Title:

Effect of Auricular Vagal Nerve Electrical Stimulation on Post-Treatment Lyme Disease Syndrome

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Pilot, Effect of Respiratory-gated Auricular Vagal Afferent Nerve Stimulation (RAVANS) on post-treatment Lyme disease syndrome (RaVLyme trial)

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Detailed Protocol

I. BACKGROUND AND SIGNIFICANCE

Overview The purpose of this pilot, double blinded, sham-controlled, randomized clinical trial is to explore the effect of Respiratory-gated Auricular Vagal Afferent Nerve Stimulation (RAVANS) on the symptoms of PTLDS using psychometric measurement, functional and cognitive test and serum biomarkers. This study consists of multidisciplinary team, including physiatrist (Dr. Qing Mei Wang), neuroimaging and Lyme disease researcher (Dr. Michael VanElzakker), RAVANS inventor (Dr. Vitaly Napodow), medical monitor (Dr. David Crandell).

Lyme Disease. Lyme disease is caused by the tick-borne spirochete bacteria *Borrelia burgdoferi* and is the most common vector borne illness in the US. The number of cases of Lyme disease reported to the Centers for Disease Control (CDC) has increased steadily over the last 25 years and the CDC currently estimates that there are 300,000 new diagnoses each year. The acute symptoms of *B. burgdoferi* infection include erythena migrans (bullseye rash), fever, fatigue, muscle and joint pain, headache, and lymphadenopathy. Prompt diagnosis of Lyme disease and treatment with oral doxycycline often results in full recovery of infected individuals. However, in over 50% of cases the characteristic bulls-eye rash is not present, and, acute Lyme disease can be mistaken for a viral illness (i.e. influenza) and go undiagnosed and untreated for weeks or months. In cases of Lyme disease where the infected individual does not receive timely antibiotic therapy, the spirochete can cause neurologic and cardiac symptoms and can be more difficult to treat than the acute infection requiring prolonged antibiotic therapy (Embers et al 2018)

Post-Treatment Lyme Disease Syndrome. It has long been recognized that a subset of individuals with confirmed Lyme disease go on to experience persistent fatigue, pain, and/or neurocognitive difficulties after treatment that are of sufficient severity to impact quality of life and physical functioning (Zubcevik et al 2020). This chronic condition has since been termed post-treatment Lyme disease syndrome (PTLDS). In 2006 the Infectious Disease Society of America (IDSA) developed the current case definition of PTLDS. According to this definition PTLDS is defined as the continuation of or new onset of fatigue, neurocognitive difficulties, and/or widespread pain 6 months from post-treatment resolution of objective signs of Lyme disease that are severe enough to impact functional status. Risk factors for the development of PTLDS include a delay in diagnosis and treatment, the severity of the initial infection, and presence of

neurological involvement. Currently, PTLDS is a diagnosis of exclusion and as such, other clinical factors which could be responsible for these chronic symptoms must be ruled out before individuals meet the definition of having PTLDS. The cause of PTLDS is not known and currently there are no recommended treatments.

We have hypothesized that some cases of PTLDS may be caused by an infection or inflammatory process on or near the neuroimmune vagus nerve, which communicates the detection of peripheral inflammation to the central nervous system and triggers the sickness response circuitry (VanElzakker 2013). The afferent vagus nerve is equipped with chemoreceptors that detect the presence of local proinflammatory cytokines, which are signaling molecules released by innate immune cells upon pathogen (or damage) detection. The vagus nerve acts as a diffuse sensory organ for the central nervous system because proinflammatory cytokines are not only released in relatively small, localized amounts but are relatively large, lipophobic polypeptide protein molecules that do not easily diffuse across the blood-brain barrier via peripheral circulating blood. Therefore, there must be other means by which to communicate the peripheral presence of infection to the central nervous system (Dantzer et al., 2000; Capuron & Miller, 2011; Watkins et al., 1995; Plotkin et al., 1996; Elmquist et al. 1997; Maier & Watkins 1998; Rivest et al., 2000; Quan & Banks, 2007; D'Mello et al., 2009; Ek et al., 1998). In addition to ongoing afferent vagus nerve signaling, another possibility for sustained microglial activation may be that an infectious “hit” triggers a primed neuroinflammatory milieu; microglia can exist in various states of “priming” which means that a given person’s microglia may activate to a relatively minor trigger. A "primed" neuroimmune milieu may also continually respond to an ongoing persistent or latent infection that would normally be asymptomatic. Whether arising from direct infection of the central nervous system, immune activation communicated from the periphery via vagus nerve, or communicated via circulating immune mediators actively transported across the blood-brain barrier, an inflammatory process in the central nervous system would include microglial activation.

Increasing evidence shows that transcutaneous auricular nerve stimulation (taVNS) can significantly reduce multiple symptoms of stress disorder including depression, fatigue, cognitive impairment, anxiety, psychomotor retardation, sleep disturbance (Kong et al, 2018; Aranow et al 2020). One of the collaborators in this study Dr. Vitaly Napadow has developed and patented RAVANS, a type of taVNS, which synchronizes stimulation to the respiratory cycle, modulate vagal systems and optimize stimulations (Napadow et al 2012; Garcia et al, 2017;Yakunina et al 2017). RAVANS has been shown beneficial effect in pain management (Napadow et al 2012; Garcia et al, 2017).

In this study, we will conduct a randomized, double blinded, sham-controlled pilot study to **explore the effect of RAVANS on the symptoms in individuals diagnosed with PTLDS using psychometric measurement, function and cognitive test, and serum biomarkers.**

II. SPECIFIC AIMS

Aim 1. Determine if Respiratory-gated Auricular Vagal Afferent Nerve Stimulation (RAVANS) can improve symptoms of Post-Treatment Lyme Disease Syndrome (PTLDS)

Aim 2: Determine if RAVANS reduces inflammation as measured by serum biomarkers (IL6, IL-10, TNF, IL-1β) after RAVANS treatment in people with PTLDS

Aim3: Determine if inflammation as measured by serum biomarkers (IL-6, IL-10, TNF, IL-1β) correlates with severity of PTLDS.

INVESTIGATORS:

Qing Mei Wang, MD, PhD, Spaulding Rehabilitation Hospital

Principal Investigator, recruitment, screening and consenting, data collection and analysis

David Crandell, MD, Spaulding Rehabilitation Hospital

*Medical Monitor

Michael VanElzakker, PhD, Massachusetts General Hospital

Co-investigator, recruitment, data analysis

Vitaly Napadow, PhD, LicAc, Massachusetts General Hospital

Co-investigator, RAVANS inventor, RAVANS technical support, data analysis,

*Responsibilities of the Medical Monitor
The medical monitor is required to review all unanticipated problems involving risk to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitor must comment on the outcomes of the event or problem and in case of a serious adverse event or death, comment on the relationship to participation in the study. The medical monitor must also indicate whether he concurs with the details of the report provided by the principal investigator.

III. SUBJECT SELECTION

<u>Inclusion criteria include:</u>

1) Adults of all genders ≥18 years

2) History of Lyme disease treated with antibiotics, and current PTLDS diagnosed by a physician

3.Evidence of past B. burgdorferi infection based on positive results from both enzyme immunoassay and Western blot testing.

4) Ability to provide informed consent,

5) Willing to maintain current PTLDS treatment regimen during participation in the study (if on long-term antibiotics or supplements for PTLDS management).

<u>Exclusion criteria</u> include:

1) History of other neurological disorder that in the judgement of the investigator could interfere with the treatment or the interpretation of the results (e.g., epilepsy, history of stroke, tumor, brain tissue damaging pathologies etc.).

2) Current psychotic disorder (e.g., schizophrenia).

3) Current acute illness or infection (e.g. cold or flu).

4) Current or past history of psychiatric illness; PTSD, depression and anxiety are exclusion criteria only if the conditions are so severe as to have required hospitalization in the past 5 years.

5)History of asystole

6)History of recurrent vaso-vagal syncope

7) Bradycardia defined as resting heart rate <50bpm

8) Implanted electronic device (e.g., pacemaker, neurostimulator)

9) Use of immunosuppressive medication such as prednisone, TNF medications within 2 weeks of the visit or anticipated use during the study

10) Current use of anti-inflammatory steroid use

11) Pregnancy

The target population is 48 individuals ≥ 18 years of age who have been diagnosed with PTLDS. All subjects receiving outpatient care at SRH may be eligible for the study and will be sought to participate through publicity flyers. The safety of RAVANS in the pediatric and pregnant population has not been assessed and therefore pregnant women will be excluded. Women of child-bearing potential will be required to take a urine pregnancy test for both study visits.

IV. SUBJECT ENROLLMENT

Potential subjects will be identified by the following sources:

1) Clinicians may refer their patients with PTLDS to the study. We will provide the clinicians with study information sheets and flyers. Prospective subjects will be encouraged to contact the study co-investigators. Flyers will be posted in the outpatient clinic area.
2) Will obtain the patient's names via medical records and RPDR

3) Will use the Mass General Brigham Research Invitations procedures for recruitment. We will send prospective subjects a letter via the Research Invitation Program.

At the first point of contact (usually a phone call), a study co-investigator will provide a description of the study, discuss study procedure, and administer a phone screening questionnaire. Once the phone screening process is complete, the information gathered by the co-investigator will be taken to the PI of the study for further review. Once the PI agrees that the subject is thus far eligible, the subject will be scheduled their first visit. Consent form will be mailed to the subject ahead of time.

Informed consent will be obtained by a license physician, PI Dr. Qing Mei Wang at SRH. The subject will meet with Dr. Wang. The test procedures will be described, and the testing equipment will be shown to the subject. Dr. Wang will clearly explain all the procedures and risks of the testing outlined in the consent form. The subject will be given an hour to consider their decision and will be encouraged to ask questions, both during the initial phone interview and throughout the study. Dr. Wang will answer any questions regarding the study at the time of the informed consent process. Once enrolled, the subject may pause or terminate his/her participation at any time during the study.

Participants will be randomized to receive active or sham RAVANS treatment 3 times per week for 2 weeks through a randomization approach with blocks of 4 (given the sample size of 48 participants, this would give 12 blocks of 4). Randomization of subjects will be blocked based on the 2 treatment options available.

V. STUDY PROCEDURES

First visit: consent, blood drawn, baseline evaluation, first RAVANS/Sham treatment

During this first visit, we will conduct the consent process. Participants that provide informed consent will be interviewed to complete inclusion/exclusion check list. The qualified subjects will be included in the study. They will complete the questionnaires (Demographic Data Questionnaire, Functional Comorbidity Index, Horowitz Lyme-MSIDS ,Sedentary Behaviors Questionnaire, Fatigue Symptom Inventory, Brief Pain Inventory, Vulnerability questionnaire, Beck Depression Inventory, Beck Anxiety Inventory, Pittsburgh Sleep Quality Index), functional test (Timed Up and Go, 4 meter usually walking speed, NIH Toolbox cognitive battery), blood drawn and they will receive 1st RAVANS or sham treatment.

Blood test
Fifteen mL of venous blood will be collected by vein for future measurement of serum biomarkers (IL-6, IL10, TNF, IL-1β).

Questionnaires/ Surveys. A standard demographic survey and PTLDS symptoms will collected using questionnaires outlined below.

1. *Demographic Data Questionnaire (DDQ).* A standard survey will be used to collect data including age, gender, racial background, marital status, education, employment and occupation, income.
2. Functional Comorbidity Index (FCI). Presence of chronic medical conditions will be measured using FCI which is a validated and reliable self-administered 18-item scale designed to examine the impact of comorbidities on physical function. Each participant will be asked to provide a list of prescription, over the counter, and recreational drugs used in the past 7 days.
3. Horowitz Lyme-Multiple Systemic Infectious Diseases Syndrome (HL-MSIDS) Questionnaire: This 55-item questionnaire evaluates the frequency, severity, and incidence of Lyme symptoms as well as assessing one's perceived overall health. A Likert scale from 0 - 3 (none – severe) is used to assess symptom frequency. The subsequent section assigns points to certain symptoms based on the answers in the first section. The third and fourth sections ask the subject assign points to symptom incidence and health perception.
4. Sedentary Behaviors Questionnaire (SBQ): This 18-item questionnaire asks about the amount of time spent engaged in sedentary behaviors on typical weekdays and weekends.
5. Fatigue Symptom Inventory (FSI): This non-diagnosis-specific questionnaire measures the severity of fatigue symptoms and how much these factors interfere with the subjects' lives. There are 14 items, most of which are 0-10 point scales, one question asking about the amount of days fatigue is present, and another is a 0-4 scale on severity of fatigue. The higher the overall score correlates with more severe fatigue symptoms.
6. Brief Pain Inventory (BPI): This 15-item questionnaire assesses the location, severity, and type of current pain, using analogue scales and body diagrams.
7. Vulnerability questionnaire (VQ) This is a medical history questionnaire to ask about possible vulnerabilities to long-term neuroinflammation. Example questions include asking about the extent of previous use of antibiotics, opioids, anti-inflammatory steroids (e.g., prednisone), and previous exposure to pollutants and toxins.
8. Beck Depression Inventory (BDI): This questionnaire evaluates current depressive symptoms. There are 21 items, each having four possible answers. Each item is scored between 0 and 3, with a higher score indicating greater symptom severity. The different items cover both psychological (e.g. concentration) and physical (e.g. appetite) symptoms of depression.

9. Beck Anxiety Inventory (BAI): This questionnaire evaluates current anxiety symptoms. There are 21 items, each having four possible answers. Each item is scored between 0 and 3, with a higher score indicating greater symptom severity. The different items cover both psychological (e.g. nervousness) and physical (e.g. hands trembling) symptoms of anxiety.
10. Pittsburgh Sleep Quality Index (PSQI): This 9-item questionnaire assesses sleep quality and patterns of sleep in adults over the past 30 days. It covers subjective sleep quality, latency, duration, habitual sleep efficiency, disturbances, use of sleeping medications, and daytime dysfunction due to poor sleep.

Objective Functional Tests. Participants will be asked to perform two functional tests; the Timed Up and Go (TUG) and the 4m usual walk speed. The Timed Up and Go test, and usual walk speed are commonly used objective tests of physical function, that independently predict poor outcomes in older adults. Minimal clinically important differences are 0.1 meters per second for gait speed, whereas a chair stand time of ≥ 12 seconds predicts a 2.4-fold increased risk of falls in older adults.

Objectively measured Cognitive Function. We will use the NIH Toolbox Cognition Battery (NIHTB-CB) to assess cognitive function in participants with PTLDS. The NIHTB-CB is a computerized adaptive test that assesses several cognitive domains. It is administered by iPAD using the NIH Toolbox™ iPAD app. The following cognitive domains are assessed: executive function (inhibitory control/attention: Flanker Task; cognitive flexibility: Dimensional Change Card Sort), working memory (List Sorting), short-term memory (Picture Sequence), and an overall composite score that combines these outcomes (Fluid Cognition Composite).

Intervention:

RAVANS Procedures

The participant will be treated with RAVANS or sham at clinic for 20 minutes 3 times per week for 2 weeks.

Respiratory-gated auricular vagus afferent nerve stimulation (RAVANS) will be performed with electrodes attached to the surface of the auricle. A small current will be delivered with a safe battery-powered portable stimulator, also used in other Partners IRB protocols (IRB#2016P000210). Current amplitude will be set between the sensitivity threshold and the pain threshold for each subject, to ensure non-noxious stimulation. Respiratory gating for stimulation will require real-time evaluation of the respiratory cycle, as described below.

For RAVANS setup, electrodes will be placed on the auricle of the left ear. Electrical stimulation to these electrodes will be provided by a current-constant stimulator (Cala

Health Inc, Burlingame, CA). Stimulation will be gated to the subject's own breathing, at a 1-second delay after peak inhalation (i.e. during exhalation.)

Sham stimulation: RAVANS device has a sham control mode on it. For sham stimulation, the device setup will be the same but no electrical current will be passed. Participant will be given instruction to use Mode 1(Active RAVANS) or Mode 2 (Sham stimulation) according to randomization. However, the participant would not know the setting of Mode 1 or Mode 2.

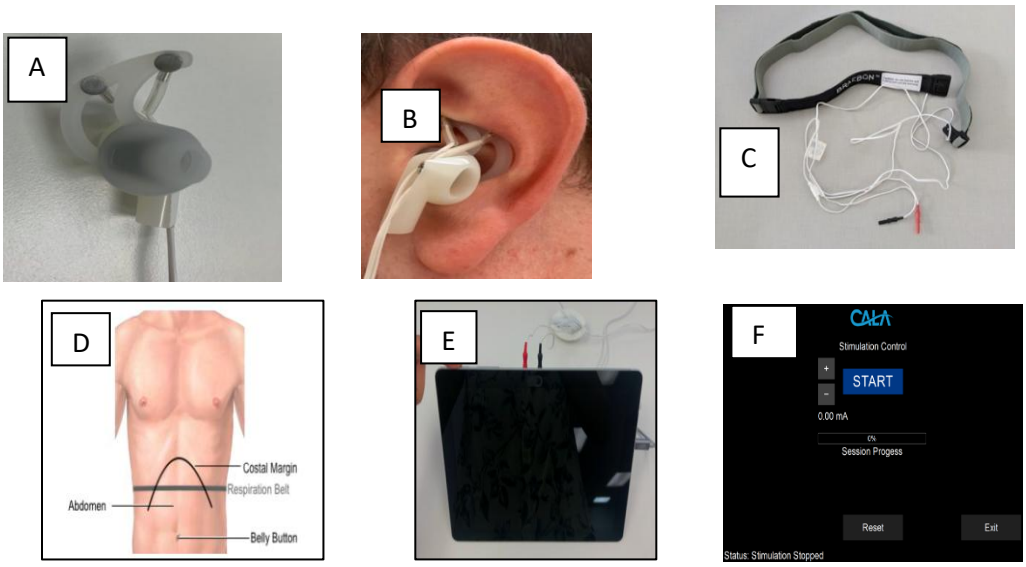

Figure 1: Setup of RAVANS: A. Electrode B. Placement of electrode on the auricle of the ear. C. Respiration belt and cables. D. Placement of respiration belt to gate the subject's breathing. E. Tablet to control the stimulation F. Cala stimulation program in the tablet

Assessments	**Visit 1**	**Visits 2-5**	**Visit 6**
DDQ	X		
FCI	X		
HL-MSIDS	X		X
SBQ	X		X
FSI	X		X

BPI	X		X
VQ	X		X
BDI	X		X
BAI	X		X
PSQI	X		X
Times Up ang Go	X		X
4 meter Walk Speed	X		X
NIHTB-CB	X		X
Blood Drawn	X		X
RAVANS/ShamTreatment	X	X	X
Urine pregnancy test for women of child bearing potential	X		X
Approximate Time	*4 hours*	*20 minutes per visit*	*4 hours*

Visit 2-5:
Subjects will receive RAVANS or sham treatment at clinic for 20 minutes.

Visit 6:
Subjects will receive RAVANS or sham treatment at clinic for 20 minutes. Subjects will complete the questionnaires (Horowitz Lyme-MSIDS, Sedentary Behaviors Questionnaire, Fatigue Symptom Inventory, Vulnerability Questionnaire, Brief Pain Inventory, Beck Depression Inventory, Beck Anxiety Inventory, Pittsburgh Sleep Quality Index), functional test (Timed Up and Go, 4 meter usually walking speed, NIH Toolbox cognitive battery), blood drawn for serum biomarkers (IL-6, IL-10, TNF, IL-1β). Total visit times will be about 4 hours.

VI. BIOSTATISTICAL ANALYSIS

Our main outcome is to assess whether there is an improvement in symptoms (comparing before and after treatment) as indexed by HL-MSIDS score in the active RAVANS group as compared with sham. We chose the HL-MSIDS as the main outcome and therefore the other scale will be the secondary outcome. For the main analysis, we will compare the differences in HL-MSIDS changes between the two groups immediately after the end of treatment. We will therefore use an unpaired t-test for such analysis. Note here that we expect similar baseline level for both groups.

Besides this analysis, we will perform several exploratory analyses as listed below: we will compare the differences in changes in SBQ, FSI, BPI, BDI, BAI, PSQI, Timed- Up

and Go, 4m walk speed, NIHTB-CB, level of serum IL-6, IL-10, TNF, IL-1β between the two groups immediately after the end of treatment. We will therefore use an unpaired t-test for such analysis.

Pearson correlation analysis will be applied to determine if serum level of biomarkers (IL-6, IL-10, TNF, IL-1β) has correlation with symptom severity as index by HL-MSIDS, SBQ, FSI, BPI, BDI, BAI, PSQI, Timed- Up and Go, 4m walk speed, NIHTB-CB.

We will use the intention-to-treat analysis and do an analysis of only subjects that completed the 2-week study. For the intention-to-treat analysis, we will use a conservative method and assume that participants will not improve from the last measured point. The last-observation-carried-forward method will be appropriate because we expect no more than 10 percent of the observations were missing in each analysis.

Power analysis (e.g, sample size)

Sample size was determined with reference to other studies that used similar endpoints. We assume that a standardized difference of 0.8 (the difference between groups divided by the standard deviation) is conservative to detect a clinically meaningful difference between groups (active RAVANS and sham). We will assume a type I error of 5% (alpha), a type 2 error of 10% (beta) and therefore the power will be 90%. Using the sample size calculation for a normal distributed population (t-test), 20 participants per arm will be necessary (total of 40 participants). Conservatively, we expect a 20% rate of dropout or loss to follow-up. We will assume that dropout participants will not improve from the last measured point; thus the sample size will be increased by 20% to 48 participants (24 per arm).

VII. RISK AND DISCOMFORTS

Blood Draws. Blood drawing may cause a small amount of pain. In addition, participants may get a temporary bruise or “black and blue mark”. Rarely, people faint when their blood is drawn. Very rarely, the vein may become red and swollen, or infected. If this occurs, we will treat the problem. Blood drawn will be performed by clinical phlebotomists to minimize the risk and discomforts.

Surveys/ Questionnaires. Some of the questions in the questionnaires may seem personal or embarrassing. Participants will be instructed that they may refuse to answer any of the questions that they do not wish to answer. If the questions make a participant very upset, we will help them to find a counselor. If participants develop fatigue during completing the questionnaire, we all ask the participant to take break.

RAVANS Risks: There is a small possibility of slight and temporary discomfort at the site where the electrodes are placed. Potential vaso-vagal reactions to stimulation

resulting in dizziness or light-headedness. The participant will be instructed to sit in chair while receiving RAVANS/Sham treatment.

The RAVANS procedure is without significant safety concerns. The electrical stimulator, checked by MGH Biomedical Engineering, has a current limiter preventing harmful stimulation levels.

Uncommon Risks. Although we have made every effort to protect participant identity, there is a minimal risk of loss of confidentiality. Data collected from individuals will be kept confidential, accessible only to trained study staff. To protect against this risk, all participants will be assigned a study ID number and all collected data will be stored with this ID number and not include name or medical record number. A link between names and ID numbers will be kept separately under lock and key.

VIII. POTENTIAL BENEFITS

As with any study focusing on basic research, the subjects will derive no direct benefit. The noninvasive techniques and paradigms employed in the current proposal do not constitute any significant risk to the subjects. It is possible that the study participant will experience an improvement in fatigue, depression, anxiety, pain and cognitive function. We envision that in the near future the information obtained from the proposed research will provide a better understanding and treatment for subjects with PTLDS.

IX. MONITORING AND QUALITY ASSURANCE

Source of Research Material: Sources of materials to be collected include data from self-report fatigue, depression, anxiety, pain, and cognitive dysfunction from measurement scales, serum level of cytokines. All materials obtained are specifically for research purposes. The final dataset will include self-reported demographic, symptom data, serum level of cytokines. The final dataset will be stripped of identifiers prior to data analysis.

Data management. Each subject will have a file that includes consent form and all source data. All subject files will be kept at a locked cabinet at the Spaulding Rehabilitation Network Research Institute. Computerized data files will be protected by passwords to protect the confidentiality of the participants and the integrity of the data. Data management will be done by the Principal investigator, Dr. Wang and by the Clinical Research Coordinator.

Prior to being added as a co-investigator, the study staff member will be trained in RAVANS as well as all aspects of the evaluations. This study staff member will then be evaluated by the PI of the study. The PI will give confirmation that the co-investigator is qualified to do the evaluations and train the participant to use RAVANS.

The PI (Dr. Wang) who is a licensed physician must always be in the building at the time of each study session.

The proposed study will be monitored for safety, with monthly staff meetings reviewing adverse events and treatment outcomes and directly reporting any adverse events. The PI will also routinely monitor and assure the validity and integrity of collected data and adherence to the IRB approved protocol. The trained staff members who carry out the procedures will also carefully monitor the study throughout its duration. The team will evaluate the progress of the study, verify that the rights and well-being of the subjects are protected, verify that the reported study data are accurate, complete and verifiable from source documents, and that the conduct of the study is in compliance with the approved protocol and amendments.

We plan on performing interim data analysis with the first block of 12 subjects. Adverse events, data, and procedures will be reviewed by Dr Wang and her team. If data are informative, we plan to publish the preliminary results. All expected and unexpected adverse outcomes will be reported to the IRB according to the Adverse Event Reporting guidelines.

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