NYU Langone Health

Official Title:	Transcranial Near Infrared Radiation and Cerebral Blood Flow in Depression (TRIADE)
NCT Number:	NCT04366258
Study Number:	20-00217
Document Type:	Study Protocol and Statistical Analysis Plan
Date of the Document:	• November 16, 2021

Tool Revision History:

Version Number	Version Date	Summary of Revisions Made
1.0	12 FEB 2020	Original version
1.1	3 MAR 2020	IRB edits incorporated
1.2	27 MAY 2020	Insertion of remote activities where possible
1.3	22 SEP 2020	Change of blinding, option to omit thermometry
1.4	17 NOV 2020	Option to omit bloodwork
2.0	11 DEC 2020	Option to modify high dose parameters for tolerability and blind preservation.
2.1	19 MAR 2021	Option for neurologist to review scan; change review from all scans to one scan per subject. Broaden antidepressant type permitted on study from SSRIs and SNRIs to SSRIs, SNRIs, and Wellbutrin (bupropion). Change of high dose parameters for tolerability and blind preservation.
2.2	3 MAY 2021	Addition of CGI to follow-up visit; addition of neuronavigation to MGH imaging protocol during experimental visits
2.3	4 NOV 2021	1. Increase number of study enrollment and screening to 62 participants; 2. Addition to study exclusion criteria to exclude participants with seizure disorders requiring treatment in the last 5 years.

TRANSCRANIAL NEAR INFRARED RADIATION AND CEREBRAL BLOOD FLOW IN DEPRESION (TRIADE)

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NYULMC Study Number:	*i20-00217*
Funding Sponsor:	National Institute of Health
IND/IDE Number:	N/A
Regulatory Sponsor:	N/A
Study Product:	LightForce® EXPi Deep Tissue Laser Therapy™ System, Transcranial PhotoBioModulation-1000 (tPBM-2.0)
Study Product Provider:	LiteCure
ClinicalTrials.gov Number	NCT04366258

Statement of Compliance

This study will be conducted in accordance with the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), 21 CFR Parts 50, 56, 312, and 812 as applicable, any other applicable US government research regulations, and institutional research policies and procedures. The International Conference on Harmonisation ("ICH") Guideline for Good Clinical Practice ("GCP") (sometimes referred to as "ICH-GCP" or "E6") will be applied only to the extent that it is compatible with FDA and DHHS regulations. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

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List of Abbreviations

AE	Adverse Event/Adverse Experience
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
AVM	Arteriovenous Malformation
BDNF	Brain Derived Neurotrophic Factor
BOLD	Blood Oxygen Level Dependent
CBF	Cerebral Blood Flow
CCO	Cytochrome c oxidase
CFR	Code of Federal Regulations
CI	Confidence Interval
CIMU	Conflict of Interest Management Unit
cm	Centimeter(s)
CNS	Central Nervous System
CREF	Clinical Research Evaluation Facility
CRF	Case Report Form
CUNY	City University of New York
CW	Continuous Wave
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
dLPFC	Dorsolateral Prefrontal Cortex
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
eCRF	Electronic Case Report Form
ECT	Electroconvulsive Therapy
EEG	Electroencephalogram
ES	Effect Size
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act (of 2007)
fMRI	functional Magnetic Resonance Imaging
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practices

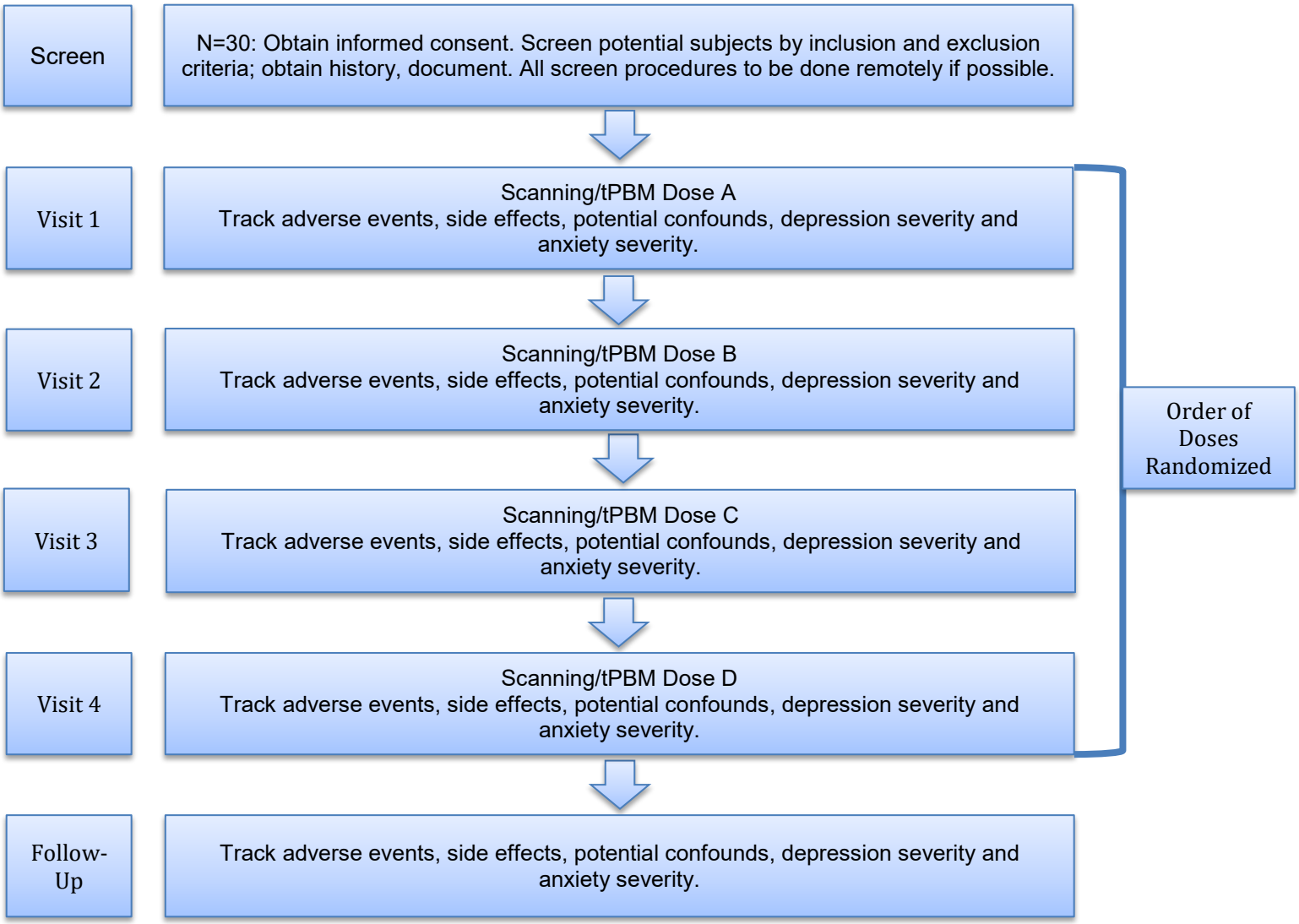
GSK-3β	Glycogen Synthase Kinase 3 Beta
HAMD-17	Hamilton Depression Rating Scale, 17-item version
HCTZ	Hydrochlorothiazide
HIPAA	Health Insurance Portability and Accountability Act
IC	Informed Consent
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IL-1β	Interleukin 1 Beta
IL-6	Interleukin 6
IL-8	Interleukin 8
iNOS	Inducible Nitric Oxide Synthase
IRB	Institutional Review Board
LED	Light Emitting Diodes
MDD	Major Depressive Disorder
MDSG	Mood Disorders Support Group
MFG	Medial Frontal Gyrus
MGH	Massachusetts General Hospital
MGH DCRP	Massachusetts General Hospital Depression and Clinical Research Program
MPE	Maximum Permissible Exposure
mPFC	medial Prefrontal Cortex
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
mW	Milliwatt
N	Number (typically refers to participants)
NAMI	National Alliance on Mental Illness
nm	Nanometer(s)
NF-κB	Nuclear Factor κB
NIH	National Institutes of Health
NIH IC	National Institutes of Health Institute/Center
NIMH	National Institute of Mental Health
NIR	Near-infrared Radiation
NKI	Nathan Kline Institute
NO	Nitric Oxide
NSMHA	North Suffolk Mental Health Association
NYU	New York University
NYUMC	New York University Medical Center

OPD	Outpatient Psychiatry Department
OPRD	Outpatient Research Department
PBM	Photobiomodulation
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
PHI	Protected Health Information
PI	Principal Investigator
PW	Pulsed Wave
QC	Quality Control
RCT	Randomized Controlled Trial
RNA	Ribonucleic Acid
ROI	Region of Interest
ROS	Reactive Oxygen Species
RF	Report Form
rTMS	repetitive Transcranial Magnetic Stimulation
SAE	Serious Adverse Event/Serious Adverse Experience
SAP	Statistical and Analytical Plan
SC	Steering Committee
SCID	Structured Clinical Interview for DSM Disorders
SD	Standard Deviation
SFG	Superior Frontal Gyrus
SNRI	Serotonin-Norepinephrine Reuptake Inhibitor
SOP	Standard Operating Procedure
SPECT	Single Proton Emission Computerized Tomography
SSRI	Selective Serotonin Reuptake Inhibitor
TBI	Traumatic Brain Injury
tDCS	Transcranial Direct Current Stimulation
TNF- α	Tumor Necrosis Factor alpha
TSH	Thyroid Stimulating Hormone
t-PBM	transcranial Photobiomodulation
UP	Unanticipated Problem
VNS	Vagal Nerve Stimulation
VRP	Volunteer Recruitment Pool
WBC	White Blood Cells

Protocol Summary

Title	Transcranial Near Infrared Radiation and Cerebral Blood Flow in Depression
Short Title	TRIADE
Brief Summary	This study will compare the effect of three t-PBM doses (high, middle, and low irradiance) to sham t-PBM on PFC CBF as assessed with fMRI (BOLD) in this multi-center, phase I, single-blind, dose-ranging, controlled, crossover study of 30 subjects with MDD. All eligible participants will undergo four sessions of t-PBM during fMRI so that they experience irradiances of 50, 300 and ≥700 mW/cm^2 as well as sham. The order of dose administration will be randomized and t-PBM will be administered with the LightForce® EXPi Deep Tissue Laser Therapy™ System, Transcranial PhotoBioModulation-1000 (tPBM-2.0).
Phase	I
Objectives	Primary: To identify the t-PBM irradiance dose (50, 300, or ≥700 mW/cm^2) with the largest impact on PFC CBF in MDD. Secondary: To compare the safety and tolerability of three t-PBM irradiance doses.
Methodology	Single-blind, dose-ranging, controlled, crossover study.
Endpoint	Primary: t-PBM effects on BOLD signal. Secondary: t-PBM effects on the frequency and severity of adverse events and side effects.
Study Duration	24 months
Participant Duration	6-12 weeks
Duration of IP administration	4 sessions
Population	30 men and women ages 18-65 with MDD
Study Sites	NYUSOM, NKI, MGH
Number of participants	30
Description of Study Agent/Procedure	Transcranial photobiomodulator, delivers laser-generated NIR to forehead.
Reference Therapy	Sham
Key Procedures	4 Sessions of t-PBM during MR scanning
Statistical Analysis	For each subject at each t-PBM dose we will model the BOLD signal at specified ROIs as a function of dose (considered a categorical variable with 4 levels) using mixed effects models, including random subject intercepts to account for the potentially correlated measures on the same subject.

Schematic of Study Design



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2 Introduction, Background Information and Scientific Rationale

2.1 Background Information and Relevant Literature

2.1.1 Problem: Unmet Need for Accessible, Scalable, and Effective Treatments for Depression
Twenty-one million Americans (~10%) suffered from a depressive episode over the past year[1]. Major depressive disorder (MDD) is the third leading cause of global disability[2]; the global cost of mental illness is expected to more than double by 2030, with depressive disorders at the forefront[3]. Sadly, 43% of primary care patients experiencing a six-month depressive episode remain untreated, with most preferring self-management[4] over professional help and/or prescription medications[5,6]. Factors limiting the adoption of firstline interventions for MDD include burdensome side effects of medications[7,8] and the time commitment for psychotherapies[9]. Somatic therapies such as electroconvulsive therapy (ECT), repetitive transcranial magnetic stimulation (rTMS) and vagus nerve stimulation (VNS) are approved for treatment resistant MDD[10]; however, they are unsuitable to become first line treatments, since they require in-office administration, and sometimes anesthesia and/or surgical procedures[10]. In contrast, the use of wellness devices is especially popular, since nearly 25% of Americans own a wearable device[11]. **A new generation of at-home devices for MDD that are safe, well-tolerated, inexpensive and easy to use, would represent a valuable firstline intervention, both accessible and scalable**.

2.1.2 Potential Solution: Transcranial Photobiomodulation (t-PBM)
Transcranial photobiomodulation (t-PBM) is a novel neuromodulation based on non-retinal exposure to light at specific wavelengths. As summarized below, a substantial body of literature proves that transcranial photobiomodulation (t-PBM) with near-infrared (NIR) light penetrates deeply into the cerebral cortex[12-14], modulates cortical excitability[15,16] and improves cerebral perfusion[17-19] and oxygenation[20]. Its safety was demonstrated in 1,410 acute stroke patients[21-23,102]. Antidepressant effects of t-PBM have been reported in both animal models[24-28] and human studies[29-34]. In a double-blind clinical trial (n=21), our team demonstrated the antidepressant effect of adjunct t-PBM NIR delivered to the prefrontal cortex (PFC) over 8 weeks[35]. Devices capable of administering t-PBM are already FDA- approved and can be manufactured with basic and relatively inexpensive lasers or light emitting diodes (LEDs). Due to its low cost, excellent safety profile, and ease of administration, PBM has the potential to become widely accessible. However, despite its clear advantages, no consensus exists on the optimal dose irradiance of t-PBM for MDD.

2.1.3 t-PBM Penetration: NIR undergoes exponential decay of the photon flux as it travels through tissues. Nevertheless, a small but significant fraction of the light delivered to the scalp reaches the cortex. Depending on parameters used, the distribution of the therapeutic dose (sufficient energy deposition for therapeutic effects) might vary from only the most superficial cortex to the whole cortical width, always in proximity of the light source[51]. While therapeutic NIR penetration varies based on wavelength[13,14], light source (LED versus laser)[12], pulsing[52] and intensity[12,13], a 2% to 3% penetration rate of NIR is attainable at target prefrontal cortex regions. This corresponds to an NIR penetration of about 2 cm of intervening skin, skull, meninges, cerebrospinal fluid and brain. The magnitude of NIR fluence (energy density, J/cm^2) to the skin surface –necessary to reach target areas of the brain therapeutically– is both safe and well tolerated. It is feasible to attain an NIR fluence on the human brain equivalent to the fluence inducing neurological benefits in animal models[53].

***2.1.4 t-PBM Effects on Cerebral Blood Flow (CBF)*:** In vivo t-PBM has robust and immediate effects on CBF. In rats, t-PBM at 660 nm with a LED device (9 mW/cm^2) was associated with increased oxygen consumption in the frontal cortex[95], while in mice NIR (808 nm) t-PBM with laser (1.6 W/cm^2) resulted in 30% increased CBF[96]. In healthy humans, t-PBM with a 3.4W laser (1064 nm) led to increased

concentration of oxygenated hemoglobin[20], while in elderly women t-PBM with a 0.2W LED (627 nm) increased blood flow in the middle cerebral and basilar arteries[19]. In chronic traumatic brain injury (TBI) patients, t-PBM with a 3.3W LED device (629 and 850nm) increased CBF (measured by SPECT)[36]. Our own preliminary data (Section 2.4) show immediate increase in CBF [measured as blood-oxygenation-level dependent (BOLD) signal on fMRI] in healthy volunteers exposed to 808 nm laser NIR. Therefore, changes in CBF during t-PBM administration, measured as changes in BOLD fMRI signal, is an excellent target engagement biomarker. Secondarily, t-PBM also has a modulatory effect on cortical excitability, reducing motor evoked potentials elicited by TMS[15] and may result in increased functional connectivity in the default mode network[97].

***2.1.5 t-PBM Cellular Mechanisms*:** The NIR delivered through t-PBM is absorbed by a mitochondrial enzyme and chromophore, cytochrome c oxidase (CCO), and is only minimally dissipated as thermal energy[53,54]. The peak absorption of light energy by CCO occurs at four different wavelengths; one of these peaks is 812–846 nm[55], overlapping with the wavelengths with best brain penetration described above.

Depression is associated with hypometabolism in specific brain areas[56-59] and mitochondrial dysfunction[60-64]. Results in cellular and animal models indicate that PBM can enhance mitochondrial activity. NIR delivers energy to the CCO and stimulates the mitochondrial respiratory chain leading to increased ATP production[54,65,66]. In addition, NIR can improve mitochondrial activity by promoting the dissociation of nitric oxide (NO) from the CCO releasing the binding site for oxygen and restoring oxidative phosphorylation[53]. The released NO may also act as a local vasodilator[67]. A study on isolated mitochondria also reported increased RNA and protein synthesis after irradiation with a low-level laser (632.8 nm)[68].

Animal research suggests that PBM might also exert, via its impact on mitochondria, beneficial effects on several other pathophysiological mechanisms implicated in MDD, such as oxidative stress[69-73], neuroinflammation[74-78], and deficits in neuroplasticity and brain derived neurotrophic factor (BDNF)[79-81], and default mode network connectivity[97]. NIR can induce short bursts of reactive oxygen species (ROS) leading to the activation of antioxidant mechanisms and the activation of the transcription factor nuclear factor κB (NF-κB), resulting in decreased overexpression of the inducible form of nitric oxide synthase (iNOS) and reduction of oxidative stress[67,82,83]. In animal models, NIR light (600 to 1000 nm) reduced neuroinflammation by decreasing proinflammatory cytokines such as IL-6, TNF-α, IL-1β, and IL-8[84-86], and decreasing the infiltration of macrophages, activated microglia and T lymphocytes to the CNS[86]. In animal studies PBM also stimulates neurogenesis and neuroprotective mechanisms in models of neuronal injury, possibly mediated by increased BDNF and by inhibition of GSK-3β and pro-apoptotic molecules[67,87-94].

***2.1.6 t-PBM Safety and Tolerability*:** The safety of one session of t-PBM was evaluated in three large RCTs with a pooled sample of 1,410 subjects with stroke[21,22,100]. No significant difference in the rate of adverse effects were observed between the group receiving laser NIR (808 nm; 5W) or sham. No serious adverse events were found in our review of the literature[33]. Two open studies using 1 and 6 sessions of t-PBM reported no treatment-emergent side-effects[29,30]. A clinical trial with 16 t-PBM sessions reported an increased number of mild side-effects in the active treatment group, the most frequent being insomnia, “seeing vivid colors”, “an ashtray-like taste”, and irritable mood[35]. Other potential risks relate to inappropriate administration and will be mitigated by the safety procedures. The risk of thermal injury from t-PBM delivered with the parameters used in the studies we reviewed is considered minimal and limited to the skin. In ten individuals treated for TBI with 10-15W lasers –a much higher power than what is used in t-PBM– the skin temperature increased to no more than 30°C with rapid cooling after removal of the NIR light. Clinically, patients reported slight skin warming, but no discomfort[101]. Laser devices have a potential risk of retinal lesions when used improperly; this is mitigated with appropriate safety eye gear and procedures.

***2.1.7 t-PBM Effects on Depression*:** Multiple pre-clinical studies support the efficacy of t-PBM, in single or repeated administration, on animal models of depression such as the forced swim test[24,27,90,98], tail suspension test[27,90], mild chronic stress[26,28], or the reserpine-induced depression[24]. In several of these studies the antidepressant effects were associated with biological effects related to mitochondrial stimulation, such as increased ATP biosynthesis and mitochondrial complex IV expression and activity in the prefrontal cortex (PFC)[27] and increased hippocampal neurogenesis[98]. All clinical studies using t-PBM

for MDD reported in the literature aimed to modulate the forebrain, consistently with the dysfunction of the prefrontal cortex associated with MDD[99]. In pilot studies with open designs, NIR was associated with an antidepressant response after a single[29] or repeated (6) administrations[30]. Our recent RCT showed t-PBM was more efficacious than sham in 21 MDD subjects receiving bilateral t-PBM at 823 nm or sham directed on dorsolateral PFC (dlPFC, EEG sites F3 and F4) twice-a-week for 8 weeks[35]. Another study suggested that t-PBM paired with a cognitive therapy may be effective in depression[34]. All these studies measured acute, short term effects; only a case report describes persistence of effects over 9 months of repeated administration in a subject with MDD[32].

2.1.8 Scales and Measures: The following clinician-administered scales and patient-reported measures will be utilized in the study.

- **(M.I.N.I)[40]** – The M.I.N.I. is a short structured diagnostic interview, developed jointly by psychiatrists and clinicians in the United States and Europe, for DSM and ICD psychiatric disorders. With an administration time of approximately 15 minutes, it is designed to meet the need for a short but accurate structured psychiatric interview for clinical trials. According to researchers at the National Institute of Mental Health's (NIMH) Division of Clinical and Treatment Research, the M.I.N.I. is a fully validated and more time-efficient alternative to the Structured Clinical Interview for DSM Disorders (SCID).

- **Inventory of Depressive Symptomatology(IDS)[41]** – This is a 30-item clinician-rated inventory of depressive symptoms such as sleep, depressed mood, appetite, concentration, suicidal ideation, interest, energy, psychomotor retardation or agitation.

- **Montreal Cognitive Assessment (MoCA**)[42]–This is a 10-item rapid screening instrument for mild cognitive dysfunction. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. This measure will be used to assess entry criteria.

- **The Massachusetts General Hospital (MGH) Antidepressant Treatment Response Questionnaire (ATRQ)[43]** – This scale is used to determine treatment resistance in major depressive disorder (MDD). The ATRQ examines the efficacy (improvement from 0% [not improved at all] to 100% [completely improved]), and adequacy (adequate duration and dose) of any antidepressant treatment in a step-by-step procedure.

- **New Immigrant Survey-Skin Color Scale (NIS-SCS)[44]** – The scale is an 11-point scale, ranging from zero to 10, with zero representing albinism, or the total absence of color, and 10 representing the darkest possible skin. The ten shades of skin color corresponding to the points 1 to 10 on the Massey and Martin Skin Color Scale are depicted in a chart, with each point represented by a hand, of identical form, but differing in color. The respondent never sees the chart. We gather this data for use as a potential covariate in analyses as skin color can affect absorption of NIR.

- **Systematic Assessment for Treatment Emergent Events (SAFTEE)[45]** – The SAFTEE is a commonly used instrument originally developed by NIMH and adapted into a self-report instrument. The version of the scale that we plan to use is the same used by the multi-center, NIMH-sponsored CO-MED trial, and it examines in a systematic fashion all possible treatment-emergent side effects and probes specific adverse symptoms, including suicidal thoughts and behaviors, and self-injurious behavior.

- **Columbia Suicide Severity Rating Scale (C-SSRS)[110]** – An instrument endorsed by the FDA for clinical trials. This instrument systematically tracks suicidal ideation and behavior (e.g., suicide attempts, wish to die, thoughts of suicide, plan and intent).

- **Clinical Global Impressions – Severity and Improvement (CGI-S, CGI-I)** – These two instruments are scored 1-7 by the clinician based on assessment of the subject's overall clinical status. They measure, based on history and scores on other instruments: (a) depressive severity (CGI-S) and (b) clinical improvement (CGI-I).

- **The Potential Confounders Questionnaire (PCQ)** –This questionnaire solicits information about sleep, presence of jet lag, diet change, and the intake of over-the-counter drugs, prescription medications, caffeine, alcohol, nicotine, marijuana, and other street drugs.

- **The Perceptions of Blinding Questionnaire (PBQ)** –**The Perceptions of Blinding Questionnaire (PBQ)** – The PBQ is a self-report questionnaire to determine the degree to which the participant believes s/he is receiving the treatment or the sham.

- **Positive and Negative Affect Schedule (PANAS)[46]** –This 20 item scale measures positive affect (e.g., excited, inspired) and negative affect (e.g., upset, afraid). Each item is rated on a five-point Likert Scale, ranging from 1 = *Very Slightly or Not at all*, to 5 = *Extremely*.

- **Montgomery-Åsberg Depression Rating Scale (MADRS)[47] –**This 10-item clinician-rated instrument measures depression severity. It will be administered with a structured interview guide. The time frame for this scale is the past 7 days

- **Symptoms of Depression Questionnaire (SDQ)[48]** – This is a comprehensive measure of depression that includes the assessment of symptoms in the anxiety–depression spectrum. It assesses irritability, anger attacks, and anxiety symptoms together with the commonly considered symptoms of depression. Analysis of the factor structure of the SDQ identified 5 subscales, including one in the anxiety–depression spectrum, with adequate internal consistency and concurrent validity.

- **Anxiety Symptoms Questionnaire (ASQ)[49] –** This, is a 17-item self-report questionnaire measuring the frequency and intensity of 17 symptoms of anxiety, including nervousness, worrying, irritability, trouble relaxing, insomnia, lack of energy, difficulty concentrating, somatic symptoms, and impairment in functioning due to anxiety.

- **Adverse Events Form-** The Adverse Events Form captures any adverse event (serious or otherwise) specifically related to the application of the t-PBM.

- **Concomitant medications and therapies form –** This form records all ongoing medications, as well as other therapies targeting depression, and will be completed at every study visit, including the screening visit, as a safety-monitoring tool.

- **Quality of Life in Neurological Disorders, Cognitive Section (Neuro-QoL)[50] -** The cognitive section of the Neuro-QoL is an 8 item self-rated measure of both executive function and general concerns. It measures perceived difficulties in cognitive abilities (e.g., memory, attention, and decision making) or in the application of such abilities to everyday tasks (e.g., planning, organizing, calculating, remembering, and learning).

- **Quality of Life, Enjoyment, and Satisfaction Questionnaire (Q-LES-Q)[111]-** Assesses subjective quality of life (i.e. physical health, subjective feelings, leisure activities and social relationships).

- **MRI Safety Checklist-** Identifies risk factors that may make MRI participation unsafe.

- **The t-PBM Self-Report Questionnaire (TSRQ)** – An open-ended questionnaire focusing on potential inconveniences and discomforts from the t-PBM.

2.2 Preclinical Data

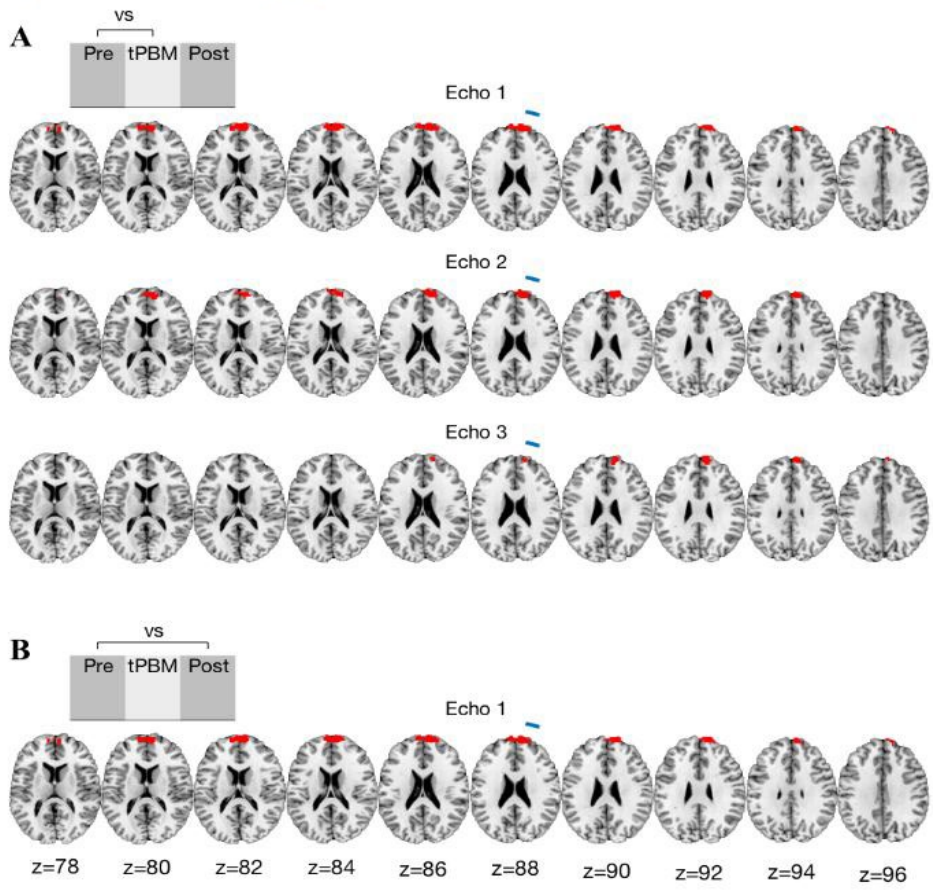
t-PBM Effects on Animal Models of Depression: Multiple pre-clinical studies support the efficacy of t-PBM, in single or repeated administration, on animal models of depression such as the forced swim test[24,27,90,98], tail suspension test[27,90], mild chronic stress[26,28], or the reserpine-induced depression[24]. In several of these studies the antidepressant effects were associated with biological effects related to mitochondrial stimulation, such as increased ATP biosynthesis and mitochondrial complex IV expression and activity in the prefrontal cortex (PFC)[27] and increased hippocampal neurogenesis[98].

t-PBM NIR with laser devices have good penetration. NIR (808 nm) was detected 4 cm below the skin in unfixed cadaver brains (n = 8) with a 5-W laser; the penetration at 808 nm (NIR) wavelength was superior to the 940 (NIR) and 660 nm (red) light[14]. Also, a 15-W laser produced a 2.9% penetration of NIR (810 nm) at 3 cm from skin surface of recently slaughtered sheep heads[12]. Moreover, pulsed wave (PW) delivery might allow higher penetration than continuous wave (CW), by increasing peak irradiance and by lowering skin warming, which would limit light exposure[52]. The penetration for LED devices has been more variable[12,13], which supports our decision to use lasers for this application.

2.3 Clinical Data to Date

Evidence of Efficacy in Depression: All clinical studies using t-PBM for MDD reported in the literature aimed to modulate the forebrain, consistently with the dysfunction of the PFC associated with MDD[99]. Dr. Cassano conducted a proof of concept, prospective trial on the safety and efficacy of adjunct t-PBM NIR (810nm) delivered to PFC, twice a week for 3 weeks in subjects with MDD, demonstrating the feasibility and safety of t-PBM in this population. In this investigation, mean HAM-D17 scores decreased from 19.8 ± 4.4 (SD) to 13 ± 5.35 (SD) after treatment (p=0.004). Patients tolerated the treatment well without any serious adverse events. Our recently published RCT showed t-PBM was more efficacious than sham in 21 MDD subjects receiving bilateral t-PBM at 823 nm or sham directed on dorsolateral PFC (dlPFC) (EEG sites F3 and F4) twice-a-week for 8 weeks[35]. Completer analyses showed a significantly greater decrease of depression scores per the HAMD-17 in the NIR-mode compared to sham [NIR (n=6) -15.7±4.41 vs. sham (n=7) -6.1±7.86; p=.031)]. Further, t-PBM was well tolerated, with no serious adverse events. The effect size was large (Cohen's d=1.5) and reached significance with an n=13

Figure 2: BOLD fMRI Imaging Pre and Post tPBM



(Figure 1). Another randomized, controlled study suggested that t-PBM paired with a cognitive therapy may be effective in depression[34]. All these studies measured acute, short term effects; only a case report describes persistence of effects over 9 months of repeated administration in a subject with MDD and anxiety[32].

Impact: *These data support the eventual clinical utility of t-PBM and highlight the importance of determining optimal parameters, as proposed in this application*.

Preliminary data on t-PBM effect on Cerebral Blood Flow (measured with fMRI-BOLD): We have collected data from n=20 subjects undergoing tPBM during resting state fMRI [103]. To disambiguate the effects of CBF from those of blood oxygenation, we employed a multi-echo MR sequence[104]. Subjects' brain activity in the form of the BOLD signal was acquired continuously for 30 minutes, with tPBM applied for 10 minutes beginning at minute 11. Stimulation was delivered with a laser (808 nm), with an intensity of 318 mW/cm^2 and a spot size of 1 cm^2 to the right forehead (standard EEG electrode location "Fp2"). Whole-brain voxel-wise statistical Chow tests[105] were conducted separately at each echo of the group-averaged BOLD in order to detect significant changes during and after t-PBM, relative to the pre-stimulation baseline period.

During t-PBM, we found a significant modulation of early-echo (13 ms) BOLD in a cluster of 214 voxels in the right medial frontal gyrus (MFG), 24 mm from the site of light incidence (Figure 2). A cluster of 154 voxels in the right MFG, 22.4 mm from incidence, was also found at echo 2 (34 ms). Four smaller clusters were detected at echo 3 (56 ms), including one in the right superior frontal gyrus (SFG), 22.5 mm from incidence. Interestingly, we found that t-PBM produced an effect on the early-echo BOLD that outlasted the stimulation. A cluster of 218 significant voxels centered in the MFG was found at a distance of 28.7 mm from incidence. The presence of significant BOLD effects at all echos, including the early-echo which is insensitive to blood oxygenation, suggests that CBF was increased during and after t-PBM.

Impact: *Thus, we are in a position to employ quantitative changes in fMRI-BOLD as a biomarker for target engagement during t-PBM.*

***Evidence of t-PBM Safety and Tolerability*:** The safety of one session of t-PBM was evaluated in three large RCTs with a pooled sample of 1,410 subjects with stroke[21,22,100]. No significant difference in the rate of adverse effects were observed between the group receiving laser NIR (808 nm; 5W) or sham. No serious adverse events were found in our review of the literature[33]. Two open studies using 1 and 6 sessions of t-PBM reported no treatment-emergent side-effects[29,30]. A clinical trial with 16 sessions reported an increased number of mild side-effects in the active treatment group, the most frequent being insomnia, "seeing vivid colors", "an ashtray-like taste", and irritable mood[35]. Other potential risks relate to inappropriate administration and will be mitigated by the safety procedures such as the use of protective eye wear.The risk of thermal injury from PBM delivered with the parameters used in the studies we reviewed is considered minimal and limited to the skin. In ten individuals treated for TBI with 10-15W lasers –a much higher power than what is used in t-PBM– the skin temperature increased to no more than 30°C with rapid cooling after removal of the NIR light. Clinically, patients reported slight skin warming, but no discomfort[101]. Inherent to the use of any laser device is the potential risk of retinal lesions resulting from improper use of the laser and from the shedding of the light beams straight through lens and from their convergence on the macula; this is mitigated with appropriate safety eyewear and procedures.

2.4 Dose Rationale

As depicted in Table 1, all t-PBM sessions will incorporate: ***1.*** laser light with **2.** a wavelength of 808nm, corresponding to the absorption peak of the primary photoreceptor cytochrome-C oxidase ***3.*** and target bilaterally the dlPFC (standard EEG electrode sites F4, F3). The total area of exposure, as a result, will also remain constant at 24cm2 (12 cm^2 bilaterally).

The irradiance will vary across three experimental doses (50, 300, and ≥700 mW/cm^2) therefore also impacting the exposure time, fluence, and total energy. The low and medium doses will both be administered in continuous wave mode; the high dose will be administered in pulsed wave mode (≥700mW/cm^2). .

Rationale for Constant t-PBM parameters: We will use a *laser source of NIR* since, in contrast to LED devices, lasers on optic fibers are MRI-compatible and can more easily produce high irradiation (e.g. 700 mW/cm^2). The 808 nm NIR wavelength is at the absorption peak of the primary photoacceptor cytochrome-C oxidase[55], has optimal penetration through the skull[12,14], and proven effects on CBF (BOLD signal)[103] and on mood[30,35].. We chose to broadly irradiate and engage the PFC, since: *1)* the aforementioned areas are all involved in emotion-regulation processes[99]; *2)* abnormalities in CBF, which are well-documented in MDD patients, involve broadly PFC[38,39,41].

Rationale for Irradiance Doses: t-PBM at irradiance doses of 50 mW/cm^2 had significant effects on mood[29,30] and cognition[108]; t-PBM at 300 mW/cm^2 have been associated with changes in CBF[103] and on cognition[107,109], while irradiance doses of 700 mW/cm^2 have been effective in depression[30] and in large studies in stroke[21,22,100]. All three doses have been safely used in adults. Therefore, the three irradiance doses proposed (50, 300, and ≥700 mW/cm^2) cover the full spectrum of doses in the literature, enabling us to determine the optimal dose for target engagement

Rationale for Wave Form Selection: We will use *continuous wave (CW)* NIR for the low and medium doses, which is supported by a preponderance of current data..

We will use a pulse wave for the high dose because pulsing can prevent skin warming and discomfort thus facilitating enrollment and retention and safeguarding the integrity of the single blind. Safety data from three previously referenced stroke RCTs including 1,410 participants[21, 22, 100] offer ample evidence that a 700 mW/cm^2 continuous wave dose is safe, However, testing during the development of our study-specific device suggests that some participants may experience it as "hot" and/or unpleasant and be able to distinguish it from the sham and lower doses. Offering the pulsed high dose instead allows us to expose participants to a similar peak irradiance while lowering the average irradiance, thus reducing the likelihood of inducing skin warming or discomfort.

Table 1. t-PBM Parameters Across 4 Sessions

		Dose			
Parameters		High	Middle	Low	Sham
Peak Irradiance (mW/cm^2)		833	300	50	0
Average Irradiance (mW/cm^2)		275	300	50	0
Time Irradiation (s)		600	333	1200	0
Average Fluence (J/cm^2)		165	100	60	0
Total energy (kJ/ cm^2)		2.0	2.4	1.4	0
Wave Mode		Pulsed	Continuous	Continuous	N/A
NIR source		Laser	Laser	Laser	N/A
Wavelength		808nm	808nm	808nm	N/A
Area of exposure		24cm^2	24cm^2	24cm^2	N/A
Anatomical targets		F4, F3	F4, F3	F4, F3	F4, F3

2.5 Rationale

Why test t-PBM parameters with BOLD fMRI

A novel or innovative therapy would not be clinically justified unless it adds significant value in terms of risk/benefit. The limitations of current treatments in MDD are well-documented [**Section 2.1.1**]. Preliminary data suggests t-PBM targeting the PFC could be a novel treatment strategy in MDD [**Section 2.2.1 and**

2.2.2]. However, the t-PBM parameters that would maximize its neural impact and therefore its antidepressant potential are not known. Therefore, this study will apply a novel measurement of changes in BOLD fMRI signal as a marker of changes in CBF in the search for optimal t-PBM parameters, with the **long-term goal of transforming clinical practice for patients with MDD.** Proof of engagement of CBF on PFC would support future clinical testing of this low-cost, time-efficient, accessible and user-friendly intervention in MDD subjects.

We considered alternative measures of CBF, including near-infrared spectroscopy (which is associated with much poorer spatial resolution and can only evaluate CBF changes in the superficial cortex in adults), or PET/SPECT (where changes in CBF over a short period of time would be difficult to quantify, and repeated weekly administrations would be limited by the total dose of radiation). We believe the method we propose offers the best balance of accuracy, spatial resolution and repeatability. In our preliminary data, we found a strong effect at echo 1 (Cohen's d = 1.54, when comparing the echo 1 BOLD immediately before light onset with the BOLD 200 seconds after onset, **(Fig.3).**

Fig. 3: Time series of BOLD changes after t-PBM was applied to the right forehead 10 min. into a 30-min. BOLD scan[103]. The duration of stimulation was 10 min. (grey area). The colored time series indicate the subject-averaged BOLD (shown here in a ROI corresponding to the right medial frontal gyrus, MFG) at echos 1 (blue, 13 ms), 2 (red, 34 ms), and 3 (orange, 55 ms). A rapid increase in the BOLD was observed shortly after stimulation onset, while a second peak emerged later in the 10 min. stimulation window.

The project is innovative because (1) it tests t-PBM with near-infrared light, an innovative technology with a well-established safety profile, and (2) it uses changes in CBF, as measured with changes in BOLD fMRI signal, as a state-of-the-art target engagement biomarker.

2.6 Potential Risks & Benefits

2.6.1 Known Potential Risks

General: By agreeing to participate in this study, subjects temporarily forgo the opportunity to receive regular psychiatric care in the community. For patients with major depressive disorder resistant to medications, alternative options would include ECT, VNS, rTMS, and additional augmentation strategies (psychotherapies, atypical antipsychotics, lithium, T3). The alternatives to this research are clearly explained to all patients, and treatment strategies that are generally used for these patients are discussed. Subjects are informed that t-PBM is not FDA approved for any psychiatric disorder and would therefore not be recommended for use after the study.

Screening and Evaluation: The risks and discomforts of the screening evaluations include discomfort or distress responding to potentially sensitive questions regarding mental illness and drug use. Research interviews are interrupted if individuals become distressed or object to answering questions.

Delay in Initiation of New, or Changes in Ongoing, Antidepressant Treatment: While this study is not testing t-PBM as a treatment for depression, an antidepressant effect of t-PBM is possible. Any delay in receiving t-PBM (while screening procedures are completed) might be considered as a delay in treatment from a patient perspective. We will emphasize that the study is not geared to test a treatment but instead the impact on cerebral blood flow of t-PBM.
Further, study participation requires stable antidepressant treatment. It is therefore possible that the participant's condition could worsen and lead to increased disturbances in mood, anxiety, sleep, appetite, energy and cognition. This could result in work loss, loss of social function, and possibly increased risk of suicide. However, the risk should be minimized as there are several safety precautions in place and participants will have frequent contact with study clinicians.

Risks of Clinical Decompensation: If a patient has worsened to such a degree that further participation would put him or her at risk, then he or she will be discontinued from the study. In such cases, the patient is immediately transferred to standard clinical treatment and exited from the research. At any point during the study, patients are told to immediately inform their study psychiatrist if they develop worsening of depression or anxiety, symptoms of mania or psychosis, or active suicidal plans.

Risks of t-PBM: The study laser device emits light with a longer wavelength than the human eye can see. The staff will be provided training on basic safety procedures relative to the use of the device. The staff administering the t-PBM will be careful not to operate the laser unless it is in direct contact with the subject's skin. Protective eyewear is required since the device is a laser. Failure of laser device, resulting in the cessation of investigative therapy can cause no adverse event to our knowledge. Delivery of the infrared laser energy to an inappropriate site, such as directly over the open eye, would pose risk to the subject, such as blindness. Based on previous observations with similar laser devices, application of the laser may result in mild thermal sensation of warmth during use. The temperature of the skin is however kept well below the level for thermal damage. Based on human clinical trial experience to date and on sale of t-PBM devices for their intended use (e.g. Omnilux New-U), each adverse event listed below has been reported by less than 0.1% of all subjects and users: erythema, pain, discomfort, warmth, headache or other reactions at the application site. In our research experience, t-PBM devices could produce insomnia, irritability and fleeting illusions. The modality of administration for the t-PBM with the study laser device is analogous to the protocols used in our feasibility studies. The dose of light delivered by the study device is analogous to our feasibility studies and to published studies on t-PBM. No serious adverse events occurred during our feasibility trials.

The MGH and NKI sites have extensive experience in transcranial photobiomodulation. The MGH Wellman Center of Photomedicine conducted the first open study on t-PBM in subject with clinically-significant mood symptoms. At the MGH DCRP, Dr. Cassano has conducted two studies on t-PBM in subjects with clinically-significant mood symptoms, also in collaboration with Dr. Iosifescu (NKI/ NYU).

Risks of MR: The risks associated with the MRI procedure are minimal. Subjects who have non-MRI compatible metal implants, cardiac pacemakers, and potential pregnancy will be excluded. Some patients experience claustrophobia (fear of being in a closed space) while inside the machine. In these cases, the examination will be terminated immediately, and the participant will be rapidly removed from the scanner.

2.6.2 Known Potential Benefits

All study participants receive without cost an extensive psychiatric and medical evaluation. No other direct benefits result from study participation. The possible benefits to the patient (remission or improvement of mood and anxiety symptoms) and to others (development of new treatments with sound target engagement of the brain) are reasonable in relation to the risks of this study. The intervention itself with tPBM may potentially provide relief of mood and anxiety symptoms, at a level equal to antidepressant medication.

3 Objectives and Purpose

3.1 Primary Objective

Our primary objective is to identify the t-PBM irradiance dose (50, 300, or ≥700 mW/cm^2) with the largest impact on PFC CBF in MDD.

3.2 Secondary Objective

Our secondary objective is to compare the safety and tolerability of three t-PBM irradiance doses.

3.3 Exploratory Objectives

Our exploratory objectives are to compare the effects of three t-PBM doses on depression symptom severity, anxiety symptom severity, and quality of life.

3.4 Description of Study Design

We will compare the effect of three t-PBM doses (high, middle, and low irradiance) to sham t-PBM on PFC CBF as assessed with fMRI (BOLD) in this multi-center, phase I, single-blind, dose-ranging, crossover study of 30 subjects with MDD. All eligible participants will undergo four sessions of t-PBM during fMRI so that they experience irradiances of 50, 300 and ≥700 mW/cm^2 as well as sham. The order of dose administration will be randomized and t-PBM will be administered with the LightForce® EXPi Deep Tissue Laser Therapy™ System, Transcranial PhotoBioModulation-1000 (tPBM-2.0).

3.5 Study Endpoints

3.5.1 Primary Study Endpoints

The primary endpoint will be t-PBM effects on BOLD signal.

The specificity of t-PBM effects on BOLD signal increases will be assessed by testing three irradiance doses covering a wide range (50, 300 and ≥700 mW/cm^2) versus sham. If a reliable effect on BOLD (compared to sham, the BOLD increase at the optimal dose will have an effect size of Cohen's d≥0.5) is seen with PBM, the study validate BOLD fMRI as a robust biomarker of target engagement for t-PBM and act as a "*go"* signal. In contrast, if none of the t-PBM doses yield reliable BOLD increases, this will fail the putative mechanism (*no go*).

Rationale for the effect size (E.S.) threshold: We have previously observed large effect size changes in BOLD (Cohen's d=0.6) after a single administration of t-PBM (at 318 mW/cm^2)[103]. In our clinical pilot study, significant, large effect size improvements in depressive symptoms were reported in MDD after 8 weeks (Cohen's d=1.5, n=21)[35]. Given these preliminary results, we will use as threshold for the Go/No-Go decision an effect of t-PBM on BOLD at one dose (but not all doses, vs. sham) of magnitude Cohen's d > 0.5 (at least medium size).

3.5.2 Secondary Study Endpoints

The secondary endpoints will be t-PBM effects on brain temperature, suicidal ideation/behavior, and the frequency and severity of adverse events and side effects occurring at the time of each t-PBM session and between each t-PBM session and the subsequent study visit. Brain temperature will be assessed with MR; suicidal ideation/behavior with the C-SSRS. Adverse events will be tracked with the adverse events log; side effects, with the SAFTEE and TSRQ.

3.5.3 Exploratory Endpoints

The exploratory end points include the total scores of the following scales as assessed at the study visit subsequent to each t-PBM session: MADRS, SDQ, ASQ, Q-LES-Q-SF, and NeuroQoL.

4 Study Enrollment and Withdrawal

4.1 Inclusion and Exclusion Criteria

Inclusion Criteria:

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Participants must be able to give written informed consent and follow study procedures.

2. Participants must be 18-65 years of age.
   Rationale: *The lower age limit for participation is 18 years old, due to diagnostic uncertainty in children and adolescents with MDD, as well as differences in response to antidepressants in this population. The enrollment of subjects older than 65 years might introduce a confound, as comorbid cerebrovascular and degenerative pathology is more frequent and might confound the impairment associated with depressive symptoms. Because even subject younger than 65 are at risk of cerebrovascular and degenerative pathology, additional provisions are adopted to exclude subjects with significant cognitive decline.*

3. Participants must have major depressive disorder; all the following conditions need to be met to ensure presence of significant depression symptoms:
   a) Meeting diagnostic criteria for Major Depressive Disorder (MDD) in the past two weeks, at the DSM-5 Mini-International Neuropsychiatric Interview (MINI)
   b) Inventory for Depressive Symptomatology Clinician-rated (IDS-C) total score ≥23 at screening
   c) Depression symptoms are the primary target of treatment or treatment-seeking.

4. Women of child-bearing potential must agree to use adequate contraception (e.g. oral contraceptives, intrauterine device, double barrier methods, or total abstinence from intercourse). Rationale: *Although t-PBM would be unlikely to have an effect on pregnancy and fetal development, the risks to a fetus are unknown; also, depression in pregnant women likely has a different biological and hormonal etiology.*

5. Participants taking medications or psychotherapy approved for the treatment of major depressive disorder will need to be stable for at least 8 weeks prior to screen. Rationale: *Other treatments initiated close to the study intervention could potentially confound the effect of t-PBM on BOLD-MR signal.*

6. Participants must be able to fit comfortably in the study scanner while wearing the study device. Rationale: *We wish to avoid enrolling subjects who will not be able to complete the study because they find the device or scannertoo uncomfortable.*

Exclusion Criteria:

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Unwilling or unable to comply with study requirements

2. Patients judged to be at serious and imminent suicidal (C-SSRS≥4) or homicide risk, or currently in crisis such that inpatient hospitalization or other crisis management should take priority. Rationale: *Suicidal thoughts are prospectively assessed at each visit throughout the study with the C-SSRS scale. Given that the t-PBM intervention is not an approved treatment for major depressive disorder and that the current study is not designed to test treatment-response in a population of major depressive disorder, and given the inclusion of sham, we considered that subjects with severe suicide risk would be better served outside the study frame.*

3. History of any or psychotic or bipolar disorder Rationale: *The aforementioned disorders require the use of specific medications (e.g. antipsychotic medications and mood stabilizers), allowing these disorders would likely increase the heterogeneity of the sample and thus obscure findings.*

4. Met diagnostic criteria for an alcohol or substance use disorder, post-traumatic stress disorder, obsessive-compulsive disorder, anorexia nervosa, or bulimia nervosa within the preceding 12 months *Rationale: We adopt a broader exclusion criterion within the past 12 months to disallow participation by individuals suffering from disorders that would likely increase the heterogeneity of the sample.*

5. History of dementia, traumatic brain injury (TBI), or neurological disorders affecting the brain, including any history of stroke or seizure disorders requiring treatment in the last 5 years (even if controlled with medications). *Rationale: The aforementioned are common, could contribute to the individual's current impairment and may be associated with a different pattern of CBF response to t-PBM.*

6. Cognitive impairment significant as determined by the Montreal Cognitive Assessment (MOCA) <22 or MOCA-Blind <19. *Rationale: We will exclude subjects with significant cognitive decline, which could be a clinical indicator of potential underlying pathology.*

7. History of antisocial personality disorder, or any clinically significant personality trait that would, in the investigator's judgment, preclude safe study participation or impair ability to remain adherent with the treatment protocol.

8. History of significant treatment non-adherence or situations where the subjects are unlikely to adhere to treatment, in the opinion of the investigator. *Rationale: In our pilot studies, subjects were very motivated and good retention rates were demonstrated. However, the study schedule with four, weekly t-PBM-MR sessions requires adequate commitment from study subjects.*

9. Pregnant (as confirmed by pregnancy test at screen) or nursing. *Rationale: The medical risk of using t-PBM during pregnancy and breast-feeding is unknown.*

10. Currently undergoing device-based treatment for depression or taking medications for depression other than SSRIs,SNRIs, or Wellbutrin (bupropion). *Rationale: We wish to minimize the biological heterogeneity of the sample (with respect to effects of t-PBM on CBF).*

11. Treatment resistance with failure to respond to more than two adequate treatments with FDA-approved antidepressant medications during current episode of major depressive disorder. *Rationale: A wide spectrum of treatment resistance would likely increase the biological heterogeneity of the sample (with respect to effects of t-PBM on CBF).*

12. History of ECT in the last 12 months; lifetime history of VNS; lifetime treatment resistance to any FDA-approved device-based treatment for major depressive disorder (such as ECT, TMS, VNS); device-based interventions for depression will need to be discontinued at least 8 weeks prior to screen. *Rationale: We will not allow ECT in the recent past in order to reduce confounding cognitive side effects. Patients previously implanted with VNS, or having failed treatment with ECT or TMS would represent a treatment refractory population which would increase heterogeneity and reduce the chance to detect an efficacy signal.*

13. Serious, unstable medical illnesses including hepatic, renal, gastroenterologic, respiratory, cardiovascular, endocrinologic, neurologic, immunologic, hematologic disease; defined as any medical illness which is not well-controlled with standard-of-care medications (e.g., insulin for diabetes mellitus, HCTZ for hypertension) *Rationale: Although t-PBM appears to be well-tolerated and safe, in subjects with unstable medical illnesses the CBF (and its response to t-PBM) may be altered secondary to their physical illness.*

14. Clinically significant abnormal findings of laboratory parameters including urine toxicology screen for drugs of abuse or at physical examination.

15. Clinical or laboratory evidence of uncontrolled hypothyroidism; if maintained on thyroid medication must be euthyroid for at least 1 month before screening. *Rationale: Hypothyroidism is sometimes associated with depression-like symptoms which would confound the diagnosis and severity ratings.*

16. Past intolerance or hypersensivity to t-PBM.

17. Significant skin conditions (i.e., hemangioma, scleroderma, psoriasis, rash, open wound or tattoo) on the subject's scalp that are found in the area of the procedure sites. *Rationale: These conditions would impede the penetration of the near-infrared (NIR) light.*

18. Any use of light-activated drugs (photodynamic therapy) within 14 days prior to study enrollment. *Rationale: The NIR light would interfere with pre-existing treatments.*

19. Any type of implants in the head, whose functioning might be affected by t-PBM (e.g. stent, clipped aneurysm, embolized AVM, implantable shunt – Hakim valve). *Rationale: The NIR light could, by increasing cerebral blood flow, induce the dislodgement of existing implants.*

20. Failure to meet standard MRI safety requirements (e.g. claustrophobia, non-removable piercings, implanted medical devices, other non-removable metals) as determined by the MRI Safety Checklist.

4.2 Vulnerable Subjects

We will not enroll vulnerable subjects. Further, we will enroll only individuals who are: 1) fluent in English because some of the instruments used in this study have not been translated and validated in other languages; and 2) and literate, as illiterate subjects may not be able to validly complete some of the assessments.

We may enroll NYU students and/or employees. However, we will not enroll any students or employees who are under the direct supervision or oversight of study team members.

4.3 Strategies for Recruitment and Retention

We intend to enroll 30 subjects over 24 months. We believe that our combined track record of recruitment in previous MDD research (see below) and our existing referral sources described below show the feasibility of recruiting sufficient numbers of MDD patients for this study.

This trial will recruit patients at two independent sites: 1) NYU and Nathan Kline Institute (NKI), acting as a single site with two locations in the greater New York City area, and 2) Massachusetts General Hospital (MGH), in Boston. MA. The two sites (NYU/NKI and MGH) will each be responsible for enrolling 50% of the total sample. We anticipate completing the R61 within 22 months, with fMRI data analyzed on a rolling basis.

At NYU, participants will be enrolled by the Mood Disorders Research Program, led by Dr. Dan Iosifescu, which has an excellent track record of recruitment in studies involving the assessment and treatment of subjects with MDD. Participants with MDD will primarily be recruited from the NYU Langone Medical Center (NYUMC) Outpatient Psychiatry Department and from the NYU Bellevue Hospital, as well as from an extensive network of community partners, supplemented with local advertising and recruitment outreach efforts. We have strong relationships with the NYU Langone OPD, where currently 500 MDD patients receive on-going treatment and approximately 300 new bipolar disorder patients are evaluated every year. NYU Bellevue Hospital, with 330 inpatient mental health beds, has ≥ 200 admissions of MDD patients per year. We will have periodic meetings with the Psychiatry staff at NYU Langone and at Bellevue Hospital to facilitate referrals to our study. We are also able to recruit through our relationship with NYU-affiliated community psychiatric clinics in Manhattan and Brooklyn, and with NYU Langone Brooklyn (formerly Lutheran Hospital in Brooklyn), where we have traditionally received referrals from clinicians and from posting NYU IRB-approved advertisements. To further increase our potential enrollment for this study Dr. Iosifescu has established relationships with the Mood Disorders Support Group of New York (MSDG/NY),

an association which provides peer support and education to several hundred of patients with mood disorders in NYC every year. MDSG/NY sees 2-4 new patients or family members in one of the two weekly support groups (2/3 of these patients or family members have an MDD diagnosis). We estimate 104 newcomers to MDSG/NY per year, 50 with an MDD diagnosis.

At NKI, participants will be recruited by the Clinical Research Division (director: Dan Iosifescu), which coordinates all outpatient recruitment for clinical trials. Participants for the proposed study will be recruited from the NKI Volunteer Recruitment Pool (VRP), a core NKI institutional resource maintained by the Outpatient Research Department (OPRD), a component of the Clinical Research Department. Approximately 1,550 individuals (patients and community controls) have been enrolled in the VRP and have undergone formal diagnostic characterization with the Structured Clinical Interview for DSM Disorders (SCID). The VRP maintains close research relationships with outpatient psychiatric aftercare centers throughout the Rockland County area. In addition, we have close relationships with private clinical practices in the area and with patient-support organizations (such as NAMI), which will provide additional referrals.

At MGH, The MGH Depression Clinical and Research Program (DCRP) is a nationally and internationally recognized center in mood disorders research. The DCRP at MGH is part of Partners Health Care (an integrated Hospital System in Boston, which includes MGH; www.partners.org). Taken together, we have access to over 9,000 patients with MDD (Source: Partners Research Patient Data Registry [RPDR]). The DCRP has a successful track record of clinical studies of treatments for all phases of MDD. A query of the Research Patient Database of Partners Healthcare System (of which MGH is a founder) performed on 10/27/18 reveals 17,089 subjects with “ever” a diagnosis of MDD (mean age = 43, 4043 females, 57%) currently followed in the Partners clinics.

For the recruitment at MGH, we will use the following strategies: 1. Advertisements (see below). 2.MGH’s RSVP for Health database. This database can be used to send out postings to volunteers who expressed interest in research. 3. Most of the participants in our feasibility study (ELATED-2 and ELATED-3) were from our general MGH DCRP advertising (e.g. radio and public transportation). The MGH DCRP program has a large volume of studies, however typically only limited device-based interventions are offered. This helps recruitment, as in our tPBM studies, since from the flow of people attracted by general advertising there is a fixed portion that is not interested in traditional interventions (e.g. medication and psychotherapy). We will potentiate this referral source as we will conduct specific advertising for our study; 4. MGH and MGH-affiliated clinics (Charlestown, Chelsea, Revere, MA) will constitute an additional source of referral; 5. Online posting (e.g., Craigslist) has been effective for our most recent MGH studies on tPBM. In both ELATED-2 and -3, we received 1-2 calls per week from Craigslist. 6. Electronic letters can be delivered within the Partners HealthCare System to inform clinicians in the greater Boston metropolitan area about the study. 7. Dr. Cassano (study PI) has also worked for the past 10 years as an attending psychiatrist at North Suffolk Mental Health Association (NSMHA) and has a network of collaborators who are favorably inclined towards clinical research.

All sites will provide local clinicians with IRB-approved materials that provide patients with the necessary information on how to contact the study investigators should they be interested in potential participation. Referring clinicians do not discuss the specific details of the study but inform their patients that this clinical trial is recruiting participants with mood symptoms.

At all sites, an additional source of referrals will be self-referral through media advertisement, and through internet postings on medical and mental health-related web sites. All advertisements will be approved by the IRB.

Interested individuals who contact the research clinic by phone are informed that the information they give over the phone is written down and discussed by the research team. They are advised that if they do not enroll in research, the information will be destroyed, and that if they do enroll, the information becomes part of their research chart, which is destroyed after seven years. An initial IRB-approved phone screening will be completed after they give verbal authorization.

On the phone screen, we have included sensitive questions related to mental illness and drug use in order to establish a general baseline of eligibility for the study over the phone before we invite potential participants to our site. Because this investigational device is being investigated for its use in MDD, we want to make sure that we only bring in individuals with MDD. That is, we do not want to invite people who meet exclusionary criteria to come all the way to our offices if they will be an instant screen fail. The same logic applies to asking about recreational drug use: we ask about drug use because our study includes multiple urine toxicity tests (including the screen visit), and recreational drug use would show on these tests and subsequently make the participant ineligible. Before starting the phone screening interview, we obtain verbal consent (we outline the scope of the interview, that it includes some personal questions related to mental illness and drug use which are necessary to establish eligibility, and that the subject can choose to not answer specific questions). We then ask the subject to confirm they are willing to proceed. For subjects who screen fail, the phone screen material will be shredded at the end of the day, to maintain confidentiality. In order to avoid duplicate screens, the names of subjects who failed the phone screen will be kept in a database hosted on secure HIPAA-compliant servers at each study site, but no confidential information will be recorded in the database (only exclusion criterion # relevant). For subjects who pass the phone screen and are scheduled for an in-person screen visit, we save the phone screen to our secure HIPAA-compliant server for use during the in-person screen visit.

If an individual meets initial enrollment criterion and is interested in participating, a face-to-face interview is conducted with a study investigator. The PI or his designees obtain written voluntary informed consent from all participants prior to any study procedure. The initial consent form describes the nature of the procedures and time requirements, potential risks, the confidentiality of information, and the rights of research participants. Following signing the initial informed consent document, medical and psychiatric screening procedures are undertaken to confirm eligibility. Participants are given a copy of the consent form to review at their leisure, and ample time is given for questions by the prospective study participant (and family member, if applicable). The original signed Consent Forms are kept in a research binder. The Consent Form makes it explicit that the protocol involves return visits at specified times. In particular, the consent form indicates that the nature of intervention is determined by the protocol, and that the study is a randomized investigation to evaluate the impact of tPBM on cerebral blood flow. Patients are informed that if their clinical condition deteriorates during the study, they may be hospitalized. The consent procedure is viewed as a process rather than a single event, and patients are encouraged to discuss the study with the research team at any time**.** Informed consent procedures, other study procedures and tPBM occur at the MGH, NKI and NYU sites.

The consent process also includes documentation of permission to obtain previous medical records, including contact with previous physicians, pharmacies, and family members. A release of information is obtained for review of any available historical and clinical data, permitting the research team to use, create, or disclose his or her PHI for research purposes. If the individual decides not to participate in this study, a staff member provides reasonable and timely assistance in obtaining an alternative referral, if so desired. The decision not to participate does not affect eligibility to participate in future studies, to receive treatment at MGH-, NKI- or NYU-affiliated hospitals, or to receive treatment on a private basis from a referring clinician. Patients are also given the opportunity to withdraw from the study prior to analysis of their data.

Inclusion of Women:
We will enroll eligible subjects regardless of gender. We will aim to enroll women and men, according to the natural prevalence of major depressive disorder (which affects larger number of women) so that we can test whether the transcranial photobiomodulation (tPBM) has the same or a different impact on cerebral blood flow across gender. In a recent study in photobiomodulation at the Massachusetts General Hospital (MGH) (2016P001490), the Depression and Clinical Research Program (DCRP), jointly with other programs, recruited 54 subjects with a range of severity of depression symptoms. Approximately 70% of this sample was female. Similarly, consistent with the prevalence of MDD in the general population, approximately 55% of participants recruited at NKI and at NYU in MDD studies have been females. Consequently, we anticipate our study sample to be approximately 60% women.

Inclusion of Minorities:

MGH: In the present study, we hope to oversample the percentage of minorities that we recruit in order to test the effects tPBM on cerebral blood flow across different races and ethnicities. The MGH DCRP has been successful in the past at recruiting a diverse participation population. In a recent NIH-sponsored study at MGH (1R01MH101486), of the 316 subjects recruited at MGH, approximately 3% were black or African American,12% were Asian, 3% were more than one race, and 10% were Hispanic or Latino.

We intend to target a minority participation rate similar to the past studies. As a result, we plan to recruit a diverse study population that is approximately 20% Hispanic or Latino, 7% black or African American, 13% Asian.

In order to ensure the recruitment of an ethnically and racially diverse study population at MGH, we plan to advertise in communities with high proportions of minority individuals such as East Boston, Somerville, Jamaica Plain, Revere, Everett and Malden (MA). These advertisements will include public transportation postings, community newspapers, and putting up flyers. In the past, these strategies have worked effectively. Another resource we will use is MGH's RSVP for Health, a database consisting of more than 8,000 volunteers who expressed interest in research. This database can be used to send out postings to volunteers based on gender, ethnicity, and race allowing for targeted recruitment of minorities and women. Further, we will recruit through MGH's community clinics (e.g., North Suffolk Mental Health, MGH Charlestown, MGH Chelsea and MGH Revere). Lastly, we will review recruitment regularly at team meetings to track progress and adjust recruitment strategies as needed.

NKI & NYU: Two of the study sites, at the New York University and the Nathan Kline Psychiatric Institute, are situated in greater New York City, one of the largest and most diverse cities in the world. It is expected that the study sample recruited at NYU and NKI will closely approximate the racial and ethnic composition of the greater New York City. The racial and ethnic distribution in Manhattan is: White, not Hispanic or Latino 46.8%; Hispanic or Latino 26.0%; Black or African American 18.2%; Asian 12.8%; American Indian and Alaska Native 1.2% (https://www.census.gov/quickfacts/table/PST045215/36061); the catchment area of the NYU affiliated hospitals in Brooklyn includes 22% Hispanics, 23% white non-Hispanics, 26% Asians and 24% blacks. The Nathan Kline Institute (NKI) is located in Rockland County, NY. It is expected that the study sample recruited at NKI will closely approximate the racial and ethnic composition of the region: White/non-Hispanic origin: 63.9%, Hispanics: 16.8%, Black: 12.9%, Asian: 6.6%, Multiracial: 1.9%, American Indian/Alaska Native 0.5%, Pacific Islander 0.1%. Prior trials conducted at NYU and NKI have consistently obtained a demographic mix that is representative of the greater New York City population. In clinical trials over the past 5 years at NYU and NKI, our gender, racial and ethnic average recruitment was as follows: Female: 55%; Caucasian (non-Hispanic): 38%; Hispanic: 23%; African-American: 25%; Asian: 13%; Other: 3%.

4.4 Duration of Study Participation

Study participation should last between six and twelve weeks. Participants are required to attend one remote screen visit and five clinic visits (second screening visit and four scanning/t-PBM sessions) and answer questions during a single follow-up phone call. If scheduled for consecutive weeks, participants will complete the study in six weeks. However, for logistical regions the time between screening completion and the first scanning/t-PBM session can be as long as four weeks; in addition, as long as two weeks may pass between each scanning/t-PMB session.

4.5 Total Number of Participants and Sites

We expect to enroll a total of 62 participants across three sights (NYUMC, NKI, and MGH). Of these, we expect 7 will be enrolled and screened at NYUMC; 14, at NKI. Of the New York cohort, we expect 15 to be eligible and subsequently scanned and treated with r-PBM at NKI. Further we expect 21 will be enrolled and screened at MGH and a subset of 15 eligible participants scanned and treated at MGH.

In summary, recruitment will end when approximately 62 participants are enrolled. It is expected that approximately 62 participants will be enrolled in order to produce 30 eligible participants.

Table 2: Expected Enrollment Per Site		
	Enrolled/Screened	Scanned and treated with t-PBM
NYUMC	12	5 (at NKI)
NKI	19	10
MGH	31	15

Site Management Plan:

The PIs will have weekly teleconference meetings during which any adverse events (AEs), unexpected problems, interim results or planned protocol modifications will be discussed. The PIs will also meet in-person at a minimum of twice yearly to review all aspects of study progress and safety.

The Innovative Clinical Research Solutions (ICRS) group at the Nathan Kline Institute (NKI) will be the Data Coordinating Center (DCC), providing a comprehensive web-based data acquisition and management system, (Acquire) to process, edit and store all study data in a centralized, secured, and password-protected database. Individual sites will enter data directly into the centralized Acquire database.

Study participant identifiers will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

4.5.1 Research Use of Stored Human Samples, Specimens, or Data

- Intended Use: Data collected under this protocol may be used to study mood and anxiety disorders. No genetic testing will be performed.
- Storage: Samples will not be stored. Data will be stored using codes assigned by the investigators. Data will be kept in password-protected computers. Only investigators will have access to the samples and data.
- Tracking: A tracking file will link identification codes with identifying information, but this file will be password-protected and stored on a computer that can also only be accessed with a password.

4.6 Participant Withdrawal or Termination

4.6.1 Reasons for Withdrawal or Termination

Participants are free to withdraw from participation in the study at any time upon request. An investigator may terminate participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Subject fails to adhere to protocol requirements so that the integrity of the data is compromised (e.g. subject fails to respond to attempts to schedule subsequent visits such that more than allotted time passes between scanning/treatment visits).

4.6.2 Handling of Participant Withdrawals or Termination

Every effort will be made to conduct the follow-up assessment via phone with all withdrawn or terminated participants. The phone assessment includes

If a site is unable to contact a subject or if the subject fails to appear for a visit, three documented phone calls should be made, followed by a letter (or its equivalent). The letter should detail the need for the subject to appear for a visit, the site's unsuccessful attempts to contact the subject, and that failure to contact the site will result in the subject being withdrawn from the study.

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

4.7 Premature Termination or Suspension of Study

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to all investigators, and the funding agency. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, data quality are addressed and satisfy the sponsor, IRB and/or FDA.

5 Study Agent (Study drug, device, biologic, vaccine etc.) and/or Procedural Intervention

5.1 Study Agent(s) and Control Description

LightForce® EXPi Deep Tissue Laser Therapy™ System, Transcranial PhotoBioModulation-1000 (tPBM-2.0)

As described in **Section 2.2**, the tPBM-2.0 is an investigational device based on LiteCure's LightForce® EXPi Deep Tissue Laser Therapy™ System. For the investigational study, the EXPi System's beam delivery - Empower™, is modified to non-invasively deliver Near-Infrared Radiation (NIR) to subjects diagnosed with MDD. The modified system is also configured to provide sham (placebo) treatment. The device is manufactured and supplied by LiteCure LLC, 101 Lukens Dr, Suite A, New Castle, DE 18720.

The tPBM-2.0 is considered a Class II medical device per 21 CFR 890.5500 and 878.4810 and is manufactured per 21 CFR 820. It utilizes a laser diode source with a maximum continuous (CW) output of ≤30 Watts at a wavelength of 808 nanometers (nm) and nominal beam diameter of 40mm at the outside aperture.

The tPBM-2.0 operates in one of five modes: NIR low irradiance (50 mW/cm^2), NIR middle irradiance (300 mW/cm^2), continuoushigh irradiance (700 mW/cm^2), NIR pulsedhigh radiance (peak irradiance 833 mW/cm^2) and sham. The device's behavior, performance/output of all visible and audible indicators including the graphic user interface, is identical for all modes, which differ only with respect to the parameters of laser emission. In simple terms, NIR modes of the tPBM-2.0 produces laser energy; sham mode does not produce laser energy. Therefore, since the laser radiation emitted during NIR mode is invisible to the naked eye, the modes are indistinguishable from one another.

The tPBM-2.0 consists of a therapeutic laser console (that produces laser energy as NIR), and an optical delivery system consisting of a flexible, double-sheathed optical fiber connected to a custom helmet (cap). The cap is configured to deliver NIR light to EEG sites F4 and F3 (or in close proximity if covered by hair), covering a total surface treatment area of approximately 24 cm^2[12 cm^2 x 2]. It also includes laser safety eye ware with an optical density rating >5.0 at 808 nm. Participants will be blinded to the order in which they experience each of the four modes. The treatment mode administered at each session will depend on a pre-determined randomization scheme

The therapeutic laser console is the only component of the device that is not MRI compatible. It will be stored in an equipment room located in Zone 2 (area with no detectable magnetic field but to which access is restricted due to scanner proximity). An MRI-compatible optical fiber will connect the laser console to the MRI-compatible cap. The safety eye wear is also MRI compatible.

Aside from the cap, the tPBM-2.0 is the same device as LiteCure's EXPi System – Model LTS-2500, which is marketed under FDA's 510(K) # K107637.

The cap is a custom 3D-printed headgear made of plastic and serves to hold the laser probe in place, between the MRI coil and the subject's head. The cap is provided by the device manufacturer (LiteCure) and is very similar to those used in our previous t-PBM studies. The laser probe (attached to the cap) will be connected to the laser console with a multimode MR-compatible optical fiber. The distal end of the optical fiber is fitted with ceramic ferrules, which will be affixed to the cap. The cap (headgear) contains clamps which secure the ferrules. The cap will be used in place of the wand. The cap is the only modification of the device delivery system, and it was required in order to do the t-PBM inside the MRI scanner.

The t-PBM-2.0 is a nonsignificant risk device because it:
(1) Is NOT intended as an implant and DOES NOT present a potential for serious risk to the health, safety, or welfare of a subject;
(2) Is NOT purported or represented to be for a use in supporting or sustaining human life and DOES NOT present a potential for serious risk to the health, safety, or welfare of a subject;
(3) Is NOT for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject; or
(4) DOES NOT otherwise present a potential for serious risk to the health, safety, or welfare of a subject.

Further,

- The t-PBM-2.0 maximum dose administered in this study does not exceed the Maximum Permissible Exposure (MPE) for skin – Center for Devices and Radiological Health (CDRH), 21CFR1040.10 and 21CFR1040.11;
- The t-PBM-2.0 is substantially equivalent to the OmniLux New U which the FDA has already categorized as a non-significant risk device;
- Currently FDA-cleared lasers are typically available over-the-counter and are considered safe to use without professional supervision.

At NKI both research coordinators and co-investigator Dr. Kate Collins, PhD, LCSW will operate the study device and perform other procedures under the close supervision of Dr. Iosifescu (PI). As in our previous studies with photobiomodulation at NKI, all study personnel involved in operating the laser will complete (and have valid training certificates for) the American Institute of Medical Laser Applications Medical Laser Safety training. In addition, they will be trained by the device manufacturer directly in device operation. Dr Iosifescu, the NKI research nurse (Sheela Sajan, RN), and one of the NKI research coordinators already have experience with the device, having administered t-PBM treatments in a prior study at NKI.

5.1.1 Acquisition

The t-PBM 2.0's will be provided and shipped to sites by LiteCure®

5.1.2 Product Storage and Stability

The device(s) will be stored in MRI equipment rooms at NKI and MGH. These rooms are secure and well-ventilated and have fire extinguishers readily available. The devices will be placed on flat hard surfaces with adequate airflow (including a minimum of 10cm clearance around the back of the device).

5.1.3 Dosing and Administration

Across the four visits participants experience one session each of: 1) sham t-PMB; 2) low-irradiance t-PBM; 3) middle-irradiance t-PBM; and 4) high-irradiance t-PBM. The sequence in which the particular t-PBM parameters (summarized in Table 1) are utilized will be random; both participant and study staff will be blind to the parameters.

5.1.4 Duration of Therapy

Participants will undergo three sessions of active and one session of sham t-PBM, all during MR scanning. The duration of irradiation is 0, 3.33 or 20 minutes, depending on randomization; however, each session will last a total of 20 minutes to maintain blinding.

5.2 Study Agent Accountability Procedures

LiteCure will ship one device each to NKI and MGH. Sites will document the date and condition of the devices at reception and eventual return (via mail) at study conclusion.

6 Study Procedures and Schedule

6.1 Study Procedures/Evaluations

Note that as many procedures as possible can and will be conducted remotely, via telephone or WebEx. Procedures marked with an asterisk (*) will require in person clinic visits.

6.1.1 Study Specific Procedures versus Standard Clinical Care

All procedures described in sections 6.2 and 6.3 will be performed as part of the study. None are part of standard clinical care.

6.2 Study Schedule

6.2.1 Screening Phase

Duration: Up to 4 weeks, usually requires 1 remote session and 1 clinic visit.
(Additional visits may be required to repeat laboratory testing in the case of suspect results, etc.)

Study staff will:

- Obtain informed consent of potential participant verified by signature on written informed consent for screening form.
    - If the first screen visit occurs remotely, the staff will obtain informed consent of the participant via WebEx (for explanation and discussion of form) and eConsenting via RedCap.
- Obtain contact and demographic information required for study participation.
- Administer the Montreal International Neuropsychiatric Interview (MINI), the Inventory for Depressive Symptomatology, Clinician-Rated scale (IDS-C), the Montreal Cognitive Assessment (MoCA), the Antidepressant Treatment Resistance Questionnaire (ATRQ), the MRI safety checklist,

and the Columbia Suicide Severity Risk Scale (C-SSRS), to determine eligibility based on inclusion/exclusion criteria.
- Administer the NIS-Skin Color Scale (NIS-SCS).
- Review medical history to determine eligibility based on inclusion/exclusion criteria.
- Review concomitant medications and therapies to determine eligibility based on inclusion/exclusion criteria.
- *Perform medical examinations needed to determine eligibility based on inclusion/exclusion criteria (includes recording vital signs).
- *Collect blood (if indicated)/urine for laboratory testing to determine eligibility based on inclusion/exclusion criteria (see section 6.2.2).
- *Test study device fit.
- Verify inclusion/exclusion criteria.

Schedule study visits for participants who are eligible and available for the duration of the study. Test Fit:Study staff will introduce the participant to the study device and confirm that they will be able to fit comfortably in the scanner while wearing it. We will exclude any participant who cannot fit.

6.2.2 Clinical Laboratory Evaluations

*Screening laboratory tests will include:
- ***Hematology:*** *hemoglobin, hematocrit, white blood cells (WBC) with differential count, platelet count. [DISCRETIONARY, may be ordered if physical exam or medical history indicates need.]*
- ***Biochemistry:*** *creatinine, total bilirubin, alanine aminotransferase (ALT), aspartate*
- *aminotransferase (AST), thyroid stimulating hormone (TSH). [DISCRETIONARY, may be ordered if physical exam or medical history indicated need.]*
- ***Urinalysis:*** *dipstick urinalysis, including protein, hemoglobin and glucose; toxicology and (when applicable) pregnancy testing.*

6.2.3 Experimental Phase

Duration: 4 visits over 4-8 weeks
Timing: No more than 2 weeks between each visit.
While the provision of study treatment requires clinic attendance, we may conduct other components of the experimental visits remotely.

At each of the four visits, study staff will:
- *Record vital signs;
- Administer
  MADRS,
  CGI-I (except Visit 1 as this will be considered a baseline rating),
  CGI-S,
  C-SSRS, and
- MRI Safety Checklist; Record concomitant medications/therapies;
- *Administer the t-PBM session during MR Scanning;
- Record adverse events as reported by participant or observed by investigator.

In addition, participants will complete:
- PANAS,
- PBQ
- PCQ
- SDQ,
- ASQ,
- SAFTEE

- Neuro-QoL
- Q-LES-Q-SF, and
- TSRQ

MGH Site Neuronavigation: The BrainSigth Neuronavigation System will be used at the MGH site to track the initial placement of the laser device on the subject's forehead and will be utilized to ensure accurate replication of positioning at each experimental visit. This is a recording device that does not involve any added risk or stimulation. Traditionally, this device is used to record precise location of EEG electrodes and transcranial magnetic stimulation (TMS) coils. In our case, we will be using it to record the position of the outlet of the laser before every PBM session. The BrainSigth Neuronavigation System is FDA approved and is used as indicated. This device bounces light off of trackers that are placed on the forehead. Study staff lightly place a wand at various spots on the head. The system correlates the position of the wand with that of the trackers to precisely record the location of the head in relation to the photobiomodulation stimulation device. This is useful for later analysis, in which we will co-register the recorded laser position to the subject's MRI.

- For the NKI/NYU scans, lipid capsules are affixed to the device for demarcation of device location in the scans.

MR: Scanning will begin with acquisition of structural images and (for most subjects) a baseline MR thermometry scan. Subsequently we will collect approximately 60 minutes of functional data collection including ~20 minutes before t-PBM, ~20 minutes coinciding with t-PBM, and ~20 minutes following t-PBM, as well as (for most subjects) a post-t-PBM MR thermometry scan. (For technical and logistical reasons, we may opt to skip the thermometry scan for some subjects.)

Note that every scan performed in this study is saved and handled under the standard PHI confidentiality restrictions and regulations employed for patients' information. At least one scan per subject is additionally reviewed by a radiologist or neurologist with experience reading MRI scans, who might then detect an abnormality. If clinically useful information is uncovered, either the Principal Investigator or another clinician on the study will speak to the subject in person or on the telephone regarding the new information. A copy of the original image report will also be provided to the subject in person and the subject will be encouraged to follow up on the discovery with his or her treating physician.

t-PBM sessions: Across the four visits participants experience one session each of: 1) sham t-PMB; 2) low-irradiance t-PBM; 3) middle-irradiance t-PBM; and 4) high-irradiance t-PBM. The sequence in which the particular t-PBM parameters (summarized in Table 1) are utilized will be random.

6.2.4 Follow-up Phase

Duration: 1 phone call
Timing: Within one week of fourth t-PBM/scan session

Over the telephone study staff will administer the

- CGI-I
- CGI-S
- MADRS, and
- C-SSRS, and
- record concomitant medications/therapies and any adverse events.

Participants will also complete the following self-report scales remotely using Acquire:

- PANAS
- PCQ
- SDQ,
- ASQ,
- SAFTEE,
- Neuro-QoL

6.2.5 Q-LES-Q-SF. Withdrawal/Early Termination Visit

If early withdrawal or termination occurs, every effort will be made to schedule and conduct all assessments initially scheduled for the **Follow-up Study Visit** (See **Section 6.2.4**).

6.2.6 Unscheduled Visit

Any unscheduled visits will be documented with notes to file to be kept in study subjects' binders.

6.3 Concomitant Medications, Treatments, and Procedures

All concomitant medications taken, and any other therapies intended to treat MDD that transpire, during study participation will be recorded on the case report forms (CRFs). For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the CRF are concomitant prescription medications, over-the-counter medications and non-prescription medications.

6.4 Prohibited Medications, Treatments, and Procedures

SSRIs, SNRIs, or psychotherapy are permitted but will need to be stable for at least 8 weeks prior to the screen. Any device-based interventions for depression will need to be discontinued, at least 8 weeks prior to screen. *Rationale: Other treatments initiated or modified close to the study intervention could potentially confound the effect of t-PBM on BOLD-MR signal.*

If, due to worsening depression or other medical event, a change in antidepressant therapies is required during the study, participants may be asked to withdraw at that time at the discretion of the principal investigators.

6.5 Prophylactic Medications, Treatments, and Procedures

N/A

6.6 Rescue Medications, Treatments, and Procedures

N/A

6.7 Participant Access to Study Agent at Study Closure

Participants will not have access to the study agent at study closure.

7 Assessment of Safety

7.1 Specification of Safety Parameters

In addition to recording and classifying adverse events (Sections 7.1 and 7.2), participants will also complete the SAFTEE and TSRQ at each visit.

7.1.1 Definition of Adverse Events (AE)

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal;
- is associated with a serious adverse event;
- is associated with clinical signs or symptoms;
- leads to additional treatment or to further diagnostic tests; and/or
- is considered by the investigator to be of clinical significance.

7.1.2 Definition of Serious Adverse Events (SAE)

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal;
- life-threatening;
- requires or prolongs hospital stay;
- results in persistent or significant disability or incapacity;
- a congenital anomaly or birth defect; and/or
- an important medical event.

Important medical events are those that may not be immediately life threatening but are clearly of major clinical significance. They may jeopardize the subject and may require intervention to prevent one of the other serious outcomes noted above. For example, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

7.1.3 Definition of Unanticipated Problems (UP)

An unanticipated problem is any incident, experience, or outcome that meets all the following criteria:

- Is unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc); and
- Is related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

7.2 Classification of an Adverse Event

7.2.1 Severity of Event

For AEs, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

7.2.2 Relationship to Study Agent

The clinician's assessment of an AE's relationship to study device is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study agent assessed. To help assess, the following guidelines are used.

For all collected AEs, the clinician who examines and evaluates the participant will determine the AE's causality based on temporal relationship and his/her clinical judgment. Evaluation of relatedness must consider etiologies such as natural history of the underlying disease, concurrent illness, concomitant therapy, study-related procedures, accidents, and other external factors. The degree of certainty about causality will be graded using the categories below.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

7.2.3 Expectedness

Principal investigators will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described in this protocol for the study agent.

7.3 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate RF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on

study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. UPs will be recorded in the data collection system throughout the study.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

7.4 Reporting Procedures – Notifying the IRB

7.4.1 Adverse Event Reporting

AEs that are unanticipated, determined to be at least "probably related" to the study intervention, and reveal a "greater risk of harm" than originally expected will be considered reportable new information and reported within 5 business days of the principle investigator(s) becoming aware of the event.

Reports, signed by a principle investigator, will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome is unanticipated, at least probably related to the study intervention, and reveals a "greater risk of harm" than originally expected;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the unanticipated adverse events.

All other adverse events will be reported annually to the IRB.

7.4.2 Serious Adverse Event Reporting

All SAEs will be reported to the IRB as soon as possible and certainly within 5 business days of the principle investigator(s) becoming aware of the event.

Reports, signed by a principle investigator, will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;

- An explanation of the basis for determining that the event, incident, experience, or outcome represents a serious adverse event;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the serious adverse event.

7.4.3 Unanticipated Problem Reporting

Unanticipated problems that are determined to be at least "probably related" to the study intervention, and reveal a "greater risk of harm" than originally expected will be considered reportable new information and reported within 5 business days of the principle investigator(s) becoming aware of problem. Other unanticipated problems will be reported within 10 business days of the principle investigator(s) becoming aware of the problem.

Reports, signed by a principle investigator, will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome is unanticipated, at least probably related to the study intervention, and reveals a "greater risk of harm" than originally expected;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the unanticipated problem.

7.4.4 Reporting of Pregnancy

Any pregnancy will be considered reportable new information and reported as soon as possible and certainly within 5 business days of the principle investigator(s) becoming aware of the pregnancy.

7.5 Reporting Procedures – Notifying the Study Sponsor

The study clinician will complete an SAE Report within the following timelines:

- All deaths and immediately life-threatening events, whether related or unrelated, will be recorded on the SAE Form and submitted to the study sponsor within 24 hours of site awareness. See Section 1, Key Roles for contact information.
- Other SAEs regardless of relationship will be submitted to the study sponsor within 72 hours of site awareness.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the adherence to be stable. Other supporting documentation of the event may be requested by the DCC/study sponsor and should be provided as soon as possible.

As a follow-up to the initial report, within the following 48 hours of awareness of the event, the investigator shall provide further information, as applicable, on the unanticipated event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Unanticipated Problem report, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing unanticipated adverse effects shall be provided promptly to the study sponsor.

The study investigator shall complete an Unanticipated Adverse Device Effect Report and submit to the study sponsor and to the reviewing IRB as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect. The study sponsor contact information is provided in Section 1, Key Roles.

As a follow-up to the initial report, within the following 48 hours of awareness of the event, the investigator shall provide further information, as applicable, on the unanticipated event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Unanticipated Problem form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing unanticipated adverse effects shall be provided promptly to the study sponsor.

The principal investigators are responsible for conducting an evaluation of an unanticipated adverse device effect and shall report the results of such evaluation to sponsor, FDA and to all reviewing IRBs and participating investigators within 10 working days after first receiving notice of the effect. Thereafter the sponsor shall submit such additional reports concerning the effect as FDA requests.

7.6 Participating Investigators

Each site will report unanticipated problems and all SAEs to the others within 10 days of site awareness.

7.7 Study Halting Rules

If at any time during the course of the study, the DSMB judges that risk to subjects outweighs the potential benefits, the DSMB shall have the discretion and responsibility to recommend that the study be terminated.

7.8 Safety Oversight

The PIs will regularly monitor potential risks and procedures for protecting risk: Drs. Iosifescu and Cassano will monitor the risks of evaluations and treatments provided at NYU, NKI, and MGH, respectively. The PIs will have weekly teleconference meetings during which any adverse events (AEs) or unexpected problems (UPs) will be discussed. The PIs will also meet in-person or via video conference at a minimum of twice yearly to review all aspects of study progress and safety. Each PI is ultimately responsible for monitoring the data and safety at that site and will provide continuous, close monitoring of adverse events. The investigators and study coordinators will evaluate the progress of the study, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the various sites, and other factors that can affect study outcome. They will also consider factors external to the study, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study.

Data and Safety Monitoring Board (DSMB)
A DSMB will be created as an independent body charged with ensuring that the safety of study subjects is protected and that the scientific goals of the study are being met. The DSMB (members and credentials listed below) will be responsible for data safety monitoring for the overall study. To support those purposes, the DSMB will review any proposed amendments to the study protocol, perform expedited monitoring of all serious adverse events, perform ongoing monitoring of drop-outs and non-serious adverse events, determine whether study procedures should be changed or the study should be halted for reasons related to the safety of study subjects, and perform periodic review of the completeness and validity of data to be used for analysis of safety and efficacy. The DSMB will also ensure subject privacy and research data confidentiality.

Membership of the DSMB
The DSMB will consist of the following three members with expertise in the following area(s): clinical trials in psychiatric populations with mood disorders, neuromodulation devices, and regulatory aspects of clinical research.

1. Dr. Cristina Cusin, M.D.,is the Director of Translational Studies in Depression at Massachusetts General Hospital and an Assistant Professor in Psychiatry at Harvard Medical School. Dr. Cusin has significant experience with clinical and translational research studies in patients with major depressive disorder, including specific expertise in neuromodulation (device-based) therapies including ECT, VNS, DBS.

2. Dr. Manish Jha, M.D., is Assistant Professor of Psychiatry and Neuroscience and Assistant Director of the Depression and Anxiety Center for Discovery and Treatment at the Icahn School of Medicine at Mount Sinai, New York, NY. Dr. Jha conducts clinical and translational research studies in patients with major depression and related disorders; he has significant expertise with neuromodulation (TMS) in clinical and research settings.

3. Dr. Menachem Krakowski, M.D., Ph.D., is a Research Scientist at the Nathan S. Kline Institute for Psychiatric Research (NKI) and a Research Associate Professor in the Department of Psychiatry at NYU School of Medicine. Dr. Krakowski has significant expertise in conducting clinical trials in subjects with severe mental illness and in the regulatory aspects of clinical research (as a long-standing member of the NKI IRB).

Monitoring of Safety Data by the DSMB
The Board will meet, by teleconference, at least bi-annually (every six months), and will have conference calls more often as needed.

There are no predetermined stopping rules for the study. Every 6 months the DSMB will review side effects and determine whether the study may continue for the next 6 months.

Dissemination of DSMB findings to Sites
Following the DSMB meetings, the chair of the DSMB will discuss the safety data with the other board members, and together they make a determination about continuation of the study. This information is communicated both verbally to the PIs (informally at the end of the teleconference), as well as in a written report prepared by the Chair and sent to the PIs of both sites following the meeting.

Unblinded Reporting
Safety information for this study will be reported to the DSMB in an unblinded manner. A statistical penalty will not be assessed for the ongoing unblinded review of safety by the DSMB. Unblinded data will not be released to the investigators unless necessary for safety reasons.

Range of Safety Reporting to the DSMB
The DSMB will review adverse events (AEs) and serious adverse events (SAEs) as recorded in the adverse events logs as well as, other data that may reflect differences in safety between treatment groups. This includes treatment retention rates, reasons for drop-out, and laboratory values reflecting potential toxicity.

Serious Adverse Events
Expedited review will occur for all SAEs. For purposes of this study, all SAEs will be reported to the DSMB, regardless of any judgment of their relatedness to the study intervention. All relevant information will be reported to the DSMB for each SAE including information about the event and its outcome, dosing history of the study intervention, concomitant medications, the subject's medical history and current conditions, and all relevant laboratory data. Notification by e-mail and fax transmittal of all related study forms shall be made to the DSMB within 7 days of the occurrence of any SAE. Information will be reviewed and a determination made of whether there was any possible relevance to the study intervention.

Non-Serious Adverse Events
At periodic intervals (biannually during the course of the study and then again at its completion), the DSMB will be provided with unblinded summaries of the numbers and rates of adverse events by treatment group. These reports will include types of events, severity, and treatment phase. Data on individual non-serious adverse events is not expected to be needed for this review.

Other Safety-Related Reports
Biannually, the DSMB will also receive unblinded summary reports of treatment retention reasons for drop-out, and laboratory values reflecting potential toxicity by treatment arm.

Monitoring of Data Quality by the DSMB

During the course of the study, the DSMB will receive reports on data quality and completeness. At a minimum, these will include an overview of the progress of patient intake and retention; summary reports describing patient adherence with visits, evaluations, and dosing as described in the protocol; and a summary of the completeness and quality of key data elements needed to characterize patients, their dosing, and their primary and secondary outcomes. These reports will be used by the DSMB to evaluate the capacity of the data capture and processing to support scientifically valid analyses.

Annual DSMB Report to Sponsor
Every twelve months, the DSMB will prepare a summary report of its findings regarding safety and quality based on data received to that point in the study. This report will include a summary of all safety findings, as well as an assessment of protocol compliance and data quality. Any recommendations to improve patient safety,

8 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s). Each clinical site will perform internal quality management of study conduct, data collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

9 Statistical Considerations

9.1 Statistical Hypotheses

Since the main goal of the study is to obtain evidence about the mechanism of action, the feasibility of implementation, and the potential for efficacy of novel treatment strategies that, if warranted, will be tested in future confirmatory studies, the emphasis of the statistical analysis will be on estimation (with effect sizes and confidence intervals) rather than on hypothesis testing.

9.2 Analysis Datasets

We will have two datasets:
1) An Intention-To-Treat (ITT) dataset including all randomized participants (those eligible after screening); and
2) A Modified Intention-To-Treat (MITT) dataset including all participants who undergo at least one scan/t-PBM session.

The MITT dataset will be used for almost all analyses. We will use the ITT dataset only to query whether there are any demographic or clinical differences between the groups of subjects that do and do not undergo at least one scan.

9.3 Description of Statistical Methods

9.3.1 General Approach

Before any statistical techniques are applied, the distribution of all variables will be investigated using descriptive statistics and outliers will be examined. Where necessary, non-parametric statistical methods will be used rather than relying on distributional assumptions. Since the main goal of the study is to obtain evidence about the mechanism of action, the feasibility of implementation, and the potential for efficacy of novel treatment strategies that, if warranted, will be tested in future confirmatory studies, the emphasis of the statistical analysis will be on estimation (with effect sizes and confidence intervals) rather than on hypothesis testing. Where formal hypothesis testing is employed, significance will be judged at 5% level, two-sided.

9.3.2 Analysis of the Primary Efficacy Endpoint(s)

For each subject at each t-PBM dose (including sham) we will obtain the BOLD signal change measured in left and right dorsolateral prefrontal cortical regions of interest. These measures will be modeled as a function of dose (considered a categorical variable with 4 levels) using mixed effects models[117], including random subject intercepts to account for the potentially correlated measures on the same subject. Since the doses will be presented to subjects in a random order, the initial model will also include order of the dose (a continuous variable) and all interactions between the dose and the order. If there is evidence for an interaction effect between dose and order, this effect will be investigated to understand how the dose effect varies as a function of week of treatment, and in such case effect size of the t-PBM doses will be estimated using only the data when the dose was given in the first week. If there is no interaction between dose and order, the order variable and the interactions will be omitted and the resulting model will be used to estimate the mean BOLD signal measures at each dose and the differences between each active t-PBM dose and sham.

9.3.3 Analysis of the Secondary Endpoint(s)

For each subject at each t-PBM dose (including sham) we will compute changes in brain temperature using data recorded just before and after the t-PBM administration with MR thermometry scans.(Note that for technical and logistical reasons, some subjects may not undergo MR thermometry.) We will quantify the frequency and severity of suicidal ideation experienced as well as the presence or absence of suicidal behaviors after each t-PBM session using the C-SSRS. Finally, we will use the SAFTEE, TSRQ, and adverse events logs to determine the frequency and severity of side effects and adverse events. We will model these measures as a function of dose (considered a categorical variable with 4 levels) using mixed effects models[117], including random subject intercepts to account for the potentially correlated measures on the same subject. Since the doses will be presented to subjects in a random order, the initial models will also include order of the dose (a continuous variable) and all interactions between the dose and the order. If there is evidence for an interaction effect between dose and order, this effect will be investigated to understand how the dose effect varies as a function of week of treatment, and in such case effect size of the t-PBM doses will be estimated using only the data when the dose was given in the first week. Whenever we find there is no interaction between dose and order, the order variable and the interactions will be omitted. The resulting models will be used to estimate the mean of each measure at each dose and the differences between each active t-PBM dose and sham.

9.3.4 Safety Analyses

See **Section 9.3.3.**

9.3.5 Exploratory Analyses

We will quantify 1) depression severity using the MADRS and SDQ scores, 2) anxiety severity using the ASQ scores, and 3) perceived quality of cognition using the Neuro-QoL scores and perceived quality of life using the Q-LES_Q-SF, as assessed at the visit after each dose of t-PBM.

We will model these measures as a function of dose (considered a categorical variable with 4 levels) using mixed effects models[117], controlling for baseline scores (from the first scan/t-PBM visit) and including random subject intercepts to account for the potentially correlated measures on the same subject. Since the doses will be presented to subjects in a random order, the initial models will also include order of the dose (a continuous variable) and all interactions between the dose and the order. If there is evidence for an interaction effect between dose and order, this effect will be investigated to understand how the dose effect varies as a function of week of treatment, and in such case effect size of the t-PBM doses will be estimated using only the data when the dose was given in the first week. Whenever we find there is no interaction between dose and order, the order variable and the interactions will be omitted. The resulting models will be used to estimate the mean of each measure at each dose and the differences between each active t-PBM dose and sham.

9.4 Sample Size

We anticipate no more than 20% dropout during the 4-week intervention, which would result in about 24 observations at each of the 4 doses. This sample size would allow us to estimate the effect size of the optimal dose within 0.37 units (80% CI), or 0.57 (95% CI), which is considered an adequate level of evidence for the goals of the grant (R61) mechanism. Note that the analytic method is mixed effects model which will provide somewhat better power than the one reported above under worst-case scenario. Also of note, we observed a large effect size for BOLD signal changes (d=1.54) during t-PBM in the right MFG in our preliminary data[103]

9.5 Measures to Minimize Bias

9.5.1 Enrollment/Randomization/Blinding Procedures

Subjects will be randomized to a specific series order of the 4 t-PBM/sham doses. There are 24 possible orders, 8 of which, (obtained from 2 different 4x4 Latin squares) will be used in each site. The study statistician (Dr. Petkova) will provide a single list to each site of block randomization of the order in which the 4 t-PBM-sham doses will be administered to each participant. This will be programmed in each site's laser device interface. Subjects and will be blind to the intervention assignment. Sham adequacy will be tested with questionnaires (PBQ) at every visit.

Blinding is maintained by including a heating element in the device which activates during sham mode, so subjects perceive a mild heating at skin level just as they would during active mode.

We expected that, by randomizing the order of the t-PBM doses we will have equal attrition in all t-PBM arms, with 24 analyzable data points in all 4 dose groups. Hence, we will not replace participants.

9.5.2 Evaluation of Success of Blinding

We will use the placebo blinding questionnaire (PBQ) to evaluate the success of blinding.

10 Source Documents and Access to Source Data/Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial. It is acceptable to use CRFs as source documents. If this is the case, it should be stated in this section what data will be collected on CRFs and what data will be collected from other sources.

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

Access to study records will be limited to IRB-approved members of the study team. The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.).

The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Quality Assurance and Quality Control

QC procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written SOPs, the data coordinating center will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements.

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

12 Ethics/Protection of Human Subjects

12.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

12.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

12.3 Informed Consent Process

12.3.1 Consent/Assent and Other Informational Documents Provided to Participants

Consent forms (submitted with this protocol) describing in detail the study agent, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study product. During the COVID-19 pandemic, online remote consenting via RedCap will be used by the study staff. No study procedures are to occur before the participant gives their informed consent, whether that is an eConsent or an in-person consent.

12.3.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families in person or via WebEx. Consent forms will be IRB-approved, and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think

about it prior to agreeing to participate. The participant will sign (e-sign if consented remotely) the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the signed informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

A copy of the signed informed consent document will be stored in the subject's research record or (if digital), on the REDCap server The consent process, including the name of the individual obtaining consent, will be thoroughly documented in the subject's research record. Any alteration to the standard consent process (e.g. use of a translator, consent from a legally authorized representative, consent document presented orally, etc.) and the justification for such alteration will likewise be documented.

12.4 Posting of Clinical Trial Consent Form

The informed consent form will be posted on the Federal website after the clinical trial is closed to recruitment, and no later than 60 days after the last study visit by any subject, as required by the protocol.

12.5 Participant and Data Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study;
- Who will have access to that information and why;
- Who will use or disclose that information ; and
- The rights of a research subject to revoke their authorization for use of their PHI.

Note that any remote data collection will be by telephone or WebEx. Webex is a HIPAA compliant platform that enables parties to communicate using a webcam. The study team member conducting the remote visits will be located in a private room to ensure confidentiality.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

Authorized representatives of the sponsor and representatives of the IRB may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at NYU Langone Medical Center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified

by a unique study identification number. The study data entry and study management systems used by clinical sites and by NYU Langone Medical Center research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the NYU Langone Medical Center. Anonymized MRI data will be shared with our collaborator and co-investigator, Jacek Dmochowski, PhD, of CUNY, such that he will analyze the imaging data we collect.

To further protect the privacy of study participants, the National Institutes of Health, as funding sponsor, will automatically generate a Certificate of Confidentiality. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

12.5.1 Research Use of Stored Human Samples, Specimens, or Data

- Intended Use: Data collected under this protocol may be used to study mood and anxiety disorders. No genetic testing will be performed.
- Storage: Samples will not be stored. Data will be stored using codes assigned by the investigators. Data will be kept in password-protected computers. Only investigators will have access to the samples and data.
- Tracking: A tracking file will link identification codes with identifying information, but this file will be password-protected and stored on a computer that can also only be accessed with a password.

13 Data Handling and Record Keeping.

13.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into Acquire, a 21 CFR Part 11-compliant data capture system provided by NKI's Innovative Clinical Research Solutions. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

13.2 Study Records Retention

Study documents will be retained for the longer of 3 years after close-out, 5 years after final reporting/publication, or 2 years after the last approval of a marketing application is approved for the drug for the indication for which it is being investigated or 2 years after the investigation is discontinued and

FDA is notified if no application is to be filed or if the application has not been approved for such indication. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

13.3 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol or GCP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site PI/study staff to use continuous vigilance to identify and report deviations. All protocol deviations will be addressed in study source documents.

Protocol deviations must be reported to the IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to IRB requirements.

13.4 Publication and Data Sharing Policy

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE policy, and the Section 801 of the Food and Drug Administration Amendments Act of 2007, requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. For interventional clinical trials performed under NIH IC grants and cooperative agreements, it is the grantee’s responsibility to register the trial in an acceptable registry, so the research results may be considered for publication in ICMJE member journals. The ICMJE does not review specific studies to determine whether registration is necessary; instead, the committee recommends that researchers who have questions about the need to register err on the side of registration or consult the editorial office of the journal in which they wish to publish.

FDAAA mandates that a "responsible party" (i.e., the sponsor or designated principal investigator) register and report results of certain "applicable clinical trials":

- Trials of Drugs and Biologics: Controlled, clinical investigations, other than Phase I investigations of a product subject to FDA regulation;
- Trials of Devices: Controlled trials with health outcomes of a product subject to FDA regulation (other than small feasibility studies) and pediatric postmarket surveillance studies.
- NIH grantees must take specific steps to ensure compliance with NIH implementation of FDAAA.

14 Study Finances

14.1 Funding Source

This study is financed through a grant from the US National Institute of Health.

14.2 Costs to the Participant

There are no costs to the participant.

14.3 Participant Reimbursements or Payments

Patients are compensated for expenses of parking and travel associated with study participation, at a rate which is reasonable, however unlikely to induce coercion for research participation. We will compensate each subject $50 for screening and $75 for each MRI they complete (up to 4 MRIs per subject for a total of $300) for time, effort and inconvenience. Subjects will also be reimbursed for transportation costs at each visit (maximum $50 per visit). Finally, each subject will receive $50 for completion of the follow-up visit, again as compensation for time, effort and inconvenience.

15 Study Administration

15.1 Study Leadership

A Steering Committee (SC), chaired by study PIs Drs. Iosifescu and Cassano and including CUNY Co-I Dr. Dmochowski, will serve as the operational governing board. . Program personnel from the NIMH and external scientists may be appointed at the recommendation of NIMH. The SC will be tasked with monitoring scientific progress, including assessing recruitment, progress of study task milestones, ensuring timely publication of abstracts and articles, and ensuring timely submission of data to ClinicalTrials.gov. The SC will meet via monthly teleconference or in-person.

16 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership in conjunction with the NIMH has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by the NYU Langone Conflict of Interest Management Unit (CIMU) with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMC investigators will follow the applicable conflict of interest policies.

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18 Schedule of Events

	Study Phase		
	Screening	**Experimental (4 visits)**	**Follow-up (1 phone call)**
Consent	X		
MINI	X		
Demographic data	X		
Inclusion/exclusion	X		
IDS-C	X		
MOCA	X		
ATRQ	X		
MRI Safety Checklist	X	X	
NIS-SCS	X		
Medical history, PE	X		
Safety labs	X		
Urine drug screen	X		
Pregnancy test	X		
Vital signs	X	X	
Concomitant Meds	X	X	X
C-SSRS	X	X	X
CGI-I,S		X	
CGI-I, I		X	
PCQ		X	X
PANAS		X	X
MADRS		X	X
SDQ		X	X
ASQ		X	X
AE Form		X	X
SAFTEE		X	X
Neuro-Qol		X	X
Q-LES-Q-SF		X	X
TSRQ		X	
PBQ		X	
Intervention tracking		X	
MRI + t-PBM/sham		**X**	