Mass General Brigham

Institutional Review Board
Intervention/Interaction Detailed Protocol

Principal Investigator: T. Atilla Ceranoglu, MD

Project Title: Evaluation of Transcranial Photobiomodulation in Autism Spectrum Disorder: Double-Blind, Placebo-Controlled, Randomized Clinical Study of a Novel Approach

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For Intervention/Interaction studies, submit a Detailed Protocol that includes the following sections. If information in a particular section is not applicable, omit and include the other relevant information.

1. Background and Significance

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by variable presentation of difficulties with socialization, reciprocal communication, and restrictive/repetitive behaviors (American Psychiatric Association, 2013). An increasingly higher prevalence of ASD is documented in each successive epidemiological survey and the disorder is now estimated to affect up to 2% of youth in the general population (Blumberg et al., 2013). This rise in prevalence is in part attributed to improved recognition of autism in intellectually capable populations (Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators & Centers for Disease Control and Prevention, 2012).

Currently, there exists no approved treatments for core features of ASD. Instead, available treatment interventions target other psychiatric disorders that frequently co-occur with ASD, including attention, anxiety, and mood disorders (Joshi et al., 2010).

Transcranial Photobiomodulation (tPBM) is a novel treatment approach based on application of an invisible, non-ionizing electromagnetic wave that results in metabolic modulation in tissues targeted. This intervention consists of exposing bilaterally the frontal brain to the electromagnetic wave that penetrates the skin and skull into brain tissue, is non-invasive and minimally dissipated as thermal energy (Mochizuki-Oda et al., 2002; Zhang, Ma, Nioka, & Chance, 2000). The benefits of tPBM are wavelength specific. Electromagnetic wave at 850nm is absorbed by cytochrome c oxidase, a specific chromophore in mitochondria and is associated with increased adenosine triphosphate (ATP) production through the respiratory chain. Ultimately, the increased ATP production leads to increased energy metabolism and activity for the cell, and it is hypothesized that a signaling cascade is also activated promoting cellular plasticity and cytoprotection (Eells et al., 2003).

These properties of the tPBM have led to novel therapeutic applications in neurology. In acute ischemic stroke subjects, acute treatment with the tPBM led to significantly better outcome as compared to sham (Lampl et al., 2007). These results were confirmed in a different cohort of stroke patients with mild to moderate severity of illness (Zivin et al., 2009). Both studies on stroke subjects showed no significant difference in rate of adverse events, as well as serious adverse events, between the tPBM and sham

treated subjects (Lampl et al., 2007; Zivin et al., 2009). The tPBM has also been used as a treatment of alopecia (Leavitt, Charles, Heyman, & Michaels, 2009) and in animal models for methanol-induced retinal toxicity (Eells et al., 2003). The tPBM is already widely used for non-invasive assessment of brain function (replacing fMRI in studies of infants and young adults, under the name of Near Infrared Spectroscopy) underscoring the relatively low risk of tPBM. The major risk of tPBM when using a laser as the light source is associated with accidental retinal exposure, when beams are projected through the lens, with increased risk of macular degeneration (Kim et al., 2007). LED light does not share the same risk level as laser light sources and in our study will have multiple protections to safeguard against this risk.

Proposed treatment with tPBM has been previously studied in patients with Major Depressive Disorder (MDD) (Cassano, Petrie, Hamblin, Henderson, & Iosifescu, 2016). MDD has been associated with deficits in brain bioenergetic metabolism. In an experimental model of depression, the mitochondrial respiratory chain (the cellular site for energy production) was found to be inhibited by chronic stress (Rezin et al., 2008). Depressed subjects have also significantly lower production of ATP (an energy vector) in their muscle tissue and greater incidence of deletions in their mitochondrial DNA (Gardner et al., 2003). Data from magnetic resonance spectroscopy in subjects with MDD showed that response to the augmentation of a selective serotonin reuptake inhibitor (SSRI) with triiodothyronine (a thyroid hormone) is associated with restoration of the levels of ATP in the brain (Iosifescu et al., 2008). A preliminary open study in 10 depressed subjects has shown that the tPBM was safe, effective and well tolerated (Schiffer et al., 2009). More recently, efficacy and safety of tPBM was also explored in treatment of ASD with promising results and no serious adverse events (Leisman, Machado, Machado, & Chinchilla-Acosta, 2018). In that study, 40 participants received eight 5-min laser light applications to the base of the skull and temporal areas across 4-week period (2 applications per week). A pulsed laser of 635nm was compared to placebo (very weak LEDs) and was shown to be associated with significant improvement in ASD symptoms. Tissue penetration varies at different wavelengths, with 800-850nm range penetrating into deep tissue compared to that of 635nm.

More recently, we completed a prospective, 8-week open-label treatment trial of tPBM in 10 adult patients with moderate to severe level of ASD. Short-term tPBM was well tolerated and was effective in reducing symptom severity of ASD and comorbid ADHD. In addition, tPBM treatment was associated with improvements in executive functions, specifically in functional domains of cognitive flexibility and emotional control, planning and organization, response inhibition and significant improvement in overall function. Treatment with tPBM was well tolerated, and there were no serious adverse events. One subject experienced headache 8 hours after first treatment, and another patient had insomnia after the first treatment episode. Both patients recovered spontaneously and required no changes to study treatments. Now, we are planning for a double-blind randomized clinical trial of tPBM in adult patients with ASD.

The main aim of this 8-week, prospective, placebo (sham) controlled study is to evaluate the efficacy, safety, and tolerability of tPBM with near-infrared light in intellectually capable adults with ASD. Because the tPBM is a non-ionizing radiation, multiple sessions are expected to be safe.

The tPBM treatment can be completed in the comfort of participants' homes, while monitoring their safety and response during scheduled visits. Our study will answer whether tPBM has an effect on ASD symptoms and whether it is safe and acceptable among our patients, for whom frequent visits otherwise would be prohibitive or render it inaccessible.

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

2. Specific Aims and Objectives

The purpose of this 8-week double-blind randomized placebo-controlled study is to assess the tolerability, safety, and efficacy of tPBM in adult patients with ASD. Based on our central hypothesis that tPBM will be safe, tolerable, and effective in improving ASD symptoms in adults, we propose to enroll up to 27 participants of both genders ages 18-50 years with intact intellectual functions satisfying DSM-5 criteria for ASD.

Primary Aims:

Aim 1. To examine the clinical efficacy and safety of tPBM in patients with ASD. We will study the short- and long-term clinical effects of tPBM in 27 adults with ASD by conducting an 8-week randomized clinical trial (RCT).

Secondary Aim:

Aim 1. Explore anti-inflammatory biomarker response to tPBM treatment. We will examine any relationship to ASD symptom severity before and after the 8-week treatment course.
Aim 2. To study the treatment effect of tPBM on mood, social cognition, executive function, ADHD, depression and anxiety symptoms in adults with ASD.

3. General Description of Study Design

This study is a double-blind, placebo-controlled, sequenced parallel study on the use of tPBM as treatment for ASD symptoms in adult patients. This study will last up to 12 weeks from enrollment (allowing up to four weeks from the date of consent to schedule and complete the initial screening process).

4. Subject Selection

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

Inclusion Criteria

Subjects may be included in the study only if they meet all of the following criteria:

- Male or female participants between 18 and 50 years of age (inclusive)
- Fulfills DSM-5 diagnostic criteria for autism spectrum disorder as established by the clinical diagnostic interview.
- Participants with at least moderately severity of ASD symptoms as demonstrated by SRS raw score ≥ 85 and CGI-ASD severity score ≥ 4

- Participants must understand the nature of the study. Participants must be deemed not to have impaired decision-making capacity and must have the capacity to provide direct informed consent. Participants must sign an IRB-approved informed consent form before initiation of any study procedures.
- Participants must have a level of understanding sufficient to communicate with the investigator and study coordinator, and to cooperate with all tests and examinations required by the protocol.
- Participant experiencing a major psychiatric disorder will be allowed to participate in the study provided they do not meet any exclusionary criteria.
- Women of child-bearing potential must use a double-barrier method for birth control (e.g. condoms with spermicide) if sexually active.
- The subject has access to Apple IOS in their household.
- The subject is willing to participate in this study.

Exclusion Criteria

Subjects will be excluded from the study for **any** of the following reasons:

- Impaired intellectual capacity (clinically determined). Participants' intellectual capacity will be assessed during the clinical evaluation and determination will be based on intact communicative language, ability to take personal care, history of holding a job and completion of high school (or equivalency credential), and no history of intellectual disability.
- Participant is unable to communicate due to delay in, or total lack of, spoken language development (grossly impaired language skills)
- Clinically unstable psychiatric conditions or judged to be at serious safety risk to self (suicidal risk) or others (within past 30 days).
- Subjects currently (within past 30 days) experiencing significant symptoms of major psychiatric disorders as clinically determined.
- Subjects with an unstable medical condition (that requires clinical attention).
- Active suicidal or homicidal ideation, as determined by clinical screening.
- The subject has a significant skin condition at the procedure sites (i.e., hemangioma, scleroderma, psoriasis, rash, open wound or tattoo).
- The subject has an implant of any kind in the head (e.g. stent, clipped aneurysm, embolised AVM, implantable shunt – Hakim valve).
- Any use of light-activated drugs (photodynamic therapy) within 14 days prior to study enrollment (in US: Visudine (verteporfin) – for age related macular degeneration; Aminolevulinic Acid- for actinic keratoses; Photofrin (porfimer sodium) – for esophageal cancer, non-small cell lung cancer; Levulan Kerastick (aminolevulinic acid HCl) – for actinic keratosis; 5-aminolevulinic acid (ALA)- for non-melanoma skin cancer)
- Current treatment with a psychotropic medication on a dose that has not been stable for at least 4 weeks prior to initiating study treatment.
- Investigator and his/her immediate family, defined as the investigator's spouse, parent, child, grandparent, or grandchild.

Source of Subjects

We plan to enroll up to 54 adults in order to achieve the goal of exposing up to 27 subjects. We plan to recruit participants from the referral pool of existing and new patients attending a specialty clinic for ASD at the Massachusetts General Hospital.

The Alan and Lorraine Bressler Clinical and Research Program for Autism Spectrum Disorder is a specialized ambulatory care program devoted to the assessment and treatment of individuals of all ages with ASD. It is one of only a few programs in the New England area to offer comprehensive assessment for individuals with ASD, including a complete psychiatric evaluation, psychopharmacological, neuropsychological, behavioral, and social services consultation, psycho-educational support and cognitive/behavioral therapies. The ambulatory care team consists of board-certified psychiatrists specialized in the assessment and management of ASD. Adults with ASD, the majority of whom are individuals with intact intellectual capacity and language skills, make up approximately one-third of the ASD population attending the clinic.

If a potential subject's clinician ascertains that the patient has an interest in study participation, the clinician will offer contact information for the study coordinator to the patient. The patient can then contact the study coordinator for more information on the trial. All subjects that enter the study will undergo standard screening and diagnostic procedures. Clinical records are not scanned in order to recruit subjects. Patients who potentially meet criteria for the study will only be contacted by their treating clinician and referred should the patient decide they wish to participate. If a patient of an investigator decides to enroll in the trial, the process of informed consent will be conducted with a co-investigator who does not treat the patient in a clinical setting. Under no circumstances will a physician investigator obtain informed consent from his or her own patient.

Subjects who have completed a previous clinical trial in our program may be eligible to participate in this study, as described in the Subject Enrollment section. Other medical records on a subject will not be used at any point during this study.

5. Subject Enrollment

Individuals who express interest in the study, will be screened for eligibility by the study coordinator or a research assistant via phone. If their answers to the IRB approved phone screen indicate that they may be eligible for the study, the potential subject will be asked to complete the Social Responsiveness Scale – Second Edition (SRS-2) online via RedCap, a secure online data capture system. The study coordinator will send a link to complete the SRS-2 electronically to the participant via REDCap. An online consent statement will appear, prompting participant to indicate their agreement to participate in the online questionnaire portion of the study. If the participant indicates their agreement, the data capture system will proceed to the SRS-2. After completing the SRS-2, eligible participants based on inclusion and exclusion criteria will either be scheduled to speak with a study clinician via phone to obtain informed consent (and assent as necessary) prior to completing further screening and baseline study procedures via virtual visit. If an additional family member of the subject is available, we will ask that family member to independently complete an SRS-2 form following the same pre-screening procedure. Both forms will be scored and the form with the higher score will be used to determine subject eligibility in order to reduce the possibility of under-recognition of social impairment.

Informed consent will be obtained remotely prior to administration of any study related procedures. If a subject expresses interest in the study, the study staff will send the consent form to subjects in PDF form ready for Adobe eSignature via email with a brief statement (email temple submitted) explaining

why they are receiving this form and further instructions. Study staff will schedule a time for the subject to meet with a study clinician via phone to complete the consent. The study clinician will have a conversation by phone with the subject to obtain consent in the usual fashion: review the consent form, review the inclusion and exclusion criteria, review risks and benefits of the study, review alternatives to participation, and answer any questions subjects may have. The informed consent documents will be used to explain, in simple terms, the risks and benefits of study participation to the subject. Only subjects who can consent for themselves will be included in this study. The nature of the study will be fully explained to the subject by a board-certified physician who is either the principal investigator or a co-investigator. The subject will be encouraged to ask questions pertaining to their participation in the study and the subject may take as much time as they feel necessary to consider their participation in the study and to consult with family members or their physician. If the subject decides to participate, they will sign the consent form using Adobe eSignature. The study clinician will also sign the consent form using Adobe eSignature prior to beginning any study procedures. Subjects will be sent a copy of the signed form by email for their records. Any subject that signs Informed Consent will be considered enrolled into the study, although they may not participate if they do not qualify for the study. Participation in this study is voluntary and subjects may withdraw from the study at any time. If a subject's ability to comprehend and communicate is compromised (per assessment of the Investigator), local regulations pertaining to Informed Consent signatures should be followed.

If a potential subject's clinician ascertains that the patient has an interest in study participation, the clinician will offer contact information for the study to the patient and/or their parent/guardian. The patient and/or their parent/guardian can then contact the study coordinator for more information on the study. If the patient's physician is a member of study staff, the patient and his/her family will also be offered the opportunity to speak with another study physician who is not their treating clinician to review study details and consider participation. Clinical records are not scanned in order to recruit subjects.

Enrollment of Female Subjects of Childbearing Potential

Female subjects of childbearing potential must consent (without any element of coercion) to use a double-barrier method for birth control (e.g. condoms with spermicide) if sexually active.

6. STUDY PROCEDURES

After providing written informed consent, all subjects will complete a clinical diagnostic interview via a virtual visit with a board-certified study clinician to assess eligibility. A study clinician will complete the Reproductive Potential Form. All subjects will be administered an assessment battery including a brief demographic interview and the SRS-2 (Pre-Screening). If an additional family member of the subject is available, we will ask that family member to independently complete an SRS-2 form. Both forms will be scored and the form with the higher score will be used to determine subject eligibility in order to reduce the possibility of under-recognition of social impairment.

We anticipate that subjects may enter this study following completion of/withdrawal from other ASD protocols in our office, and that there may be procedural overlap. So as to not burden subjects/parents/guardians with redundant time commitments, we will use the following diagnostic data previously collected: If a subject has completed an evaluation with one of the study clinicians and/or the structured diagnostic assessment in the three years prior to entrance into this study, he/she will not be asked to repeat any overlapping diagnostic procedures. We will use the study diagnostic data that had been previously collected so as to not burden the subject with redundant time commitments.

However, the study clinician will review the interval time period to assess for clinically significant medical or psychiatric history, to ensure that the subject meets appropriate study entrance criteria.

All female subjects of childbearing potential will have a urine pregnancy test that will be mailed to their address prior to Screening visit. If a participant has a positive urine pregnancy test, she will not be able to take part in the study. The study doctor will inform the participant of any positive test results.

Upon completion of Informed Consent, subjects will be randomized to receive either tPBM or sham and the respective device will be mailed. This study will include up to 27 subjects randomized at 1:1 ratio into 2 groups: tPBM and Sham.

Treatment group assignment will be decided by randomization and managed by the study statistician. Randomized codes will be generated by the study statistician. When the study statistician is not avalaible, randomization will be managed by the department's clinic manager. For each randomized subject, a sealed envelope with treatment assignment will be available at the study site. Both subjects and study staff (except the study statistician and clinic manager) will be blind to treatment group assignment. The sham treatment will consist of applying all the procedures for the delivery of tPBM, whicle actually delivering no light. The blinding of the sham will be effective because the radiation is not visible, and because the active therapy wil not produce any patient discernable difference from sham, e.g., skin warming. The sham treatment will mimic tPBM procedure as described below (Devices and Dosing).

All participants will receive daily tPBM/sham treatments for 8 weeks. All subjects and investigators (except the study statistician) will be blind to treatment sequence assignment.

Subjects will be assessed on measures of efficacy and safety at regular scheduled visits throughout the study. Study clinicians will complete a chart note at Weeks 0, 1, 2, 3, 4, 6, and 8. For a complete schedule of assessments, please refer to Table 2. The screening process may take place over multiple days, as necessary. All study visits will be conducted via Telemedicine platform.

All data will be collected and entered into Research Electronic Data Capture (REDCap), an electronic data capture system that streamlines data collection and ensures data integrity. REDCap software allows researchers to design and implement study surveys electronically for collecting, storing, retrieving, and manipulating data.

Subjects, family members or friend when available, and research staff will enter survey responses into the electronic assessment forms on REDCap. A member of study staff will send forms on REDCap to subjects via the REDCap email function. The responses will be then transmitted securely via an encrypted connection and stored in a secured database. Electronic data capture eliminates the need for subsequent data entry by staff, this minimizing human error. However, if REDCap is unavailable or malfunctioning, study staff will print or download PDFs of all study instruments and mail or securely email (or email normally if given verbal consent) the instruments to subjects (and optional informants), thus study data will be collected in paper or PDF form.

Subjects will be instructed to administer daily tPBM treatments for 8 weeks. Each subject will be mailed a device to keep at home during study participation and return to study staff upon completion. At the

baseline visit, the subject will be instructed on how to accurately place the device. Written instructions are also provided with the device.

Devices and Dosing

Each subject will receive daily tPBM or sham treatments for 8 weeks.

There will be 10 Niraxx G1 Headbands to allow up to 10 subjects simultaneously receiving treatment at any given time. Five of these devices will be designed to be sham device. The treatment will be bilateral and applied to the frontal areas with application sites on the left side and on the right side [left and right forehead centered on EEG sites on F3, Fp1 and F4, Fp2 and Fz, Fpz]. The probes delivering the LED light are embedded on the inside of the headband and are activated by a button press once accurate placement is ensured (Appendix 1: Niraxx G1 Headband Quick Start Guide). Energy is administered with a radiation wavelength of 850 nm. Following the Week 0 (baseline) study visit, the duration of irradiation is started at 20 min per day for the first three days, increased to 30 min per day on day four of treatment, and to 40 min per day on day eight of treatment. At day 15 (Week 2), the clinician will recommend 50 min daily in the context of good tolerability (Table II). The dose will be escalated to 50 min daily if no side-effects occurred. In the case of an adverse event, the tolerated lower dose will be kept. Therefore, the duration of irradiation is 20-50 minutes at each application site (all sites are irradiated at the same time which is equivalent to 20-50 minutes of total time) (Lampl et al., 2007; Schiffer et al., 2009; Zivin et al., 2009). Each subject will receive specific instructions on appropriate application of tPBM treatment device at the Baseline visit. Written instructions are also provided with the device. All staff who deliver instructions must pass training that is approved by the MGH Laser Safety Committee.

The treatment device is classified as an “Exempt” device in accordance with the requirements of the international standard EN 62471 – Photobiological safety of lamps and lamp systems and, when used correctly, the output is less than the maximum permissible exposure (MPE) no special controls are needed for the environment it is to be used in. At the time of study implementation, the staff will be provided training on basic safety procedures relative to the use of the device. The staff will then instruct the subject on the administration of the tPBM such that the headband is appropriately placed. Written instructions are also provided to the participant with the device. The delivery of the tPBM is expected to last 20-50 minutes total. The subject will be asked to rest for five minutes after the delivery of tPBM. Sites of application will be inspected at each study visit. At all visits, study staff will confirm with the participant that they understand and are following the provided instructions. Trained study staff will review the instructions with participants if it is necessary as to ensure all subjects understand and adhere to instructions.

Flexible Titration Schedule

Titration of tPBM will follow a flexable titration schedule with an option for slower titration, holding at lower dose, or lowering the dose based on tolerability and response to the tPBM as per clinician judgment. Dose will be adjusted upward per the flexible titration schedule provided the treatment is well tolerated. Titration will be adjusted if a participant experiences a treatment limiting side effect related to tPBM, such as scalp discomfort, headaches, or any side effects reported on TSRQ. We will plan to titrate to 50 minutes for all participant unless a participant experiences a side effect at 40 mins, in which case, we will continue at 40 minutes. Week 2 and onwards, participants will be maintained on maximum achieved dose with a one-time option to decrease the dose of the tPBM to the next lowest

available dose per clinician judgment based on tolerability. For the detailed titration schedule, please refer to Table II.

Concomitant Medications/Treatments

As part of the initial psychiatric evaluation, a detailed history of past and present treatments (pharmacological and non-pharmacological) will be obtained. At each study visit, subjects will be assessed for the use of concomitant medication. The guidelines for use of concomitant medications/treatments during the study are as follows:

- Participants may continue treatment with concomitant psychotropic medications (provided no exclusion criteria are met) and must remain on a stable dose during the course of the trial.
- Subjects requiring initiation of acute or chronic medication treatment may be discontinued from the study if treatment is judged by the investigator to interfere with the assessment of study drug effect.
- Non-pharmacological treatments such as supportive individual, family, or group therapy will be permitted provided they were in place for a substantial period of time (>1 month) prior to study enrollment and remain unchanged during the course of the trial.
- No new non-pharmacological treatments may be initiated during the course of the trial.

Study Visits

Screening Visit (Week 99)

The screening Visit will last up to 2 hours in total (involving up to 2 visits) and includes the following components:

- The subjects will meet with a study clinician for a psychiatric evaluation and review of medical history.
- The study clinician will ask the participant about their symptoms of autism spectrum disorder.
- Female participants of childbearing potential will complete a urine pregnancy test. If a participant has a positive pregnancy test, she will not be able to take part in the study. The study doctor will inform the participant and discuss the clinically appropriate course of action. The participant will be offered 3 follow-up visits.
- The participant will be asked complete a questionnaire about their ASD symptoms (SRS-2). This questionnaire takes approximately 5-10 minutes to complete.
- The participant will be asked to complete a brief demographic interview collecting information regarding socioeconomic status and history of head injury or trauma. This will take about 5-10 minutes to complete.
- Optional: if a family member is available, we will ask them to complete one to three observer-rated measures on REDCAP.

The following assessments will be performed at the Screening Visit:

- Demographic Interview
- Psychiatric Evaluation and Medical History
- Urine pregnancy test (females only)
- Pregnancy potential monitoring form
- Concomitant Medications
- CGIs
- GAF
- SRS-2 (Pre-Screening)

- ABCL
- MGH-SECS-C
- MGH-SECS-I

Study participants may request the results of their scales and assessments. In this case, the subject will receive a letter providing a basic interpretation of the results and will be available for any questions or concerns.

Washout Period

After the screening period, subjects who are currently taking prohibited medications as noted in exclusion criteria, and willing to discontinue the use of their medication may do so in order to be eligible for participation in this study, if clinically appropriate per clinician judgment. If appropriate, medication washout will be recommended by our clinicians to participants and current providers. Clinicians will determine a washout schedule based on the half-life of the medication, the adverse effects associated with treatment and withdrawal, and an assessment of individual factors including duration on drug and dose. No subjects will be asked to discontinue ongoing exclusionary medications to participate in the study; however, if the subject is already tapering the medication off for clinical reasons at the time of screening and evaluation for participation, they will be considered eligible to participate only after the washout period as described. Our office does not take over care for the patient, but remains available during this time period. The washout schedule will be discussed with the participant and current providers.

Study Treatment Phase (Weeks 0-8)

Pregnancy potential will be monitored at screening, baseline, Week 2, Week 4, and Week 6 by a clinician-rated reproductive potential form. If, during any of these visits the participant is deemed to have pregnancy potential, a member of study staff will contact the participant to complete a phone screen to ensure the participant understands why a urine pregnancy test is necessary, explain the required procedures, and obtain verbal consent from the subject to send the urine pregnancy test to their home address. If the participant provide verbal consent, a pregnancy test will be overnight shipped using study funds and sent directly to the subject's home address within 24 hours. The participant will be asked to stop treatment until the urine pregnancy test is performed. In addition, a phone call will be set up at a specified time the next day. During this call, the study coordinator will review the procedure for completing the urine pregnancy test while on the phone and have the subject complete the test while remaining on the line. The subject will verbally report the result to the study coordinator, and will also send visual confirmation (i.e. a photo of the test with results send via secure email) prior to continuing the study treatment. If the participant decides that they no longer feel comfortable completing the urine pregnancy test, they will be withdrawn from the study.

Subjects will be instructed to administer the treatment sessions daily for eight weeks, with each session lasting 20-50 minutes. Subjects will be instructed to contact the investigator or a member of his staff at any time between visits concerning adverse events or worsening of symptoms.

Baseline Visit (Week 0)

The overall baseline session is estimated to last about 60 minutes. The subject will be examined to inspect the sites of application (left and right forehead) for any possible skin lesions (e.g. laceration or signs of inflammation) which would contraindicate the treatment. The site of the test treatment will also be examined. The subject will then be instructed on the appropriate administration of tPBM.

Following assessments will be performed at the Baseline Visit:

- Virtual examination of exposure sites
- Urine pregnancy test (females only)
- Pregnancy potential monitoring form
- CGIs
- GAF
- CTAE
- Concomitant Medications
- Ham-A
- Ham-D
- AISRS
- BRIEF-A
- ASRS
- Q-LES-Q

Study Treatment: Virtual Clinical Monitoring Visits (Weeks 1-6)

There are 5 monitoring visits scheduled at Week 1, Week 2, Week 3, Week 4 (Midpoint), Week 6, and Week 8.

Participants will be prescribed to administer study treatments at home daily for a period of 8 weeks. Study visits will have a visit window of +/-3 days to facilitate scheduling.
During the visits, the participants will be asked questions about the symptoms of ASD and general health in addition to any present side effects or changes in their medications. At weeks 99, 0, 1, 2, 3, 4 (Midpoint), 6, and 8 (endpoint) the participant will be asked to fill out questionnaires.

Following assessments will be performed at the Week 1, Week 2, Week 3, and Week 6 Visits:

- Virtual examination of exposure sites
- Urine pregnancy test (if necessary)
- Pregnancy potential monitoring form (Week 2 and Week 6)
- CGIs
- GAF
- CTAE
- Concomitant Medications
- TSRQ

Following assessments will be performed at the Midpoint (Week 4) Visit:

- Virtual examination of exposure sites
- Urine pregnancy test (if necessary)
- Pregnancy potential monitoring form
- MGH-SECS-C
- CGIs
- GAF
- CTAE
- Concomitant Medications
- TSRQ

- AISRS
- Ham-A
- Ham-D
- SRS-2
- ASRS
- Q-LES-Q
- BRIEF-A
- ABCL

Study Endpoint Visit (Week 8 or Endpoint)

At the conclusion of the endpoint visit, the subject will be asked to mail the Niraxx G1 Headband device back to the primary investigator.

Following assessments will be performed at the Week 8/Endpoint Visit:

- Virtual examination of exposure sites
- Progress Note
- MGH-SECS-C
- AISRS
- HAM-A
- HAM-D
- CGIs
- GAF
- CTAE
- Concomitant Medications
- SRS-2
- MGH-SECS-I
- BRIEF-A
- ABCL
- TSRQ
- Q-LES-Q
- ASRS

Study Discontinuation

We will encourage subjects to continue enrollment in the study via follow-up visits regardless of the nature of discontinuation of the tPBM. At the end of the study, we will refer the subjects to their counselor and/or psychiatrist and will communicate clinical data upon signed authorization. If the CGI-S increases more than 2 points during the study, the subject will be counseled to start or change to an FDA-approved medication treatment. If the CGI-S increases more than 5 points or if a subject becomes actively suicidal as per clinician judgment, termination from the study will be mandated. Subjects who are deemed actively suicidal and are in imminent danger will be directed to Acute Psychiatric Service (the local psychiatric Emergency Department at Massachusetts General Hospital) for further evaluation, or to call 9-1-1. If the subject is appropriate for outpatient monitoring, the subject will be followed with

frequent appointments outside the study. Each subject will have the Investigator's contact information as well as instructions on how to call for emergency services, if needed.

A participant may withdraw consent at any time for any reason (e.g., lack of efficacy, adverse events, etc.). A subject may be withdrawn from the study at any time if any of the following conditions are met:

- Worsening of ASD or current/emergent major psychiatric disorder.
- Subjects who experience intolerable adverse effects as determined by the PI.
- Emergent suicidality
- Pregnancy
- Failure to keep study appointments for more than 2 consecutive visits
- Clinical judgment of the investigator

Those subjects who terminate study participation before the completion of the study will be asked to complete all tasks scheduled to take place on the final study visit at the time of study discontinuation.

If study participation is discontinued due to safety reasons, participants will receive up to three follow-up visits, allowing adequate time for appropriate psychiatric referrals to treaters in their communities, or if present, appropriate psychiatric follow up access is assured with their treating provider. If emergent suicidality were to occur during the course of the study, the supervising clinician will then directly assess the level of risk and take the appropriate action (including contacting treatment providers, working on a safety plan, arranging for emergency evaluation via an ER, calling 9-1-1, etc.). The clinician will document the actions taken, and it will be noted in the participant's file. Subjects who discontinue due to non-compliance with the protocol will receive a referral to treaters in the area.

Follow-up visits are optional and are at no cost to participants. These visits are not part of the clinical trial and no research data will be collected during follow-up. Subjects who fail to keep study appointments, or are non-compliant may be dropped from the study. These study subjects will be given a referral to treaters in their area but will not be offered three follow-up visits.

If a subject would like us to forward their clinical history to his/her primary care physician, or a new clinician, we will forward any pertinent information with the proper completed release of information authorization form. If a subject who has come from the clinic of the investigator happens to drop out of the study, he or she will return to his or her treating physician.

Lost to Follow-Up

If a clinical staff is unable to contact a subject or if the subject fails to appear for a visit, three documented phone calls should be made, followed by a certified letter. The certified letter should detail the need for the subject to appear for a visit, the site's unsuccessful attempts to contact the subject, and that failure to contact the site will result in the subject being withdrawn from the study.
If the certified letter is returned to the site as undeliverable or the letter is delivered but the subject does not contact the site and no other contact is made with the subject or the subject's caregiver, then the subject will be considered Lost to Follow-up and discontinued from the study. All attempts to contact the subject will be documented.

STUDY ASSESSMENT SCALES AND FORMS

Subjects will be evaluated at scheduled intervals. At each visit, measures of safety and efficacy will be obtained using assessments of psychiatric symptoms and functioning (CGIs, GAF) and measures of adverse effects (CTAE). At the midpoint (end of week 4) and final study visits (Week 8 or Endpoint), additional clinician- and subject-rated assessments will be repeated (see below for details). Optional observer-rated assessments will be completed at the screening (week 99) and final study visits.

All data will be collected and entered into REDCap. REDCap is an electronic data capture system that streamlines data collection and ensures data integrity. REDCap software allows researchers to design and implement study surveys electronically for collecting, storing, retrieving, and manipulating data. Participants will enter survey responses into electronic assessment forms using REDCap. Participants will be sent the forms via REDCap. The REDCap email will not allow the participant to access any other data stored on the password-protected database. Only those subjects who receive the link via REDCap email will be able to access the questionnaires .The responses will then be transmitted securely via an encrypted connection and stored in a secured database. Electronic data capture eliminates the need for subsequent data entry by staff, thus minimizing human error. However, in the event that REDCap is malfunctioning, study staff will print or download PDFs of all study instruments and study data will be collected in paper or PDF form.

Clinician-Rated Behavioral Measures

(Administered at all visits)

- Clinical Global Impression Scale (CGI; (National Institute of Mental Health, 1985)): The CGI is a measure of illness severity, improvement, and efficacy of treatment. The score for severity ranges from 1 (normal, not at all ill) to 7 (among the most extremely ill subjects). Improvement ranges from 1 (very much improved) to 7 (very much worse). The effectiveness index measures to what extent the subject is experiencing therapeutic effects in conjunction with the level of adverse events they are experiencing. CGI scales will be used for the assessment of *ADHD* and *ASD*.
- Global Assessment of Functioning Scale (GAF; (Endicott, Spitzer, Fleiss, & Cohen, 1976)): composite rating of an individual's overall level of functioning (1= worst to 100 = best).

(Administered at baseline and/or screening, midpoint, and endpoint)

- MGH Social-Emotional Competence Scale [Clinician-Rated Measure] (MGH-SECS-C): This is a 37-item scale that assesses change in the frequency and severity of core and associated symptoms of ASD.
- Adult ADHD Investigator Symptom Report Scale (AISRS;(Spencer et al., 2010)): The AISRS, shown to be sensitive to drug effects in adult populations, assesses each of the 18 individual criteria symptoms of ADHD in DSM-IV on a severity grid (0=not present; 3=severe; overall minimum score=0; maximum score=54).
- Hamilton Anxiety Scale (HAM-A; (M Hamilton, 1959)): a rating scale used to assess anxiety. This questionnaire consists of 14 questions assessing these symptoms. Clinicians are asked to rate each symptom as absent, mild, moderate, severe, or very severe.
- Hamilton Depression Scale (HAM-D; [(M. Hamilton, 1960)): a rating scale used to assess depression. This is a multiple-item questionnaire used to rate the severity of depression in adults. Clinicians are asked to rate each symptom as absent, mild/trivial, moderate or severe.

Clinician-Rated Safety Measures

(Administered at baseline, all treatment visits and at final visit)

- Clinician-Rated Treatment Emergent Adverse Events Log (CTAE): to record any spontaneous adverse health events experienced during the study, along with duration, severity, cause, treatment, and outcome.
- Concomitant Medications: to record additional medications taken during the study.

Subject-Rated Measures

Subject-Rated Behavioral Measures

(Administered at screening evaluation)

- Demographic Interview: This brief interview will collect information regarding socioeconomic status and history of head injury or trauma (Hollingshead, 1975).

(Administered at baseline, midpoint, and final study visits)

- Social Responsiveness Scale-Second Edition Self-Report (SRS-2; (Constantino & Gruber, 2012)): a 65-item rating scale completed by subjects that is used to measure the severity of autism spectrum symptoms as they occur in natural settings.
- Adult ADHD Self-Report Scale (ASRS; (Adler et al., 2006)): The 18-item ADHD Rating Scale will be completed to evaluate frequency of ADHD symptoms on a scale of 0 to 4.
- Behavior Rating Inventory of Executive Function-Adult Self-Report Version (BRIEF-A; (Roth, Isquith, & Gioia, 2004)): a 78-item rating scale to assess level of executive function deficits.
- Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q; (Endicott, Nee, Harrison, & Blumenthal, 1993)): a 16-item questionnaire to evaluate the degree of enjoyment and satisfaction experienced in eight areas of daily functioning.

Subject-Rated Safety Measures

(Administered at week 1, week 2, week 3, Midpoint, week 6, Endpoint, and Follow Up)

- The tPBM Self-Report Questionnaire (TSRQ) – An open-ended questionnaire focusing on potential inconveniences and discomforts related to the tPBM. It will be offered at week 3 and week 6 of the study (after at least two tPBM sessions have been delivered).

Observer-Rated Behavioral Measures

(Administered at baseline and endpoint)

- Social Responsiveness Scale-Second Edition Informant-Report (SRS-2; (Constantino & Gruber, 2012)): a 65-item rating scale completed by parents/caregivers that is used to measure the severity of autism spectrum symptoms of the subjects as they occur in natural settings.
- MGH Social-Emotional Competence Scale-Informant (MGH-SECS-I): This is a 37-item scale that assesses change in the frequency and severity of core and associated symptoms of ASD.
- Adult Behavior Checklist (ABCL; (Achenbach & Rescorla, 2003)): An observer-rated assessment of maladaptive behavioral and emotional problems, social competence and substance use in adults ages 18-50.

Data Collection Forms

Several procedures are in place to assure data integrity and protocol adherence. We will use Research Electronic Data Capture (REDCap) to support direct data entry by patients and study staff. REDCap is a free, secure, HIPAA compliant web-based application hosted by the Partners HealthCare Research Computing, Enterprise Research Infrastructure & Services (ERIS) group. REDCap is an electronic data capture system that streamlines data collection and ensures data integrity. REDCap software allows researchers to design and implement study surveys electronically for collecting, storing, retrieving, and manipulating data.

Participants will be sent the RedCap link via the RedCap email function. The link will not allow the participant to access any other data stored on the password-protected database. Only those subjects who receive the link via email will be able to access the questionnaires. The responses will then be transmitted securely via an encrypted connection and stored in a secured database. Electronic data capture eliminates the need for subsequent data entry by staff, thus minimizing human error. However, if REDCap is malfunctioning, study staff will print or download PDFs of all study instruments and the study data will be collected via telemedicine and recorded on paper/ PDF forms.
Self-report measures will be completed by participants on a computer, directly into REDCap, thus minimizing errors due to data entry. For clinician-administered measures, all clinicians, and independent evaluators (IEs) will enter responses directly in REDCap.

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

DATA ANALYSIS

Primary outcome measure

Primary outcome measure of efficacy will be reduction in ASD symptoms as measured by change from baseline on the clinician-rated SRS-2. Responders will be defined as those who demonstrate a ≥25% reduction in SRS-2 total score *and* a score of 2 or 1 on the CGI-ASD-Improvement subscale ("much" or "very much improved").

Secondary outcome measure:

1. Treatment-related changes in associated psychopathology, social cognition, executive functions will be assessed by:
   - MGH Social-Emotional Competence Scale (MGH-SEC Scale; clinician- &
   - observer-rated)

- Social Responsiveness Scale-Second Edition (SRS-2; self- & observer-rated)
- Adult Behavior Checklist (ABCL; self- & observer-rated)
- Behavior Rating Inventory of Executive Function-Adult Self Report Version (BRIEF-A) or parent version (BRIEF-P) for youth
- Adult ADHD Self-Report Scale (ASRS) & clinician-rated Adult ADHD Investigator Symptom Report Scale (AISRS) and CGI-ADHD scale
- Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q)
- Hamilton Anxiety scale (HAM-A) & clinician-rated CGI-Anxiety scale
- Hamilton Depression scale (HAM-D) & clinician-rated CGI-Depression scale

2. Safety and tolerability will be assessed by:
    - The monitoring of spontaneous treatment-emergent adverse events with the Clinician-rated Treatment-emergent Adverse Events Log (CTAE)
    - tPBM Self-Report Questionnaire (TSRQ)

SAFETY

Consistent with good clinical practice, safety will be monitored at each study visit by a subject's assigned clinician. This clinician will be available 24 hours a day by page. The principal investigator will supervise all study activities including ratings and reported adverse events. All procedures have been designed to minimize subject discomfort, and no subject will be asked to engage in research procedures not outlined in the consent form.

If study participation is discontinued due to safety reasons, participants will receive three follow-up visits, giving adequate time for appropriate psychiatric care to be arranged.

Study Treatment

Safety will be monitored through treatment-emergent adverse events and subject rated TSRQ responses. Subjects will be monitored for adverse events at each visit. All adverse events will be recorded. A subject may be dropped from the study or dosage may be decreased at any time due to adverse events. All adverse events will be reported to the PHRC according to PHRC guidelines. All concomitant medications will be assessed at every study visit.

Accountability and Investigational Product Control

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

The Principal Investigators or designated study site personnel who verify the receipt of the devices/device accessories will notify Niraxx, Inc. via email and record the correspondence in a note to file. Device Accountability and Acknowledgment Logs will be maintained on secure servers. These logs will list all equipment received, the receiving date, the serial number of each device. Treatment compliance will be reviewed with the participant at each study visit and documented on REDCap.

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

CONFIDENTIALITY
All research-related records initiated as a result of a subject's participation in this study that reveal the subject's identity will remain confidential except as may be required by law. Subjects will only be contacted regarding future studies if they indicate that they are interested in being contacted by initialing in the specific section of the consent form.

Subject Confidentiality

Any information and data collected during any subject's participation in this investigation will be considered confidential. The Investigator will need to ensure that all subjects' anonymity will be maintained on all documentation by completely redacting (eliminating or "blacking out") each subject's name and/or other identifying information. The identifying information will be replaced with the subject's study number. For the purpose of internal montoring, Niraxx Inc. will use participants' email addresses to access treatment adherence and compliance data, which will be sent directly to the study team on a weekly basis via secure email and stored on a secure server at MGH. To protect subject confidentiality, Niraxx Inc. follows the General Data Protection Regulation (GDPR)— a European Union data protection and privacy regulation (Directorate-General, 2022). Further, Niraxx Inc. will only be provided the email addresses of subjects' who provided their consent to allow Niraxx to access treatment adherence data. With the exception of providing email addresses to Niraxx Inc. for the purpose of montoring treatment adherence, the investigational staff is not to provide to Niraxx Inc. information such as subject's telephone numbers, home address, personal identification numbers such as passport numbers, etc. Care must be taken by investigational staff when communicating with representatives from Niraxx Inc. in the form of telephone or electronic correspondence in not providing information (except for the email addresses of consenting subjects) that may disclose a subject's identity.

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

7. Risks and Discomforts

Potential Adverse Events

Risks to the subject may include but are not limited to the following:
The Niraxx device emits light with a longer wavelength than the human eye can see. This device is classified as an "Exempt" device in accordance with the requirements of the international standard EN 62471 – Photobiological safety of lamps and lamp systems and, when used correctly, the output is less than the maximum permissible exposure (MPE) no special controls are needed for the environment it is to be used in. The staff will be provided training on basic safety procedures relative to the use of the device. The subject administering the tPBM will be instructed on safe application of it, specifically not to operate the Niraxx device unless it is in direct contact with the subject's skin. Each subject will receive specific instructions on appropriate application of tPBM treatment device at the Baseline visit. Written instructions are also provided to the participant with the device. At all visits, study staff will confirm with the participant that they understand and are following the provided instructions. Trained study staff will review the instructions with participants if it is necessary as to ensure all subjects understand and adhere to instructions. Sites of application will be inspected at each study visit. Subjects will be instructed to contact the investigator or a member of his staff at any time between visits concerning adverse events or worsening of symptoms.

Failure of the Niraxx device, resulting in the cessation of investigative therapy can cause:

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

Delivery of the infrared LED energy to an inappropriate site, such as directly over the open eye, is not recommended but should pose no risk to the subject. The prominent medical concern is accumulative infrared radiation damage to the cornea that may be associated with cataracts in a 10-20 year time frame. To protect against this risk, the Niraxx tPBM device has a sensor installed which prevents infrared radiation from shining lights to the eyes by turning off the device if it does not detect the skin contact.

Based on previous consumer observations, application of the Niraxx device may result in mild thermal sensation of warmth during the use. The temperature of the skin is well below the level for thermal damage.

Based on human clinical trial experience to date and sale of the device for its intended use of treating periorbital wrinkles since March 2008, each adverse event listed below has been reported by less than 0.1% of all subjects and users:

- Application Site Erythema
- Application Site Pain
- Application Site Discomfort
- Application Site Warmth
- Application Site Reaction
- Headache

Additional information on adverse events and prior clinical experience can be found in the User Guide. Other potential risks are described below:

- Risk of Depression, Suicidality & Manic Switch: Worsening of depression and increased suicidality are possible complications of antidepressant treatments which could be prescribed to subjects with ASD and mood disorders. We will minimize this risk by selecting only subjects who do not present active suicidal ideation at screening. We will also discontinue any subject who develops active suicidal ideation during the course of the study and arrange for appropriate levels of care and standard treatment. Manic switches are possible adverse events and will be closely monitored during treatment as well. Regularly scheduled clinical study visits will allow early recognition of treatment-emergent suicidal ideation or prodromal hypomanic signs. Subjects who develop mania or hypomania will be discontinued and provided appropriate level of care.

Risks of Assessments/Questionnaires

Answering detailed questionnaires may create a mild degree of inconvenience for the subjects and will be monitored by study clinician. Some questions may make subjects feel uncomfortable because of the nature of the question topics. Some questions ask about possibly sensitive information, including questions pertaining to alcohol and drug abuse. Subjects may refuse to respond to any questions they do not feel comfortable answering.
Adverse events and unanticipated problems will be reported to the PHRC according to current guidelines. We will follow and adhere to all guidelines as defined and outlined on the Partners Human Research Committee web site: http://healthcare.partners.org/phsirb/adverse_events.htm

Other Adverse Events Related to Study Treatment

Problems and side effects not listed above and not known at this time could occur. Subjects will be informed of any newly discovered risks as investigators come to learn of such knowledge, if applicable.

Subject Disposition Criteria

Withdrawal from the Study

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

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

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

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

- Virtual examination of exposure sites
- Progress Note
- MGH-SECS-C
- AISRS
- HAM-A
- HAM-D
- CGI
- GAF
- CTAE
- Concomitant medications form
- SRS-2
- BRIEF-A
- TSRQ
- MGH-SECS-I
- ABCL
- Q-LES-Q
- ASRS

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

If study participation is discontinued due to safety reasons, participants will receive three follow-up visits, giving adequate time for appropriate psychiatric care to be arranged.

8. Benefits

There may be no direct benefit to subjects participating in this study. Participants may benefit by potentially experiencing improvement in ASD symptoms and by gaining knowledge about ASD.

Study subjects will receive comprehensive clinical assessment of their psychiatric condition. This information will be readily available to their counselors if the subjects agree to disclose. This information might guide counseling in the long-term treatment. In the short-term, the subject will receive close and systematic monitoring, beyond current standards of care. Easy access to routine physical exams are also a potential benefit in the community population, which is often underserved. The subjects will have access to a different modality of treatment if counseling was not sufficient and if medications were not acceptable to them. However, there may be no improvement at all, or benefit to participant.

9. Statistical Analysis

54 subjects will be randomized (1:1) into the tPBM or placebo/sham group and be treated for 8 weeks post randomization. This design largely protects against the bias introduced by confounding factors.

We will rely on comparisons of the participants' performances in each study arm (placebo/sham and tPBM) at baseline prior to the initiation of treatment relative to their scores at the last assessment (completion/drop-visit). Since we are following subjects over a short period, missing data are not expected to impact our analyses such that standard statistical analyses will be employed. Specifically, we will employ longitudinal mixed-effects regression models (RM) with random effects for the subjects using Stata 16.0 (StataCorp, 2019). The RMs will use robust standard errors to account for the repeated measures on each subject. We will test binary data using mixed-effects logistic RMs, count data with mixed-effects Poisson RMs, and normally distributed data with mixed-effects linear RMs. Each model will predict outcome scores from treatment group (placebo/sham or tPBM), study visit (continuous predictor), and the treatment group-by-study visit interaction. All analyses will be intent to treat (ITT).

10. Monitoring and Quality Assurance

Consistent with good clinical practice, safety will be monitored at each study visit by a subject's assigned clinician. This clinician will be available 24 hours a day by page. The principal investigator will supervise all study activities including ratings and reported adverse events. All procedures have been designed to minimize subject discomfort, and no subject will be asked to engage in research procedures not outlined in the consent form.

Safety will be monitored through treatment-emergent adverse events. Subjects will be monitored for adverse events at each visit. All adverse events will be recorded. A subject may be dropped from the study or dosage may be decreased at any time due to adverse events. All adverse events will be reported to the PHRC according to PHRC guidelines. All concomitant medications will be assessed at every study visit.

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

Overview and Definitions

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

- ***Adverse Event*** is defined as any untoward/undesirable clinical occurrence in a clinical investigation of a subject using a device and/or product and which does not necessarily have a causal relationship with this treatment. An Adverse Event can therefore be any unfavorable and/or unintended sign, symptom, or disease temporally associated with the use of a device product, whether or not considered related to the device product. Only abnormal laboratory values that are deemed clinically significant by the investigator will be classified as adverse events.
- ***Serious Adverse Event*** is defined as any untoward/undesirable adverse experience that results in any of the following outcomes: 1) death; 2) a life-threatening adverse experience; 3) inpatient hospitalization or prolongation of existing hospitalization; 4) a permanent/persistent or significant disability/incapacity or a congenital anomaly/birth defect; 5) important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
- ***Anticipated Adverse Device Effect*** is defined as any adverse effect related to the device or procedure, which is identified in the protocol.
- ***Unanticipated Adverse Device Effects*** is defined as any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Safety Monitoring

The study subjects will undergo frequent clinical evaluations including depressive scores, concomitant medications, adverse events, and serious adverse events and unexpected device events will be recorded from study entry through completion. Additionally, doctors must monitor a subject's safety by asking the subjects frequently about the subject's own comfort during treatment application.
If skin erythema is present, treatment will be suspended. Patients will be instructed to contact the study site principal investigator or a member of his staff at any time between visits concerning adverse events or worsening of symptoms. If at any study visit the subjects' clinical condition is significantly worsened from baseline (operationalized as the clinical global impression improvement score, CGI-I, of 6 or higher) or if a subject becomes actively suicidal with intent and/or plan, based on the clinical interview, the subject will be offered to start an antidepressant medication. If the subject were deemed at imminent danger as a result of suicidality, s/he would be discontinued from the study and referred to appropriate clinical treatment (see 6.1.5 Study Discontinuation).
Study staff responsible for data collection and storage will be aware of and comply with all regulatory requirements related to adverse events. If a patient becomes ill or is injured as a direct result of study participation, medical care will be made available. All adverse events (and device events) will be followed to resolution and reported to the MGH IRB as serious if,

1. They are unanticipated and possibly related to the study (same reporting as SAE) or
2. They meet any one of the following criteria:
    - Any fatal event,
    - immediately life-threatening event,

- permanently or substantially disabling event,
- event requiring or prolonging inpatient hospitalization, or any congenital anomaly.
- This also includes any event that a study investigator judges to impose a significant hazard, contraindication, side effect, or precaution.

The Principal Investigator (Dr. Ceranoglu) will have weekly meetings with study staff; during these meetings, they will discuss all Adverse Event reports to identify any safety concern. Based on such concerns they will be able to decide whether a temporary discontinuation of study enrollment, modifications of the study protocol, or to terminate the study is necessary.

Data Safety Monitoring Board

Additionally, A Data and Safety Monitoring Board (DSMB), consisting of at least 2 clinicians and one biostatistician not directly involved in the study (Table 1). The DSMB will review SAEs every six months during the study. The DSMB will be provided with unblinded data from the study so as to examine whether the study should be stopped because the study interventions are either clearly beneficial or harmful to participants. In addition to IRB, the Chairman of DSMB will be informed of any serious adverse events occurring during the study. If at any time during the study, more than 40% of the patients in the active treatment arm of the study have had a serious adverse event, the study will be stopped. The primary concern of the investigators of this study is the safety of the participants. Members of DSMB will include the following clinicians and biostatistician:

Table 1. Data Safety Monitoring Board

Member Name	Specialty	Institution
Janet Wozniak, MD (Chairperson)	Psychiatry, Child and Adolescent Psychiatry, Clinical Research	Massachusetts General Hospital, Boston
Mai Uchida, MD	Psychiatry, Child and Adolescent Psychiatry, Clinical Research	Massachusetts General Hospital, Boston
J. Michael Murphy, EdD	Psychology, Clinical Research, Statistician	Massachusetts General Hospital

Reporting Procedures for All Adverse Events

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

- Description of event
- Date of onset
- Date of resolution (if applicable)
- Seriousness
- Relationship to the study device and/or procedure(s)
- Intensity
- Action(s) taken
- Outcome(s)

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

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

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

If the Adverse Event is of such intensity in the Investigator's judgment that it warrants withdrawal from the study, the subject should be withdrawn from treatment. The subject should be given appropriate care under medical supervision until symptoms resolve.
The relationship of an Adverse Event to the study device or procedure will be graded as follows:

- ***Unrelated.*** The Adverse Event is not associated with the study device use.
- ***Possible.*** This causal relationship is assigned when the Adverse Event:
    - Follows a reasonable temporal sequence from device use,
    - Could have been produced by the subject's clinical state or other modes of therapy administered to the subject, or
    - Cannot be reasonably explained by known characteristics of the subject's clinical state.
- ***Definite.*** This causal relationship is assigned when the Adverse Event:
    - Follows a reasonable temporal sequence from device use;
    - Abates upon discontinuation of the treatment; and
    - Is confirmed by the reappearance of the Adverse Event on repeat exposure.

Serious Adverse Events

All Serious Adverse Events must be reported to the overseeing Institutional Review Board (IRB) and Niraxx, Inc as required.
If the Investigators are notified by Niraxx, Inc or its designee of any Serious Adverse Events that are considered to be Unanticipated Adverse Device Effects, the Investigators must notify his/her own IRB/EC as required.

Deaths

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

Withdrawals for Adverse Events

All Adverse Events which result in the subject's withdrawal from the study must be reported immediately by telephone to Niraxx Inc.
The Investigator may be asked to provide detailed follow-up information. The Investigator will determine the reportability of the event on a case-by-case basis, and will report to the appropriate regulatory authorities evaluating the study device as necessary.

11. Privacy and Confidentiality

- ☒ Study procedures will be conducted in a private setting
- ☒ Only data and/or specimens necessary for the conduct of the study will be collected

- ☒ Data collected (paper and/or electronic) will be maintained in a secure location with appropriate protections such as password protection, encryption, physical security measures (locked files/areas)
- ☐ Specimens collected will be maintained in a secure location with appropriate protections (e.g. locked storage spaces, laboratory areas)
- ☒ Data and specimens will only be shared with individuals who are members of the IRB-approved research team or approved for sharing as described in this IRB protocol
- ☒ Data and/or specimens requiring transportation from one location or electronic space to another will be transported only in a secure manner (e.g. encrypted files, password protection, using chain-of-custody procedures, etc.)
- ☒ All electronic communication with participants will comply with Mass General Brigham secure communication policies
- ☒ Identifiers will be coded or removed as soon as feasible and access to files linking identifiers with coded data or specimens will be limited to the minimal necessary members of the research team required to conduct the research
- ☒ All staff are trained on and will follow the Mass General Brigham policies and procedures for maintaining appropriate confidentiality of research data and specimens
- ☒ The PI will ensure that all staff implement and follow any Research Information Service Office (RISO) requirements for this research
- ☐ Additional privacy and/or confidentiality protections

12. References

Achenbach, T. M., & Rescorla, L. A. (2003). *Manual for ASEBA Adult Forms & Profiles*. Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families.

Adler, L. A., Spencer, T., Faraone, S. V., Kessler, R. C., Howes, M. J., Biederman, J., & Secnik, K. (2006). Validity of pilot adult ADHD self report scale (ASRS) to rate adult ADHD symptoms. *Ann Clin Psychiatry, 18*(3), 145-148. doi:10.1080/10401230600801077

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (5th ed.)* (5th ed.). Arlington, VA: American Psychiatric Publishing.

Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators, & Centers for Disease Control and Prevention. (2012). Prevalence of autism spectrum disorders--Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2008. *MMWR Surveill Summ, 61*(3), 1-19. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22456193

Blumberg, S. J., Bramlett, M. D., Kogan, M. D., Schieve, L. A., Jones, J. R., & Lu, M. C. (2013). Changes in prevalence of parent-reported autism spectrum disorder in school-aged U.S. children: 2007 to 2011-2012. *Natl Health Stat Report*(65), 1-11, 11 p following 11. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24988818

Cassano, P., Petrie, S. R., Hamblin, M. R., Henderson, T. A., & Iosifescu, D. V. (2016). Review of transcranial photobiomodulation for major depressive disorder: targeting brain metabolism, inflammation, oxidative stress, and neurogenesis. *Neurophotonics, 3*(3), 031404. doi:10.1117/1.NPh.3.3.031404

Constantino, J. N., & Gruber, C. P. (2012). *The Social Responsiveness Scale Manual, Second Edition (SRS-2)*. Los Angeles, CA: Western Psychological Services.

Directorate-General, C. (2022). European Commission Website: Rules for business and organisations Retrieved from https://commission.europa.eu/law/law-topic/data-protection/reform/rules-business-and-organisations_en

Eells, J. T., Henry, M. M., Summerfelt, P., Wong-Riley, M. T., Buchmann, E. V., Kane, M., . . . Whelan, H. T. (2003). Therapeutic photobiomodulation for methanol-induced retinal toxicity. *Proc Natl Acad Sci U S A, 100*(6), 3439-3444. doi:10.1073/pnas.0534746100

Endicott, J., Nee, J., Harrison, W., & Blumenthal, R. (1993). Quality of life enjoyment and satisfaction questionnaire: A new measure. *Psychopharmacol Bull, 29*(2), 321-326.

Endicott, J., Spitzer, R. L., Fleiss, J. L., & Cohen, J. (1976). The global assessment scale. A procedure for measuring overall severity of psychiatric disturbance. *Arch Gen Psychiatry, 33*(6), 766-771.

Gardner, A., Johansson, A., Wibom, R., Nennesmo, I., von Dobeln, U., Hagenfeldt, L., & Hallstrom, T. (2003). Alterations of mitochondrial function and correlations with personality traits in selected major depressive disorder patients. *J Affect Disord, 76*(1-3), 55-68. doi:10.1016/s0165-0327(02)00067-8

Hamilton, M. (1959). The assessment of anxiety states by rating. *Br J Med Psychol, 32*, 50-55.

Hamilton, M. (1960). A rating scale for depression. *Journal of Neurological and Neurosurgical Psychiatry, 23*, 56-62.

Hollingshead, A. B. (1975). *Four Factor Index of Social Status*. New Haven, CT: Yale Press.

Iosifescu, D. V., Bolo, N. R., Nierenberg, A. A., Jensen, J. E., Fava, M., & Renshaw, P. F. (2008). Brain bioenergetics and response to triiodothyronine augmentation in major depressive disorder. *Biol Psychiatry, 63*(12), 1127-1134. doi:10.1016/j.biopsych.2007.11.020

Joshi, G., Petty, C., Wozniak, J., Henin, A., Fried, R., Galdo, M., . . . Biederman, J. (2010). The Heavy Burden of Psychiatric Comorbidity in Youth with Autism Spectrum Disorders: A Large Comparative Study of a Psychiatrically Referred Population. *J Autism Dev Disord, 40*(11), 1361-1370. doi:10.1007/s10803-010-0996-9

Kim, E. A., Kim, B. G., Yi, C. H., Kim, I. G., Chae, C. H., & Kang, S. K. (2007). Macular degeneration in an arc welder. *Ind Health, 45*(2), 371-373. doi:10.2486/indhealth.45.371

Lampl, Y., Zivin, J. A., Fisher, M., Lew, R., Welin, L., Dahlof, B., . . . Oron, U. (2007). Infrared laser therapy for ischemic stroke: a new treatment strategy: results of the NeuroThera Effectiveness and Safety Trial-1 (NEST-1). *Stroke, 38*(6), 1843-1849. doi:10.1161/STROKEAHA.106.478230

Leavitt, M., Charles, G., Heyman, E., & Michaels, D. (2009). HairMax LaserComb laser phototherapy device in the treatment of male androgenetic alopecia: A randomized, double-blind, sham device-controlled, multicentre trial. *Clin Drug Investig, 29*(5), 283-292. doi:10.2165/00044011-200929050-00001

Leisman, G., Machado, C., Machado, Y., & Chinchilla-Acosta, M. (2018). Effects of Low-Level Laser Therapy in Autism Spectrum Disorder. *Adv Exp Med Biol, 1116*, 111-130. doi:10.1007/5584_2018_234

Mochizuki-Oda, N., Kataoka, Y., Cui, Y., Yamada, H., Heya, M., & Awazu, K. (2002). Effects of near-infra-red laser irradiation on adenosine triphosphate and adenosine diphosphate contents of rat brain tissue. *Neurosci Lett, 323*(3), 207-210. doi:10.1016/s0304-3940(02)00159-3

National Institute of Mental Health. (1985). CGI (Clinical Global Impression) Scale - NIMH. *Psychopharmacol Bull, 21*(8), 839-844.

Rezin, G. T., Cardoso, M. R., Goncalves, C. L., Scaini, G., Fraga, D. B., Riegel, R. E., . . . Streck, E. L. (2008). Inhibition of mitochondrial respiratory chain in brain of rats subjected to an experimental model of depression. *Neurochem Int, 53*(6-8), 395-400. doi:10.1016/j.neuint.2008.09.012

Roth, R. M., Isquith, P. K., & Gioia, G. A. (2004). Brief-A^{tm} Self report form. In: Psycholigcal Assessment Resources, Inc.

Schiffer, F., Johnston, A. L., Ravichandran, C., Polcari, A., Teicher, M. H., Webb, R. H., & Hamblin, M. R. (2009). Psychological benefits 2 and 4 weeks after a single treatment with near infrared light to the forehead: a pilot study of 10 patients with major depression and anxiety. *Behav Brain Funct, 5*, 46. doi:10.1186/1744-9081-5-46

Spencer, T. J., Adler, L. A., Meihua, Q., Saylor, K. E., Brown, T. E., Holdnack, J. A., . . . Kelsey, D. K. (2010). Validation of the adult ADHD investigator symptom rating scale (AISRS). *J Atten Disord, 14*(1), 57-68. doi:10.1177/1087054709347435

StataCorp. (2019). Stata Statistical Software: Release 16. College Station, TX: StatCorp LLC.

Zhang, Q., Ma, H., Nioka, S., & Chance, B. (2000). Study of near infrared technology for intracranial hematoma detection. *J Biomed Opt, 5*(2), 206-213. doi:10.1117/1.429988

Zivin, J. A., Albers, G. W., Bornstein, N., Chippendale, T., Dahlof, B., Devlin, T., . . . Safety Trial, I. (2009). Effectiveness and safety of transcranial laser therapy for acute ischemic stroke. *Stroke, 40*(4), 1359-1364. doi:10.1161/STROKEAHA.109.547547

APPENDIX A

Data Monitoring Committee / Data and Safety Monitoring Board Appendix

- *To be completed for studies monitored by Data Monitoring Committee (DMC) or Data and Safety Monitoring Board (DSMB) if a full DMC/DSMB charter is not available at the time of initial IRB review.*
- *DMC/DSMB Charter and/or Roster can be submitted to the IRB later via Amendment, though these are not required.*

A Data Monitoring Committee (DMC) or Data and Safety Monitoring Board (DSMB) will be convened for safety monitoring of this research study. The following characteristics describe the DMC/DSMB convened for this study (Check all that apply):

☒ The DMC/DSMB is independent from the study team and study sponsor.

☒ A process has been implemented to ensure absence of conflicts of interest by DMC/DSMB members.

☒ The DMC/DSMB has the authority to intervene on study progress in the event of safety concerns, e.g., to suspend or terminate a study if new safety concerns have been identified or need to be investigated.

☒ Describe number and types of (i.e., qualifications of) members:
Janet Wozniak, MD
Mai Uchida, MD
J. Michael Murphy, EdD

☒ Describe planned frequency of meetings:
Once every six months.

☒ DMC/DSMB reports with no findings (i.e., "continue without modifications") will be submitted to the IRB at the time of Continuing Review.

☒ DMC/DSMB reports with findings/modifications required will be submitted promptly (within 5 business days/7 calendar days of becoming aware) to the IRB as an Other Event.

Appendix B
Table II. Study Schema

Weeks	99	0	1	2	3	4	6	8
Visits	Screen	Baseline	W1	W2	W3	MP	W6	EP/ ET
Informed Consent	X							
tPBM Test Dose								
tPBM Device Subject Training		X						
tPBM Initial Titration: Day 1-3, 20 min/day Day 4-7, 30 min/ day		X						
tPBM Treatment - 40 min/day			X					
tPBM Treatment - 50 min/day				X	X	X	X	X
Diagnostic Assessments								
Inclusion/Exclusion Criteria	X							
Psychiatric Evaluation & Medical History	X							
Physiological Procedures								
Virtual Examination of Exposure Sites		X	X	X	X	X	X	X
Urine pregnancy test	X*	X*	X*	X*	X*	X*	X*	
Clinician-Rated Assessments								
Pregnancy Potential/Monitoring Form	X	X		X		X	X	
MGH-SECS-C	X					X		X
AISRS		X				X		X
HAM-A		X				X		X
HAM-D		X				X		X
CGIs	X	X	X	X	X	X	X	X
CTAE		X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X
GAF	X	X	X	X	X	X	X	X
Progress Note		X	X	X	X	X	X	X
Subject-Rated Assessments								
Demographic Interview	X							
SRS-2	X					X		X
ASRS		X				X		X
BRIEF-A		X				X		X
Q-LES-Q		X				X		X
TSRQ			X	X	X	X	X	X
Observer-Rated Assessments								
SRS-2	X							X
MGH-SECS-I	X							X
ABCL	X							X

Appendix C

Table III. Flexible Titration Schedule

Day	Maximum Total Dose (minutes)
1-3	20
4-7	30
8	40
15	50