionary defined by members of the research team with planning assistance from Pthera LLC. A research associate (RA) will oversee the automated export of study data from the web-based surveys to a relational study database in structured query language (SQL), allowing for systematic data query and verification.

Participants will complete the physical self-report measures, which will then be transcribed into the data collection form by the person responsible for data entry on the research team. For doctor-administered measures, all doctors will enter responses directly into the database.

To minimize missing data from the self-report forms, we will program unanswered question warnings into the FCRe that will alert participants in real time if they inadvertently skip a question. Then, participants can go back and answer the missed questions or, if they intentionally skipped questions, they can ignore the warning message and continue answering the remaining questions. We will also program real-time range checks in FCRe 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 visit date. By recording study data in this manner, the information can be considered "de-identified" and, therefore, complies with the Health Insurance Portability and Accountability Act of 1996 (HIPAA) Privacy Standards for Individually Identifiable Health Information ("Privacy Rule"). Any information transmitted electronically will be fully encrypted and password protected. Subjects' names will not be entered into the database; each will be identified only by an identification number. Consent forms, any printed PHI, and any study measures completed on paper will be stored and filed in locked office cabinets.

4.12 Data Management

The study biostatisticians will oversee the management of the study database. The principal investigators, Dr. TBD (PI) is ultimately responsible for the quality of data collection and the overall conduct of the study, and directly supervises the study coordinators and data management staff. Data management will include doctor- and patient-rated assessments (see assessments), screening data, fidelity data, visit compliance data, and rater reliability data and safety reports. Weekly database reports will monitor enrollment, completion, attrition, dropout, and individual subject progress, as well as completion of critical assessments. Additional reports will be done as needed to monitor baseline characteristics, protocol adherence, and other issues of interest.

All measurements that are completed on paper will be entered by the RA into the database, and a two-step verification system will be used to minimize data entry errors. All records in the database have a form completion status that can be Incomplete (appears as a red circle in the Record Status Panel), Not Verified (yellow circle), or Verified. The RA initially entering the data will save each record entered as Unverified (at which point it will appear as a yellow circle in the Record Status Panel). Then, a second or the same RA at each center will go to each unverified record, compare each entered value to the paper source document, make corrections, and then re-save the record as verified. The unverified and verified identifiers will be unambiguous and easy to evaluate.

4.13 Training

Study-trained doctors who are unaware of treatment randomization will administer diagnostic assessments and rating scales. All raters will be experienced doctors who will have received criteria-specific training in the use of the study measures. Any new evaluators will receive training. Periodically, all study doctors will evaluate an audiotape during a staff meeting. These duplicate evaluations will be used both for calculating kappa coefficients and for monitoring. Differences between raters will be discussed during monitoring to identify reasons for disagreement and improve inter-rater reliability. These procedures will help us ensure that study doctors refine their diagnostic skills and will also establish common guidelines for continued use in diagnostic decision making. Inter-rater agreement will be assessed by evaluating 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 doctors will be retrained.

4.14 Compensation

Participants will receive 15.00 soles for each study visit (total expected visits = 20 for those who completed the study, including screening and follow-up visits, maximum total compensation per patient is 300.00 soles).

The sponsor will guarantee transportation, in coordination with the participant and members of the research team, from the research center to the PET scan center and back to the starting point. This applies to participants who are included in the exploratory target research subgroup.

The amount indicated does cover travel and transportation expenses, but should these costs exceed this amount, and in order to protect the rights of the participants, the amount will be reimbursed taking into account the personal and specific considerations of each of these expenses.

4.15 Accountability / Investigational Product Control

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

Principal Investigators or designated study site personnel verifying receipt of devices/device accessories must complete the Device/Accessory Acknowledgement Form and fax a copy to Pthera LLC. Device accountability and acknowledgement records will be maintained at each study site. These records will list all equipment received, date received, serial number of each device. Study site personnel will initiate the log each time the device is used. The use of tPBM-2.0 will also be recorded in the appropriate CRF.

Malfunctioning devices and device accessories, including all components, will be returned to Pthera LLC for investigation at Pthera LLC's expense.

4.16 Publishing

Once the preliminary results for the country and globally are available, they will be communicated to each of the principal investigators.
The principal investigators are responsible for informing each of the subjects enrolled in the study (both those who completed the study and those who were discontinued) of the results of the study. Likewise, the ethics committee and the national health institute (INS) will be informed.
The sponsor agrees to publish the results at the end of the study, whether the results are negative or positive. If authorized by the sponsor, this information may also be published by third parties.

4.17 Clinical Adverse Events

4.17.1 Overview and Definitions

All adverse events will be recorded from the time of Informed Consent until completion or completion of the study. The adverse event CRF must be completed and submitted to the IRB, FDA and Pthera LLC as required. Adverse event management regulations and reporting contained in the FDA and ICH Guidelines will be adhered to.

Consideration of adverse events will hereinafter consist of adverse events, serious adverse events, and adverse device effects, including anticipated and unanticipated adverse device effects.

- **Adverse event** is defined as any unfavorable/undesirable clinical event in a clinical investigation of a subject using a device and/or product that does not necessarily have a causal relationship with this treatment. Therefore, an adverse event can be any unfavorable and/or unintended sign, symptom or disease temporally associated with the use of a product or device, whether or not considered related to it. Only abnormal

laboratory values that the investigator considers clinically significant will be classified as adverse events.

- **Serious Adverse Event** is defined as any adverse/undesirable experience that results in any of the following outcomes: 1) death; 2) a life-threatening adverse experience; 3) hospitalization or prolongation of existing hospitalization; 4) a permanent/persistent or significant disability/incapacity, or congenital anomaly/birth defect; 5) major medical events that may not be life-threatening, life-threatening, or require hospitalization may be considered a serious adverse experience when, based on appropriate medical judgment, they may endanger the subject and may require medical or surgical intervention to prevent any 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** are defined as any serious adverse health or safety effect or any life- or death-threatening problem caused by or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of occurrence in the research or implementation plan (including a supplemental plan or implementation), or any other serious unanticipated problem associated with a device that relates to the rights, safety, or welfare of subjects.

4.17.2 Safety Monitoring

Study subjects will undergo frequent clinical assessments including depressive scores, concomitant medications, adverse events, and serious adverse events and unexpected device events will be recorded from study entry to completion. In addition, clinicians should monitor the safety of subjects by frequently asking them about their comfort during treatment delivery.

If skin erythema occurs, treatment will be discontinued. Patients will be instructed to contact the principal investigator of the study site or a member of his or her staff at any time between visits regarding adverse events or worsening of symptoms. If at any study visit, the subjects' clinical status significantly worsens from baseline (operationalized as the clinical global impression severity score, CGI-S, of 6 or greater) or if a subject develops active suicidal intent and/or plan, based on the C-SSRS scale and/or the clinical interview, the subject will be referred to the treating doctor to start an antidepressant medication or make appropriate changes in the treatment regimen. If the subject were considered to be in imminent danger as a result of suicidality, he/she would be discontinued from the study and referred to appropriate clinical treatment (see 4.9 Termination).

The two site principal investigators (Dr. TBD, PI) will have bi-weekly conference calls (which will include all study staff); during these conference calls, they will discuss all adverse event reports to identify any safety concerns, based on such concerns, they may decide to temporarily discontinue study enrollment, modifications to the study protocol or terminate the study.

In addition, a Data and Safety Monitoring Board (DSMB), composed of at least 3 members not directly involved in the study, will review the SAEs annually. The 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 injured as a direct result of study participation, medical care will be provided. All adverse events (and device events) will be tracked to resolution and reported to the MGH or NKI IRB as serious in the event that:

1. Not anticipated and possibly study-related (same report as SAE) or
2. If any of the following criteria are encountered: any fatal event, immediately life-threatening event, permanently or substantially disabling event, event requiring or prolonging 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.

An expedited review will be conducted for all events that meet the FDA definition of serious adverse events (SAE), i.e., any fatal event, immediately life-threatening, permanently or substantially disabling event, event requiring or prolonging 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 the purposes of this study, all SAEs should be reported to the DSMB, regardless of any judgment about their relationship to the study device. All relevant information will be reported to the DSMB for each SAE, including event and outcome information, dosing history of all study TLT applications, concomitant medications, subject medical history and current conditions, and all relevant laboratory data. Per IRB protocol, the principal investigator at each site will report all unanticipated serious adverse events to the IRB within 5 working days / 7 calendar days after 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 will 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 study site personnel, all adverse events occurring during the study, whether or not attributed to the tPBM-2.0 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 should be assigned:

1. Description of the event
2. Start Date
3. Date of resolution (if applicable)
4. Severity
5. Relation to the study set-up and / or procedures
6. Intensity
7. Action(s) taken
8. Resolution

Intensity is defined as a measure of the severity of a reaction, effect or experience. Measures are described as mild, moderate or severe. However, the event itself may be of relatively minor medical importance.

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

Mild
The adverse event is transient, does not require treatment and does not interfere with the subject's daily activity.

Moderate
The adverse event introduces a low level of inconvenience or concern for the subject and may interfere with daily activities, but is usually ameliorated by simple therapeutic measures.

Severe
The adverse event disrupts the subject's usual daily activity and requires systematic therapy or other treatment.

If the adverse event is of such intensity in the judgment of the investigator that it warrants withdrawal from the study, the subject should be withdrawn from treatment. The subject should receive appropriate care under medical supervision until the symptoms disappear.

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 use of the study device.

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) Follow a reasonable time sequence of device usage, but
b) It could have been produced by the subject's clinical condition or other methods of therapy administered to the subject.

Likely
This causal relationship is assigned when the adverse event:

a) Follow a reasonable time sequence of device use;
b) It decreases when treatment is suspended;
c) It cannot be reasonably explained by the known characteristics of the subject's clinical condition.

Definitive
This causal relationship is assigned when the adverse event:

a) Follow a reasonable time sequence of device use;
b) Decreases when treatment is discontinued; and
c) It is confirmed by the recurrence of the adverse event on repeated exposure.

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

4.17.4 Serious Adverse Events

All serious adverse events should be reported to the Institutional Review Board (IRB) oversight, the FDA and Pthera LLC, as required.

If Pthera LLC or its designee notifies Investigators of any serious adverse event that is considered an unanticipated adverse effect of the device, Investigators should notify their own IRB/EC as necessary.

The investigator should report serious adverse events, serious adverse effects and serious and unexpected suspected adverse effects to the ethics committee when the event occurs or becomes known within a period of no more than one (1) calendar day. Likewise, it must report to the INS OGITT within seven (7) calendar days of the occurrence of the event or as soon as it becomes aware of the event, through the Sistema de Reporte Virtual de Eventos Adversos Serios (REAS-NET).

4.17.5 Death

Deaths reportable to Pthera LLC include all deaths during study participation.

4.17.6 Withdrawal for Adverse Events

All adverse events resulting in subject withdrawal from the study should be reported immediately by telephone to Pthera LLC.

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 report to the appropriate regulatory authorities evaluating the study device as necessary.

4.18 Measures to Ensure Subject's Safety

The investigator will be responsible for monitoring the safety of subjects entering this study and for alerting Pthera LLC of any study-related events that appear unusual and/or unanticipated to their facility.

The investigator will be responsible for the proper medical care of subjects during the study in relation to the protocol procedures for their center.

The investigator will remain responsible for providing appropriate medical care options after a subject terminates or the study is discontinued 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 ineligible according to the inclusion and exclusion criteria of the study.
3. The subject develops an adverse event that would not allow continuation in the study.
4. The subject has an adverse event that, in the opinion of the investigator, warrants withdrawal from the study. Pthera LLC must be notified within two business days.
5. The subject and/or the investigator make the decision that the subject should withdraw from the study.
6. Subject to death of participant.
7. Positive pregnancy test during eligibility procedures.
8. The subject misses one of the 3 scheduled sessions per week and it is not possible to make it up before the end of the week. When a subject withdraws or is withdrawn from the study, the following will be performed, if possible, at the study termination (exit) visit:

- HAM-D-17
- QIDS-C
- CGI
- C-SSRS
- ADVERSE EVENT FORM
- Concomitant Medication Form
- ASQ (self-assessment)
- SAFTEE-SI (self-assessment)
- SDQ (self-assessment)

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

4.19.2 Loss of Follow-up

If a center is unable to contact a subject or if the subject fails to appear for a visit, three documented telephone calls should be made, followed by a certified letter (or its equivalent, such as an e-mail). The certified letter should detail the need for the subject to appear for a visit, the center's failed attempts to contact the subject, and that failure to contact the center will cause the subject to be withdrawn from the study.

If the certified letter is returned to the center as undeliverable or the letter is delivered but the subject does not communicate with the center and no further contact is made with the subject or the subject's caregiver, then the subject will be considered lost to follow-up with the subject and suspended from the study. All attempts to contact the subject will be documented.

A delay in the application of the laser, not exceeding 72 hours, will be tolerated for reasons of major cause. Provided that this delay allows to comply with the 3 administrations corresponding to the week of the intervention under study. If the subject does not comply with the 3 administrations of the treatment, he/she will be withdrawn from the study.

4.20 Summary of Risks and Benefits

4.20.1 Potential Adverse Events

Risks to the subject may include, but are not limited to, the following:

The EXPi device emits light with a wavelength longer than the human eye can see. Personnel will be trained in basic safety procedures related to the use of the device. Personnel administering the NIR-TLT will be careful not to operate the EXPi device unless it is in direct contact with the subject's skin. Both the subject receiving the NIR-TLT and the study doctor present in the NIR-TLT room will wear eye protection in the form of goggles or pads. The goggles provided with the EXPi device 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 have an adhesive to adhere to the subject's eyelids, so there is a possibility of an allergic reaction.

EXPi device failure resulting in cessation of investigational therapy may cause:

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

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

The application of the EXPi device may cause a slight thermal sensation of heat during use. The skin temperature is well below the thermal damage level.

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

1. Erythema at the application site
2. Pain at the application site
3. Discomfort at the application site
4. Heat at the application site
5. Reaction at the application site
6. Headache

Additional potential side effects of NIR-TLT, documented in previous trials, include:

- See bright colors, abnormal taste
- Feeling "out-of-body" experiences
- Insomnia, restless sleep, erratic sleep, early morning awakening.
- Vivid dreams
- Irritability
- Difficulty finding words
- Abdominal distention

Other potential risks are described below:

Risk of worsening depression, suicidality and manic change: Worsening 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 exhibit 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 considered to be in imminent danger; we will then coordinate appropriate levels of care and standard antidepressant treatment (see 4.9 Termination). Manic changes are potential adverse events and will also be closely monitored during antidepressant treatment. Our strict 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 with the appropriate level of care.

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

Duration of testing for cognitive circuitry: The total testing duration takes a total of 40 minutes at baseline and 40 minutes at weeks 5 and 9. There is no expected risk to patients beyond the 40 minutes of sitting at the computer for testing and possible startle experience. Every effort will be made to ensure that patients are comfortable and safe during this period, and the study can be stopped at any time. Beyond these problems, there is little or no risk to patients.

Risk of bruising: venous puncture may cause accidental bruising when blood is drawn from the forearms.

4.20.2 Benefits:

Study subjects will receive a systematic MINI assessment of their DSM comorbidity. This information will be readily available to their treating doctors if subjects so desire and agree to disclose. This information can guide long-term treatment. In the short term, the subject will receive close and systematic depression follow-up and formalized cognitive assessments beyond current standards of care. Easy access to routine physical examinations is also a potential benefit. Subjects will have access to a different treatment modality if counseling and/or medications were not sufficient or not acceptable to them. The treatment itself with transcranial laser therapy (TLT) can potentially alleviate depressive symptoms on a level equal to antidepressant medication.

4.20.3 Allowed and prohibited concomitant interventions:

No concomitant prohibited interventions have been reported during treatment with this device. Contraindications for use:

- Do not apply infrared light to abdominal or lumbosacral points in pregnant women.
- Do not apply infrared light to epiphyseal lines in children.
- Do not apply infrared light in the thoracic region or on the pacemaker itself in patients with pacemakers.
- Do not apply infrared light to the thyroid gland, ovaries or testicles.

- Do not apply infrared light to patients who are taking
- drugs that have contraindications that are sensitive to heat or heat sensitive.
- light, such as, among others, certain types of steroids.

5. Data Analysis Methods

5.1 General Considerations

It is expected that multiple sites, randomized, sham-controlled, double-blinded, double-blinded trials will include 112 patients, randomized into four groups. Each group will receive a different dose of NIR-TLT. Group tasks and dosing will be blinded to doctors, patients and outcome assessors. Device operators will be aware of the group assignments.

The purpose of this study is to compare the antidepressant effect of four different doses of NIR-TLT and to correlate this effect with the effects of the individual dose on brain electrophysiology and mitochondrial function.

5.2 Aims of the Study

Primary Aims:

A. To compare the effect(s) of each of the four doses of NIR-TLT on depression symptomatology: antidepressant effect.

Hypothesis 1: Total HAM-D 17 scores will vary in a NIR-TLT dose-dependent manner, with BPW dose being more effective.

We also expect to be able to estimate the effect size of the antidepressant action as a function of the irradiance applied to the NIR-TLT skin, with the BPW dose being more effective.

B. Compare the safety and tolerability of each dose.

Hypothesis 2: The doses of NIR-TLT tested in this study will be safe and well tolerated by patients with MDD, as assessed by SAFTEE-SI and serious adverse events.

We do not anticipate dose-dependent differences in reported side effects.

Secondary Aims:

C. To compare the effect(s) of each of the four doses of NIR-TLT on brain electrophysiology.

D. To compare the effect(s) of each of the four doses of NIR-TLT on mitochondrial function.

E. To study the correlations (correlating) antidepressant effects, brain electrophysiology and mitochondrial function of each of the NIR-TLT doses.

5.3 Sample Size

The sample size was determined using G Power software version 3.1, considering a calculation by double mean difference with common standard deviation, assuming a significance level of 0.05 and 90% power. The parameters used were obtained from page 7 (section "Antidepressant effect according to clinician-rated measurement") of the previous ELATED-2 study (Cassano et al., 2018), where the mean difference of the HAM-D17 score for the experimental group was -10.8± 7.55 and for the control group 4.4±6.65; such data identify a double difference of 6.4 with common standard deviation of 7.091. With this information, a total sample of 112 participants is obtained, taking into consideration a 20% loss rate. Each group will have 28 participants.

With n=112, in 4 groups in a longitudinal design, we estimated a power of 90% to differentiate (alpha=0.05) the therapeutic effect of the dose, giving an antidepressant effect and an effect variance equivalent to that obtained in participants who completed the ELATED-2 study for all groups (Cassano et al., 2018b)

5.4 Baseline and Demographic Characteristics of the Subjects

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

A t-test or chi-squared test, as appropriate for quantitative or categorical data, will be used to determine whether there were differences in the baseline demographic profiles of the groups. Differences were considered significant at p values <0.05.

5.5 Efficacy and Safety Analysis for the Aims

It will be carried out as follows:

A. Antidepressant effect of NIR-TLT doses:
To compare the antidepressant effects of NIR-TLT doses, mean changes in HAM-D 17 scores (for evaluation of the Primary Assessment Criteria) and QIDS-C and CGI-S (for evaluation of the Secondary Assessment Criteria), will be performed for each dose at 3 time points (visit 1, visit 9 and visit 18) will be compared using a repeated measures ANCOVA. A significant ANCOVA interaction in mean score changes between groups will be taken as indicating that the groups have different antidepressant efficacy. If significant ANCOVA interactions are found, paired samples t-tests will be used to explore differences between scores/groups.

Both the full analysis and the analysis by intention will be performed to analyze the severity of depression at the endpoint. Patient attrition and dropout will be analyzed. The possible

differences between treatment groups in dropouts will be examined with Fisher's exact test.

B. Brain electrophysiological effect of NIR-TLT doses:

To compare the effects of NIR-TLT doses on brain electrophysiology, changes in EEG spectral power in all spectral bands for each dose at 3 time points (visit 1, visit 9, and visit 18) will be compared using a repeated measures ANCOVA. A significant ANCOVA interaction in mean score changes between groups will be taken as indicating that the groups have different effects. If significant ANCOVA interactions are found, paired samples t-tests will be used to explore differences between groups.

C. Safety and tolerability of NIR-TLT doses:

To compare the safety and tolerability of NIR-TLT doses, mean changes in SAFTEE-SI scores at 3 time points (visit 1, visit 9, and visit 18) will be compared using a repeated measures ANOVA. A significant ANOVA interaction in mean score changes between groups will be taken as indicating that the NIR-TLT doses have different tolerability. If significant ANOVA interactions are found, paired samples t-tests will be used to explore differences between groups.

In addition, the frequency and severity of side effects and adverse events reported for each dose will be compared using a Fisher's exact test. The corrective actions taken by the doctors as well as the clinical judgment on the likelihood of association of the side effect/adverse event with the NIR-TLT dose will also be reported.

D. Findings visualized in the PET *scan*:

The analysis of the results obtained in the PET will be for exploratory purposes to study the brain metabolism of the participants, before and at the end of the study intervention in the 4 groups. Following the recommendations of Daniel et al. (Daniel, 2012), for the exploratory analysis we will work with a minimum number of 5 participants per group, with a total of 20 participants.

The results obtained in the PET will be presented in a descriptive manner.

5.6 Safety Analysis

The number and percentage of adverse events until the end of the study will be presented for each treatment group according to adverse event.

5.7 Additional Safety Analysis

Descriptive safety statistics for each treatment group will include the number of subjects, mean, standard deviation, minimum and maximum median 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 approval of the protocol and informed consent form from the IRB. Additional documentation may be required pending applicable local requirements. At least the following documentation should be obtained:

1. IRB / EC protocol approval.
2. IRB/EC approval of the informed consent form.
3. Approval of the annual renewal of the IRB/EC protocol (or at any other frequency when applicable, i.e. quarterly, semi-annually in accordance with the local IRB/EC standard operating procedure).
4. 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 Good Clinical Practice (GCP) guidelines and other applicable regulatory requirements including, but not limited to:

- The Food and Drug Administration's (FDA) Investigational Device Exemption Regulations (21 CFR 812),
- FDA's Human Research Regulations (21 CFR 50, 54 and 56),
- Health and Human Services Regulations (DHHS) on human subjects research (45 CFR 46 Subpart A, B, C and D) and
- The International Conference on Harmonization (ICH) "Good Clinical Practice Guideline for Industry-E6: Consolidated Guideline".
- The regulation of clinical trials approved by DS 021-2017-SA.

6.3 ClinicalTrials.gov

The study will be registered and published on ClinicalTrials.gov (www.clinicaltrials.gov).

6.4 Monitoring Procedures

Monitoring will be conducted by the PI, who will periodically review laboratory results and clinical information. The PI will coordinate the different phases of protocol implementation, such as application and follow-up. The PI will be in frequent contact with the other staff members responsible for the delivery of the NIR-TLT and with Pthera LLC, who will loan the NIR-TLT device. We have minimized the risks potentially associated with NIR-TLT use by providing subjects and the operator with safety goggles or eye pads during NIR-TLT application and by requiring two methods of contraception (although NIR-TLT has not been associated with a teratogenic effect). We have also included treatment discontinuation rules based on the development of skin erythema or discomfort lasting more than 24 hours at TLT delivery sites (to avoid the risk of the subject having unknown skin photosensitivity)

Treatment will end for participants who experience temporary pain or discomfort during a treatment session that is present up to 24 hours after the end of the session.

6.4.1 Security Monitoring

Study subjects will undergo frequent clinical assessments including depressive scores, concomitant medications, adverse events, and serious adverse events, and unexpected device events will be recorded from study entry to completion. In addition, clinicians should monitor a subject's safety by frequently asking them about the subject's comfort during treatment delivery.

If skin erythema occurs, treatment will be discontinued. Patients will be instructed to contact the study site principal investigator or a member of his or her staff at any time between visits regarding adverse events or worsening symptoms. If at any study visit, the subjects' clinical status significantly worsens from baseline (operationalized as Clinical Global Impression Severity Score, CGI-S, of 6 or greater) or if a subject becomes actively suicidal with intent and/or plan, as per On the C-SSRS scale and/or clinical interview, the subject will be referred to the treating doctor to start an antidepressant medication or make appropriate changes in the treatment regimen. If the subject were considered to be in imminent danger as a result of suicidality, he/she would be discontinued from the study and referred to appropriate clinical treatment (see Section - 4.9 Termination).

The site will have bi-weekly conference calls with a designated Pthera LLC study monitor (which will include study RA's); During these conference calls, they will discuss all adverse event reports to identify any safety concerns, based on such concerns, they may decide to temporarily discontinue study enrollment, modifications to the study protocol or terminate the study.

In addition, a DSMB, consisting of at least 3 members not directly involved in the study and not employed by Pthera LLC, will review SAE quarterly. 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 injured as a direct result of study participation, medical care will be provided. All adverse events (and device events) will be tracked to resolution and reported to the DSMB as serious in the event that they were not anticipated and possibly related to the study (same report as SAE) or meet any of the following criteria, any:

(1) fatal event,
(2) immediate life-threatening event,
(3) event of permanent or substantial disability,
(4) event requiring or prolonging hospitalization, or any congenital anomaly,
(5) If a study investigator judges to impose a significant hazard, contraindication, side effect, or precaution.

An expedited review will be conducted for all events that meet the FDA definition of Serious Adverse Events (SAE), i.e., any fatal event, immediately life-threatening event, permanent or substantial disabling event, event that requires or prolongs 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 the purposes of this study,

all SAEs should be reported to the DSMB, regardless of any judgment about their relationship to the study device. All relevant information will be reported to the DSMB for each SAE, including event and outcome information, dosing history of all NIR-TLT applications in the study, concomitant medications, medical history and current conditions of the subject, and all relevant laboratory data. The principal investigator will report all unanticipated serious adverse events to the site IRB within 5 working days / 7 calendar days after 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 will have the discretion and responsibility to recommend that the study be terminated.

6.4.2 Measures to ensure the safety of the subject

The investigator will be responsible for monitoring the safety of subjects entering this study and for alerting Pthera LLC of any study-related events that appear unusual and/or unanticipated for their site.

The investigator will be responsible for the proper medical care of the subjects during the study in relation to the protocol procedures for their site.

The investigator will remain responsible for providing appropriate medical care options after a subject terminates or the study is discontinued due to adverse events.

6.5. Training of the center's study personnel

Training will begin before the protocol is implemented. Training will consist of lecture and hands-on practice. Application of the NIR-TLT procedure will be performed only by PI or his/her designee trained by the Sponsor (or his/her designee) to perform the procedure.

6.6 Informed Consent

The Principal Investigator will be responsible for developing the Informed Consent Form to be prepared in accordance with FDA 21 CFR Part 50 for all U.S. sites. The Informed Consent Form will be used to explain in simple terms, before the subject enters the study, the potential risks and benefits to the subject. The Informed Consent Form will contain a statement that 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 dated personally by the subject or by an individual authorized to sign on behalf of the subject in accordance 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 discussion of the Informed Consent Form. After the Written Informed Consent Form and any other written information provided to the subject is read and explained to the subject or legal representative, and the subject has given oral consent for participation in the study, the witness will sign and date the consent form.

A witness in the consent process is considered an unbiased individual independent of the study.

Note: ***The investigator should document the acquisition of the written informed consent form in the subject's medical records, and the subject or legal representative should receive a copy of the informed consent form document prior to enrollment in the study.***

6.7 Adherence to Protocol.

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

6.8 Data Collection

Data will be captured on Case Report Forms and kept locked at the center.

6.9 Record Retention

The investigator should maintain a file of all documents and records related to the conduct of the study. Subject files and other source data should be retained 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.

6.10 Accountability / Investigational Product Control

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

Principal investigators or designated study site personnel verifying receipt of devices/device accessories must complete the device/accessory acknowledgement form and fax a copy to Pthera LLC. Device accountability and acknowledgement records will be maintained at each study site. These records will list all equipment received, date received, serial number of each device. Study site personnel will initiate the log each time the device is used. EXPi device usage will also be recorded in the appropriate CRF.

Malfunctioning devices and device accessories, including all components, will be returned to Pthera LLC. for investigation at Pthera LLC.'s expense.

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8. Table of Assessments

STUDY WEEK	Week 0	Week 1	Week 1 to 3	Week 3	Week 4 to 6	Week 6	Week 8
VISIT PROCEDURE	V0 Selection	V1	V2 to 8	V9	V10 to 17	V18	V19 Follow-up
Consent	■						
M.I.N.I.	■						
PET *scan* in study subgroup	▲					▲	
Concomitant Medication	■	■	■	■	■	■	■
Physical Examination / Lab	■ / ▲						■
Progress Note		■	■	■	■	■	■
Vital Signs	■	■	■	■	■	■	■
Adverse Events Form		■	■	■	■	■	■
ASQ	● / ▲	● / ▲	● / ▲	● / ▲	● / ▲	● / ▲	● / ▲
CGI	■	■		■		■	■
C-SSRS	■	■	■	■	■	■	■
HAMD-17	■	■		■		■	■
QIDS-C	■	■		■		■	■
SAFTEE-SI	● / ▲	● / ▲	● / ▲	● / ▲	● / ▲	● / ▲	● / ▲
SDQ	● / ▲	● / ▲	● / ▲	● / ▲	● / ▲	● / ▲	● / ▲
EEG		■		■		■	
Treatment- TX		▲	▲	▲	▲	▲	

Legend

■ DOCTOR
▲ RESEARCH ASSOCIATE
● PATIENT