AUTOIMMUNITY CENTERS OF EXCELLENCE

AJA01

USING THE CHOLINERGIC ANTI-INFLAMMATORY PATHWAY TO TREAT JUVENILE IDIOPATHIC ARTHRITIS

TCVNS IN JIA

VERSION 2.0 /APRIL 9, 2024

National Clinical Trial #NCT05710640

Study Sponsor: The National Institute of Allergy and Infectious Diseases (NIAID)

NIAID Funding Mechanism: Autoimmunity Centers of Excellence (ACE) Cooperative Agreement

Investigational Devices/Manufacturers: Active Transcutaneous Vagus Nerve Stimulation (tcVNS) System; Sham tcVNS System/Feinstein Institutes for Medical Research (FIMR)

Consortium/Network: Autoimmunity Centers of Excellence

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Confidentiality Statement

NIH National Institute of Allergy and Infectious Diseases

SITE INVESTIGATOR SIGNATURE PAGE	
Protocol Number: AJA01 (tcVNS in JIA)	**Version Number/Date: v2.0 09Apr2024**
Site Principal Investigator:	
Protocol Title: Using the Cholinergic Anti-Inflammatory Pathway to Treat Juvenile Idiopathic Arthritis	
Study Sponsor: The National Institute of Allergy and Infectious Diseases (NIAID)	
Return Signed Form to: *The original signature page must be kept for your records. Return an electronic PDF copy of the signed signature page (*as described below) to the DAIT Regulatory Management Center via the applicable DAIT RMC email address for the protocol/network.* *If PPD is not the Regulatory Management Center for this protocol, e.g., for an Investigator Initiated clinical trial, contact the DAIT Regulatory Officer for instructions on where to send the completed signature page.*	
I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 45 CFR part 46 and 21 CFR parts 50, 56, and 312, 812 and in the International Conference for Harmonisation (ICH) document entitled Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2). Further, I will conduct the study in keeping with local legal and regulatory requirements. As the site Principal Investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without the written permission of the IRB and NIAID. **The site Principal Investigator should sign and date at the indicated location below. A written signature/date is acceptable (e.g., scanned and sent via email as a PDF version). An electronic signature is also acceptable (e.g., sent via email as a PDF version).* _____ **Site Principal Investigator (Print)** _____ _____ **Site Principal Investigator (Signature)** **Date**	

Protocol Synopsis

Title	AJA01: Using the Cholinergic Anti-Inflammatory Pathway to Treat Juvenile Idiopathic Arthritis
Short Title	tcVNS in JIA
Clinical Phase	II
Number of Sites	Approximately 11 Pediatric Sites in the United States
Study Sponsor	The National Institute of Allergy and Infectious Diseases (NIAID)
Study Objectives	Primary Objective The primary objective of this study is to determine the effect of tcVNS on disease activity in participants with active JIA as assessed by the JIA ACR 50 response. Secondary Objectives The secondary objectives of this study are to determine the effect of tcVNS on disease activity in participants with active JIA assessed by the JIA ACR 30 response, JIA ACR 70 response, and Juvenile Arthritis Disease Activity Score in 27 Joints (JADAS-27) response. Exploratory Objectives Clinical Exploratory Objectives The clinical exploratory objectives of this study are to determine the effect of tcVNS on the following aspects of JIA in participants with active JIA: • Pain • Functional status • Overall health and health-related quality of life • Arthritis Mechanistic Exploratory Objectives The mechanistic objectives of this study seek to understand the mechanisms by which tcVNS may reduce inflammation and decrease musculoskeletal pain in participants with active JIA by examining serum, cellular and transcriptomic biomarkers of clinical response.
Study Design	AJA01 is a multicenter, double-blind, sham-controlled, 16-week trial to evaluate the safety and effectiveness of tcVNS for the treatment of JIA. A total of 100 participants will be randomized 1:1 to treatment with active tcVNS at the cymba concha or SS at the neck for 5 minutes once a day for 8 weeks. During this time, participants, guardians, and participant assessors will be blinded to treatment assignment; treatments on clinic visit days will be conducted in the clinic under the supervision of a trained, unblinded staff member, and participants will only discuss the stimulation procedure with this staff member.

	Additional information on blinding can be found in Section 3.6, *Randomization, Stratification, and Blinding/Masking*. The double-blind, sham-controlled 8-week period will be followed by an 8-week open-label period in which all participants will receive treatment with active tcVNS at the cymba concha once a day for 5 minutes. This will allow for an evaluation of response in responding participants at week 8, and sustainability after 8 weeks of tcVNS. Additionally, an open-label period will facilitate enrollment. Consenting individuals meeting all entry criteria will have a Baseline/Day 0 Visit including vital signs, patient-reported outcomes (PROs), clinician-reported outcomes (ClinROs) including physical and joint exams conducted by blinded personnel and blood draws. All participants will be instructed not to discuss the study or their treatment experience with other participants or people outside of the study Participants, with guardian assistance as needed, will then administer 5 minutes of active tcVNS at the cymba concha or SS at the neck, followed by repeat vital signs, under the supervision of an unblinded coordinator/nurse. The treating site staff member will work with participants to ensure that they can properly administer future stimulations at home. Participants will then be sent home with the stimulation system for self-administration. Starting on Day 1, participants (with guardian assistance as needed) will self-administer stimulation at home once a day for 5 minutes, recording information in a diary, including any difficulties or side effects encountered. Follow-up visits will occur at Day 1 (telemedicine to confirm proper technique) and Weeks 1, 4, and 8. At the Week 8 Visit, all participants will receive an active tcVNS system with appropriate settings and other necessary items. Prior to receipt of the active tcVNS system at the Week 8 Visit, participants will be asked what treatment they believe that they received during the double-blind, sham controlled 8-week period. A similar schedule will be followed for the open-label extension period. Follow-up visits will occur at Day 57 (telemedicine to confirm proper technique) and Weeks 9, 12, and 16. At each follow-up visit, participants will perform transcutaneous stimulation in the clinic followed by vital signs readings, and the voltage of the device will be assessed by oscilloscope. PROs will be administered, ClinROs will be conducted, safety data will be collected, and central clinical and mechanistic labs will be assessed intermittently according to Table 8.1, *Schedule of Events*.
Primary Endpoint(s)	The primary endpoint is the proportion of participants achieving ≥50% improvement in clinical status as defined by JIA ACR 50 at Week 8. This primary endpoint will be compared between the active tcVNS and SS treatment arms. The JIA ACR 50 is a validated composite response consisting of 6 core criteria: • number of joints with active arthritis • number of joints with limited motion • physician's assessment of disease activity (measured on a 10 cm visual analog scale (VAS)) • parent/patient assessment of overall well-being (measured on a 10 cm (VAS) • a validated measure of physical function (Childhood Health Assessment Questionnaire {CHAQ})

	• a laboratory measure of inflammation (CRP) The JIA ACR 50 is achieved if 3 of any 6 core set variables improve by at least 50% from the baseline/Day 0 visit, and no more than 1 variable worsening by >30%. [1]
Secondary Endpoint(s)	<u>Efficacy:</u> The following secondary efficacy endpoints will be compared between the active tcVNS and SS treatment arms: • Proportion of JIA ACR 50 responders at Weeks 4, 12, and 16 using Day 0 as Baseline. • Proportion of JIA ACR 30 responders at Weeks 4, 8, 12, and 16 using Day 0 as Baseline. • Proportion of JIA ACR 70 responders at Weeks 4, 8, 12, and 16 using Day 0 as Baseline. • Proportion of JIA ACR 50 responders at Weeks 12, and 16 using Week 8 as Baseline. • Proportion of JIA ACR 30 responders at Weeks 12, and 16 using Week 8 as Baseline. • Proportion of JIA ACR 70 responders at Weeks 12, and 16 using Week 8 as Baseline. • Change in JADAS-27 at Weeks 4, 8, 12, and 16 compared to Day 0. • Change in JADAS-27 at Weeks 12, and 16 compared to Week 8. • Longitudinal trends in JADAS-27 from Day 0 to Week 16. <u>Safety:</u> The following safety endpoints will be summarized in the active tcVNS and SS treatment arms: • Incidence of specific grade 1 AEs (described in Section 12.3.1, *Grading Criteria*) plus all grade 2 or higher AEs. • Incidence of SAEs. <u>Exploratory:</u> Clinical Exploratory Endpoints: The following exploratory efficacy endpoints will be compared between the active tcVNS and SS treatment arms: • Change in each of the following endpoints at Weeks 1, 4, 8, 9, 12, and 16 compared to Day 0: ○ Pain Assessment by Wong-Baker FACES Scale ○ CHAQ ○ Patient-Reported Outcomes Measurement Information System (PROMIS) Mobility Questionnaire ○ Patient-Reported Outcomes Measurement Information System (PROMIS) Upper Extremity Questionnaire

	○ Global Assessments by VAS ○ Physician Global Assessment (PGA) ○ Joints with Active Arthritis ○ Joints with Limited Motion • Change in each of the following at Weeks 9, 12, and 16 compared to Week 8: ○ Pain Assessment by Wong-Baker FACES Scale ○ CHAQ ○ PROMIS Mobility Questionnaire ○ PROMIS Upper Extremity Questionnaire ○ Global Assessments by VAS ○ PGA ○ Joints with Active Arthritis ○ Joints with Limited Motion Mechanistic Exploratory Endpoints: The following endpoints will be compared between the active tcVNS and SS treatment arms: 1. Change in serum levels of soluble mediators regulated by the CAP at Weeks 8 and 16 compared to Day 0 and at Week 16 compared to Week 8. 2. Change in serum levels of substance P at Weeks 8 and 16 compared to Day 0 and at Week 16 compared to Week 8. 3. Peripheral blood cell production of soluble mediators after 24 hours of stimulation by LPS at Week 8 compared to Day 0. 4. Change in transcriptomics and gene expression of select genes such as TNF, IL-6, and IL-1 at Week 8 compared to Day 0. 5. Change in composition of circulating blood cells at Week 8 compared to Day 0.
Accrual Objective	100 randomized (1:1) to Active tcVNS or SS Randomization will be stratified by: • Use of biologic and • Number of active joints at the Baseline/Day 0 visit (3-4 joints vs 5 joints or more)
Study Duration	Participants will be engaged in the study for a maximum of 16 weeks in addition to the screening period. We anticipate 2 years to achieve the target number of randomizations.
Treatment Description	<u>Weeks 0-8:</u> Participants will treat themselves for 5 minutes daily with either active tcVNS at the cymba concha or SS at the neck.

	• The active tcVNS system will be placed on the left cymba concha, with the stimulator component programmed with a 300-microsecond pulse at frequency of 30 Hz. • The sham tcVNS system will be placed on the left side of the neck, with the stimulator component programmed with a 50-microsecond pulse at frequency of 2 Hz. <u>Weeks 9-16:</u> All participants will treat themselves for 5 minutes daily with active tcVNS at the cymba concha as outlined above.
Inclusion Criteria	Individuals who meet all the following criteria are eligible for enrollment as study participants: 1. Participant is 5 through 18 years of age (inclusive) at screening. 2. Regarding informed consent and compliance: a. If 5 through 6 years of age, the participant's guardian is willing and able to understand and provide informed consent and comply with study protocol. b. If 7 through 17 years of age, the participant is willing and able to sign assent and comply with study protocol, and the participant's guardian is willing and able to understand and provide informed consent and comply with study protocol. c. If 18 years of age, the participant is willing and able to understand and provide informed consent and comply with study protocol. 3. The participant has a JIA diagnosis meeting ILAR classification criteria with one of the following subtypes: • rheumatoid-factor negative polyarthritis. • rheumatoid-factor positive polyarthritis. • persistent oligoarthritis. • extended oligoarthritis. • psoriatic arthritis. • enthesitis-related arthritis • systemic arthritis. 4. The participant has ≥ 3 joints with active arthritis at screening. 5. If the participant is receiving therapy for JIA at screening, that therapy is stable for the time period outlined below and is expected to remain stable for the duration of the study: • Stable dose for at least 1 week prior to screening: o Oral steroids, ≤ 0.2 mg/kg/day with a maximum 10 mg/day dose • Stable dose for at least 2 weeks prior to screening:

	○ NSAIDs • Stable dose for at least 8 weeks prior to screening ○ adalimumab ○ anakinra ○ canakinumab ○ certolizumab pegol ○ etanercept ○ golimumab ○ infliximab ○ leflunomide ○ methotrexate ○ tocilizumab • Stable dose for at least 12 weeks prior to screening ○ abatacept 6. If a female of child-bearing potential, the participant has a negative urine pregnancy test at screening. 7. If of reproductive potential, must agree to abstinence or effective methods of birth control for the duration of the study.
Exclusion Criteria	Individuals who meet any of these criteria are not eligible for enrollment as study participants: 1. Other than NSAIDs or intra-articular injections, participant has been treated for JIA with lack of efficacy with: a. More than 2 different classes of therapies or b. More than 3 medications in total 2. Participant has received high-dose steroids (≥0.2 mg/kg/day) within the 28 days prior to screening. 3. Participant has had active systemic disease (fever, systemic rash) within the 3 months prior to screening including any of the following lab manifestations at screening: a. Ferritin >1000 ng/mL b. WBC ≥15,000/mm^3 4. Participant has had an active acute systemic infection within 2 weeks of screening involving fever (100.4^oF or higher) for more than 24 hours, requirement for systemic antibiotics or antivirals, GI symptoms lasting 48 hours or more, or the need to hold second line medications for JIA (methotrexate or biologic). 5. Participant has a history of arrhythmia. 6. Participant has been diagnosed with postural orthostatic tachycardia syndrome (POTS).

	7. Participant has received an intra-articular cortisone injection within the 28 days prior to screening. 8. Participant has received treatment with an investigational drug or device during the 28 days prior to screening or within five half-lives of the investigational drug prior to screening/baseline, whichever is the greater length of time. 9. Participant has received chronic treatment with an anti-cholinergic medication, including over the counter medications. 10. Participant has received treatment with rituximab: a. Within one year of screening b. At any time previously without documented B cell repletion 11. Participant has a comorbid disease that has required treatment with corticosteroids within the past year. 12. Participant has an implantable electronic device such as a pacemaker, defibrillator, hearing aid, cochlear implant, insulin pump or deep brain stimulator. 13. Participant has used cutaneous Vagus Nerve Stimulation (VNS) within 12 weeks prior to screening. 14. Participant has received a live attenuated viral vaccine within 28 days prior to screening or is expected to receive one during the study. 15. Participant has any condition which, in the opinion of the investigator, would jeopardize the participant's safety following exposure to a study intervention. 16. Participant has any past or current medical problems or findings from a physical examination or laboratory testing that are not listed above but which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements, or may impact the quality or interpretation of the data obtained from the study.
Study Stopping Rules	There are no pre-specified criteria that will stop the study, but the Data and Safety Monitoring Board (DSMB) will be convened for an ad hoc meeting should any of the following events occur: 1. Any death (regardless of relationship to investigational device), 2. Any National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE) Grade 4 or higher AE in a single participant, 3. The occurrence of NCI-CTCAE Grade 3 or higher AEs (including SAEs) that are related to investigational device in 2 of the first 10 participants or 20% of all participants at any time, 4. At the request of the DSMB/NIAID.

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Glossary of Abbreviations

α7nAChR	alpha7 Nicotinic Acetylcholine Receptor
ACh	Acetylcholine
AChE	Anti-Cholinesterase
ACR 20/30/50/70	American College of Rheumatology Response Criteria 20/30/50/70
ADE	Adverse Device Effect
AE	Adverse Event
BP	Blood Pressure
CAP	Cholinergic Anti-Inflammatory Pathway
CBC	Complete Blood Count
CCP	Cyclic Citrullinated Peptide
CFR	Code of Federal Regulations
CHAQ	Childhood Health Assessment Questionnaire
CMP	Comprehensive Metabolic Panel
CRF	Case Report Form
CDAI	Crohn's Disease Activity Index
ClinRO	Clinician-Reported Outcome
CRP	c-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DAS28	Disease Activity Score in 28 Joints
DMARDs	Disease-modifying Anti-rheumatic Drugs
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
eCRFs	Electronic Case Report Forms
ELISA	Enzyme-Linked Immunosorbent Assay
ERA	Enthesitis-Related Arthritis
EULAR	European League Against Rheumatism
FDA	Food and Drug Administration
FLS	Fibroblast-like Synoviocytes
FIMR	Feinstein Institutes for Medical Research
GCP	Good Clinical Practice

HR	Heart Rate
HRV	Heart Rate Variability
ICH	International Conference on Harmonization
IDE	Investigational Device Exemption
ILAR	International League of Associations for Rheumatology
IND	Investigational New Drug
IRB	Institutional Review Board
JADAS	Juvenile Arthritis Disease Activity Score
JIA	Juvenile Idiopathic Arthritis
JPsA	Juvenile Psoriatic Arthritis
LPS	Lipopolysaccharides
MAUDE	Manufacturer and User Facility Device Experience
mITT	Modified Intent to Treat
MOP	Manual of Procedures
NCI-CTCAE	National Cancer Institute's Common Terminology Criteria for Adverse Events
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NSAIDs	Non-Steroidal Anti-Inflammatory Drug
OA	Osteoarthritis
PBMCs	Peripheral Blood Mononuclear Cells
PGA	Physician Global Assessment
PI	[Site] Principal Investigator
PRO	Patient-Reported Outcome
PROMIS	Patient-Reported Outcomes Measurement Information System
RA	Rheumatoid Arthritis
RF	Rheumatoid Factor
SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
sIRB	Single Institutional Review Board
SS	Sham Stimulation
SLE	Systemic Lupus Erythematosus

SOP	Standard Operating Procedure
SUSAR	Serious Unexpected Suspected Adverse Reaction
taVNS	Transcutaneous Auricular Vagus Nerve Stimulation
tcVNS	Transcutaneous Cervical Vagus Nerve Stimulation
TENS	Transcutaneous Electrical Nerve Stimulation
UADE	Unanticipated Adverse Device Effect
VAS	Visual Analogue Scale
VNS	Vagus Nerve Stimulation

1. Background and Rationale

1.1 Background and Scientific Rationale

1.1.1 Juvenile Idiopathic Arthritis (JIA)

Juvenile idiopathic arthritis (JIA) is a chronic autoimmune inflammatory joint disease with an age of onset of ≤16 years and a prevalence ranging from 15-150 per 100,000, with nearly 300,000 total children diagnosed in the United States. It is the most common rheumatic disease occurring in childhood [2] and most children continue to experience active inflammatory disease into adulthood. Seven subtypes have been described based upon the presentation: oligoarticular, seropositive polyarticular, seronegative polyarticular, systemic onset, enthesitis-related arthritis (ERA), juvenile psoriatic arthritis (JPsA), and undifferentiated [3]. Disease-modifying anti-rheumatic drugs (DMARDs), both traditional and biologic, have been increasingly utilized as the pediatric rheumatology community has become aware of the benefits of early aggressive intervention both for control of the existing inflammatory manifestations and for advantageous effects on long-term prognosis [4]. However, these agents are expensive, do not adequately control disease in approximately one-third of patients, and have noteworthy toxicities including risks of infection, malignancy, or development of a new autoimmune disease. Many patients lose their response to these medications. Furthermore, DMARDs and biologics often add significant burden for patients and families. Unpleasant, often treatment limiting side effects occur and require changes in the therapeutic plan. Biologic treatment most often requires injections to be given by the guardians at home or intravenous administration of the medication resulting in significant anxiety for the child and guardian, and days away from school and work in the case of infusion medications. Children with oligoarthritis that is refractory to nonsteroidal anti-inflammatory medications or repeated intra-articular steroid injections may need to escalate to a second-line medication even though they do not have widespread or polyarticular disease.

1.1.2 Cholinergic Anti-inflammatory Pathway

This project represents the intersection of two critical scientific lines of investigation. In the first, a “cholinergic anti-inflammatory pathway” (CAP) was recently defined as a neuro-immune pathway capable of modulating inflammatory and autoimmune disease [5],[6]. This mechanism, studied in detail by Dr. Kevin Tracey at the Feinstein Institute, New York implicates brain cholinergic signaling as a key regulator of peripheral cytokine release and inflammation through the efferent vagus nerve. The critical mediator, acetylcholine (ACh), interacts with a specific cholinergic receptor, the alpha7 nicotinic acetylcholine receptor (α7nAChR), through a multicellular pathway and ultimately suppresses cytokine production in macrophages [7], [8]. The CAP originates in the brainstem, the site of origin of the vagus nerve. Action potentials are transmitted through the cholinergic vagus nerve to terminate in the celiac ganglion. Neurons within the adrenergic splenic nerve originate in the celiac ganglion and terminate in the spleen where they release norepinephrine. A subset of CD4+,CD44hi,CD66low T cells within the red pulp and marginal zones are in close proximity to the splenic nerve fiber endings, express the norepinephrine receptor and produce ACh in response to norepinephrine [9]. In turn, Ach modulates and suppresses inflammatory cytokine production by macrophages. The in vivo significance of the pathway has been demonstrated in animal models of Rheumatoid Arthritis (RA)[10].

There are clearly additional pathways synchronized with VNS. In the rat adjuvant animal model of arthritis, inhibition of p38 MAP kinase within the spinal cord diminishes peripheral arthritis [11]. Although the exact mechanism is unknown, subsequent studies have demonstrated that it does involve activation of vagus nerve outflow [12]. It is also important to note that VNS is an effective modality for pain reduction. How it does this is not clear, but perhaps by decreasing inflammatory mediators that activate neurons. Additionally, VNS results in changes in functional brain imaging; although mechanisms are not known, peripheral effects of VNS may be mediated by these central alterations.

Children with polyarticular JIA are faced with treatments that have significant toxicities, resulting in anxiety of painful injections or infusions. For children with oligoarticular arthritis, the decision to add a second-line medication is not straightforward. One common modality of treatment that has been utilized for joints not responsive to NSAIDS is intra-articular injection with corticosteroids. This procedure requires sedation in younger children, is painful, and has a variable duration of response often only lasting several months. In addition, intra-articular injection is not feasible when several joints are affected. For these reasons, an alternative treatment particularly for active oligoarthritis would be desirable. If effective, VNS would provide a painless treatment with few potential adverse effects (AEs) for children. It would provide an excellent alternative for oligoarticular arthritis when second-line medications may not be warranted but a treatment more

than NSAIDs or intra-articular injection is required. Furthermore, it is a treatment that can easily be performed at home by patients in a short time with inexpensive equipment.

1.2 Rationale for Selection of Investigational Device

Promising preliminary data described below (see Section 1.4, *Clinical Studies*) suggests that VNS may be beneficial for the modulation of inflammatory autoimmune diseases i.e., RA, Crohn's disease and Systemic Lupus Erythematosus (SLE) with minimal toxicity and without immunosuppression. As the CAP can be engaged non-invasively by stimulating the auricular branch of the vagus nerve, we will assess the effects of transcutaneous vagus nerve stimulation (tcVNS) in JIA. Both the cymba concha and the tragus of the outer ear are innervated by the auricular branch of the vagus nerve, an afferent sensory nerve. tcVNS at the cymba concha will be utilized as 1) this location is innervated solely by the auricular branch of the vagus nerve, 2) multiple studies have shown beneficial clinical effects of VNS at this site and 3) previous experience and familiarity of the Feinstein Institutes for Medical Research (FIMR) with using this anatomic site to conduct studies of tcVNS. A double-blind, sham-controlled trial of short duration is planned to test the engagement of the CAP by tcVNS which will result in diminished inflammation and a clinical response in children with active JIA. Previous trials of tcVNS conducted by investigators at the FIMR have shown mechanistic and clinical effects of tcVNS applied to the cymba concha for 5 minutes. This trial will extrapolate from these previous studies and evaluate the effects of tcVNS for 5 minutes daily in children with active JIA. Additionally, potential molecular mechanisms and pathways by which tcVNS decreases inflammation in JIA will be studied and verify that it will be through reduction of serum inflammatory markers. The levels of inflammatory markers are anticipated to correlate with JIA disease activity. Potential early biomarkers of future clinical response will be explored.

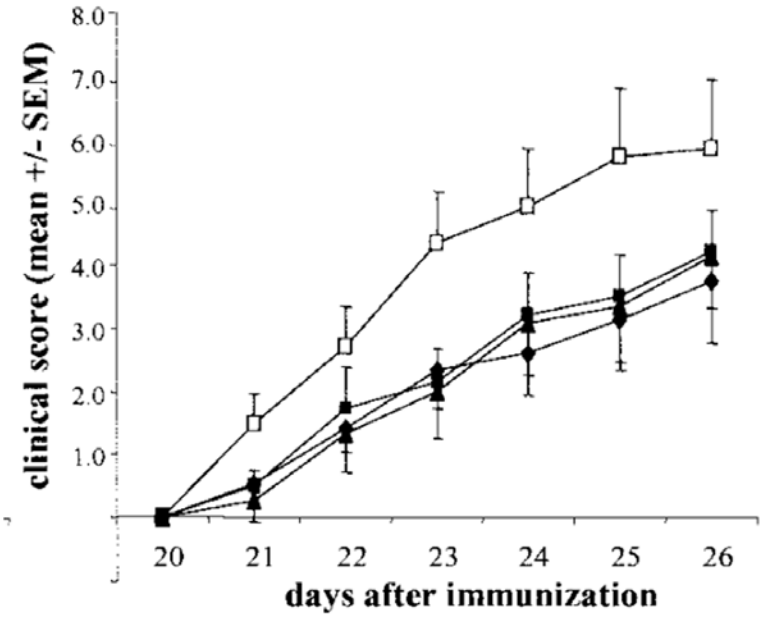
1.3 Preclinical Experience

Given the "anatomy" of the CAP, the pathway can be engaged by 3 potential interventions to potentially suppress inflammatory disease: 1) stimulation of the vagus nerve, 2) enhancement of cholinergic signaling by suppression of anti-cholinesterase (AChE) activity: AChE is an enzyme that degrades ACh and terminates its signaling in the cholinergic synapse, or 3) directly stimulating monocytes with α7nAChR agonists. Peripheral administration of galantamine, a centrally acting AChE inhibitor, significantly suppresses serum tumor necrosis factor (TNF) levels and improves survival during lethal inflammation in mice [8]. The effect of galantamine is dependent on the vagus nerve, as cutting the vagus nerve significantly reduces the magnitude of the suppressive actions of the drug on TNF levels [13]. The mediating role of the CAP is also supported by the absence of an effect of galantamine on serum TNF levels in mice lacking the α7nAChR [7], [14]. In the rat carrageenan paw edema model, an animal model of acute inflammation, galantamine significantly decreases paw swelling and decreases levels of systemic inflammatory mediators [13].

The beneficial potential of the CAP has been demonstrated in collagen induced arthritis, a mouse model of RA [10]. Vagotomy worsens the clinical arthritis; in contrast, increased cholinergic activity secondary to administration of nicotine, or AR-R17779, a specific α7nAChR agonist, results in clinical improvement, reduction of histologic synovitis, and reduced synovial tissue expression of TNF transcript and plasma TNF protein levels (Figure 1) [10]. Furthermore, synovial inflammation and serum levels of TNF are significantly worsened in α7nAChR knock-out mice in this arthritis model [15].

Figure 1. Improvement in Clinical Arthritis Scores in Mice Treated with AR-R17770

Scores from mice treated with saline (open circles) were significantly higher than scores in mice given AR-R1779 (1, 2.5 or 5 mg/kg). P= 0.05 using AUC from d 20-26. N=15 per dose. [10]



Similarly, administration of nicotine to Lewis rats with adjuvant induced arthritis, a different model for inflammatory arthritis, resulted in a diminution of the arthritis score, and reduction of IL-1β and TNF production by synovial cells when the agent was given after the induction of the arthritis [16]. If given before the induction of arthritis in this model, the arthritis score was increased. Anti-CCP titers correlated with disease activity and were increased in rats that were pretreated with nicotine while they were reduced in rats treated with nicotine after the onset of arthritis. While nicotine does engage the α7nAChR, it also engages numerous other cholinergic receptors, leading to numerous off target side effects including addiction (and withdrawal symptoms), increased risk of malignancy and atherosclerosis.

Importantly and of relevance to this proposal, direct electrical stimulation of the vagus nerve is a potent means to engage the CAP and results in decreased production of pro-inflammatory mediators. Peripheral blood mononuclear cells (PBMCs) obtained from normal dogs after 1 minute of VNS, administered through an implanted device, were stimulated with lipopolysaccharides (LPS). LPS-stimulated TNF release was suppressed, and the suppression of TNF release was maintained for at least 3-4 days following a single stimulation (Figure 2) (unpublished data from FIMR). Consecutive stimulations have more potent effects. After 2 stimulations given one week apart, the return of TNF secretion by LPS-stimulated PBMCs to baseline levels was observed after 12 days. Thus, the time taken for the response to normalize increases after the second VNS. These data demonstrate that intermittent VNS results in a durable suppression of TNF secretion by LPS-activated monocytes. Direct stimulation of the vagus nerve has been performed in the collagen-induced arthritis murine model (Figure 3) [17]. Disease progression was inhibited in Lewis rats receiving stimulation after immunization with collagen but before onset of arthritis compared to sham stimulation with significantly reduced joint swelling and histopathologic scores. Pannus formation, cartilage destruction and bone erosion were all significantly less in animals receiving VNS. Furthermore, serum levels of IL-1β, IL-6, TNF, and IFNγ were reduced in the treated rats [17].

Figure 2 Persistence of VNS Effect Beyond VNS Stimulation Period in Canine Model

Dogs were implanted with cuffed electrode and an external stimulator. Vagus nerve stimulation was applied on Days 21 and 28. Whole blood ex-vivo LPS stimulated TNF release its shown (unpublished data from FIMR).

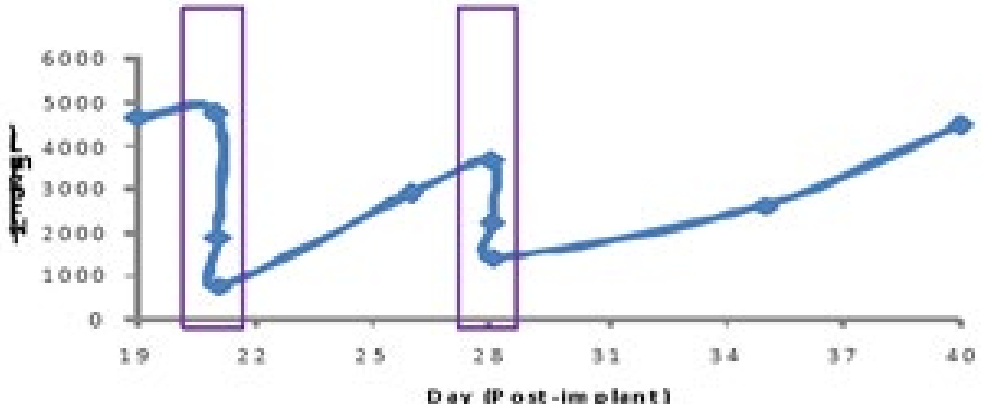
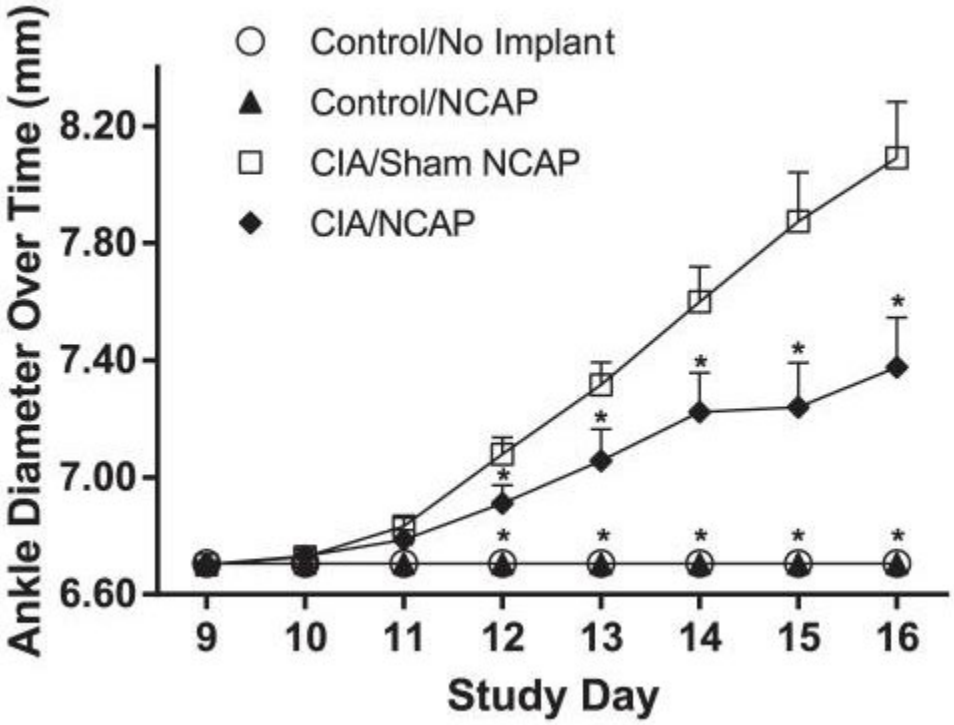


Figure 3. Vagus Nerve Stimulation in Lewis Rats with Collagen-induced Arthritis

Lewis rats were injected with bovine type II collagen and Freund's incomplete adjuvant on days 0-6. Sham or direct vagus nerve stimulation was conducted from days 9-15. Animals were sacrificed on day 16. [17]



The CAP can also be engaged by stimulating the auricular branch of the vagus nerve which innervates the concha as well as the tragus of the outer ear. The concha is innervated solely by the auricular branch of the vagus nerve. Electrical stimulation of either the concha, i.e. tcVNS or the vagus nerve directly, significantly reduced serum levels of TNF, IL--1 and IL-6 in rats ($p<0.01$, $p<0.05$ respectively) in rats pretreated with LPS [18]. Vagotomy prevented the amelioratory effects of tcVNS in this model. Additionally, pretreating rats with an α7nAChR antagonist (thereby blocking the CAP) abolished the effects of tcVNS or VNS reduction on LPS mediated pro-inflammatory cytokine release.

Synovial tissue contains the necessary components to observe the anti-inflammatory effects of ACh. Local production of ACh occurs within synovial tissue as choline acetyltransferase (ChAT), the enzyme that synthesizes ACh from choline and acetyl-CoA, is observed in mononuclear cells and fibroblast-like synoviocytes (FLS) within synovial tissue from patients with osteoarthritis (OA) or RA [19]. Additionally, the α7nAChR is expressed in human synovial tissue, predominantly in the synovial intimal lining of affected joints of patients with OA and RA [20]. The α7nAChR is also expressed in cultured FLS. Administration of ACh to IL-1 stimulated FLS in culture, significantly reduces production of IL-6; methyl lycaconitine, an α7nAChR antagonist or α7nAChR specific siRNA blocked the suppression of inflammatory mediators by ACh [21]. PNU-282,987, a selective ?7nAChR also decreased IL-6 production by IL-1 stimulated FLS [20]. In other experiments, IL-6 and IL-8 secretion was reduced by nicotine or AR-R17779 in TNF stimulated FLS [21], [10].

Human CD14+ monocytes secrete TNF and other pro-inflammatory cytokines upon stimulation with LPS which may be attenuated by engagement of the α7nAChR [22]. Similarly, monocytes from patients with RA produce inflammatory cytokines in response to ex vivo LPS stimulation which is also attenuated by exposure to GTS-21, an α7nAChR agonist [22], [23]. Inflammatory cytokine production by human monocytes from RA patients and controls following activation of TLRs 2, 3, 9 and RAGE, is additionally diminished by GTS-21 [22]. In summary, engaging the CAP demonstrate anti-inflammatory effects on multiple cell types and multiple potential pathways that contribute to the pathogenesis of JIA.

1.4 Clinical Studies

Vagus Nerve Stimulation via Implanted Devices in Autoimmune Disease

Preliminary studies of the potential benefits of the CAP for treatment of human inflammatory autoimmune disease were conducted at the Feinstein Institute, NY. Direct stimulation of the auricular branch of the vagus nerve was performed in 6 healthy subjects. Decreased secretion of TNF, IL-6 and IL-8 by ex vivo LPS stimulated PBMCs was observed following a single auricular VNS (Figure 4). Subsequently, a pilot study examining the effects of VNS in 11 patients with RA was completed. Following administration of twice daily VNS for 2 days, the mean Disease Activity Score 28 (DAS 28) score fell from 3.7 to 2.8 ($p = 0.002$) corresponding to a moderate response using the European League Against Rheumatism (EULAR) response criteria. Subjects with greater baseline disease activity had a significantly greater response to VNS ($r = 0.87$, $p = 0.01$)(Figure 5)[24]. For active subjects not in clinical remission (baseline DAS 28 >2.6), the difference in mean DAS 28 before and after VNS was -1.1; mean DAS 28 fell from 4.19 ± 0.33 [3.16–5.96] to 3.12 ± 0.25 [2.43–4.37], $p < 0.05$, RMANOVA, Friedman test). Additionally, disease activity remained significantly reduced following the 2-day intervention (7 days post-stimulation DAS28 score = 2.79 ± 0.21 [2.14–3.96]; $p < 0.01$, RMANOVA, Friedman test). Of note, 4 of the 11 patients were receiving treatment with a biologic (i.e., TNF blockade;) and also benefited from VNS. Of particular importance in this non-placebo-controlled study, DAS improvement reflected improvement in objective parameters i.e., numbers of swollen and tender joints and c-reactive protein (CRP).

Figure 4. Decreased IL-6 Production Following a Single Articular Vague Nerve Stimulation

Vagus nerve stimulation was performed at time 0 in 6 normal subjects. Blood was collected at times shown and incubated with endotoxin (10ng/m) for 4 hrs. Plasma was collected an IL-6 measured by ELISA (30 min vs -1hr: p= .04; 2 hr vs -1hr p = .02; 24 hr vs -1hr: p = .04 (unpublished data from FIMR).

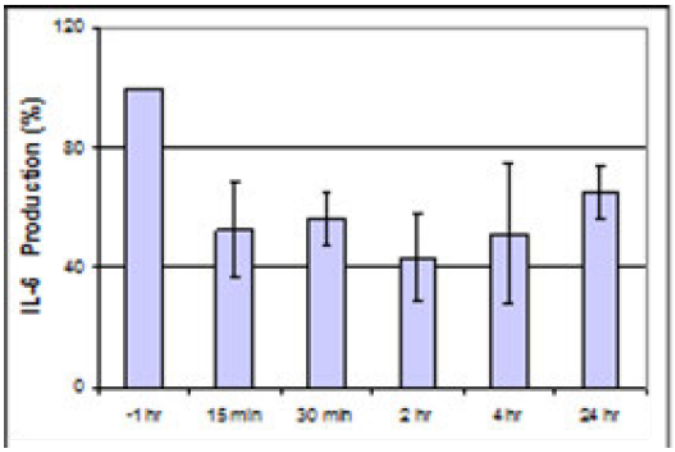
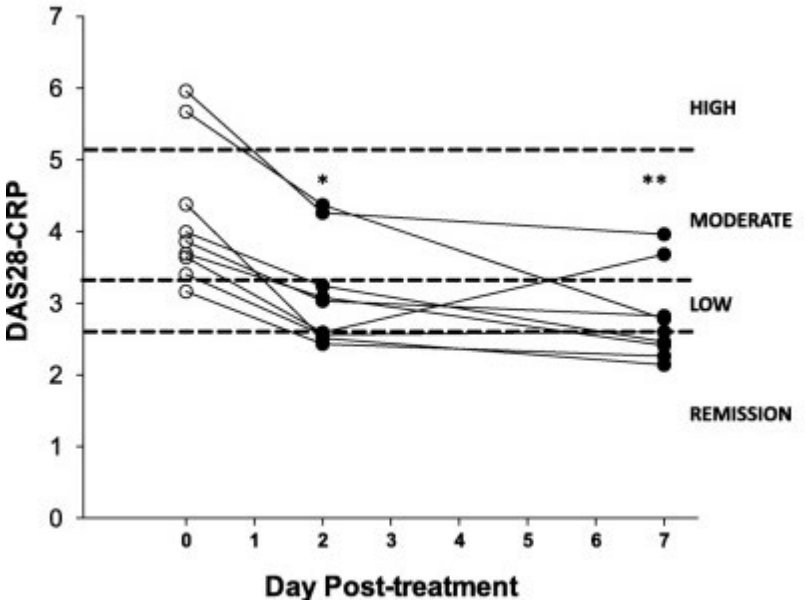


Figure 5. The effect of vibrotactile treatment at the cymba concha in patients with rheumatoid arthritis.

The DAS28-CRP Scores in nine RA patients enrolled in the prospective interventional study.[24]

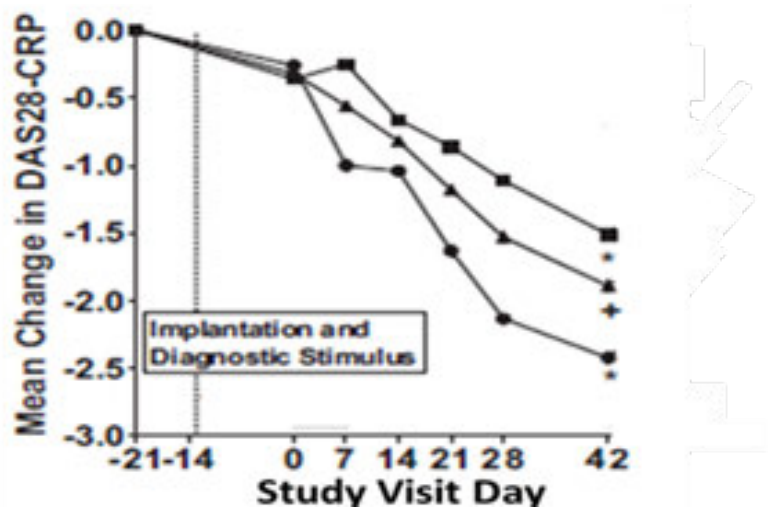


Administered by a surgically implanted cuff, VNS has been an FDA approved for treatment of refractory epilepsy since the late 1990's. Beneficial effects have additionally been reported in depression (with FDA approval), tinnitus, headache, and pain. In a study of epilepsy patients receiving the implanted cuff, cytokine production (TNF, IL1 and IL6) by LPS stimulated whole blood, was markedly reduced following VNS compared to stimulated cytokine release at baseline [25]. Clinical efficacy of VNS has been shown in inflammatory bowel disease. In one small open label pilot study, 5 of 7 biologically naïve patients receiving daily VNS for active Crohn's disease, a TNF mediated condition, showed significant improvement on the Crohn's Disease Activity Index (CDAI). Moreover, CDAI remission was achieved in 4 of the 5 patients at 6 months and only 1 patient required ongoing immunosuppressive medication. Inflammatory markers, i.e., serum CRP and fecal calprotectin were also significantly diminished [26] A second pilot study included 8 subjects with active Crohn's disease who were refractive to biologic treatment and concluded after 16 weeks of daily VNS. At week 16, CDAI scores were significantly reduced meeting a predefined target reduction of 70 in 6 of 8 patients; 3 patients achieved CDAI remission. Inflammatory markers (CRP and fecal calprotectin) were reduced in patients who had responded clinically.

Recently, an open-label study of VNS given by an implanted device was completed in 18 patients with RA. Subjects (8 subjects non-responsive to methotrexate and 10 non-responsive to biologics) received daily stimulation. At day 42, significant improvement of disease activity was observed in both cohorts and a EULAR response was achieved in 7of 8 and 6 of 10 patients in each group (Figure 6). TNF secretion by ex vivo LPS-stimulated whole blood was attenuated by daily VNS. Moreover, a significant reduction in serum IL-6 was demonstrated in those patients with a EULAR response [25]. The treatment was well tolerated, and the observed AEs were those known to associate with an implanted device (transient hoarseness and events related to the actual surgery). Importantly, no infections were observed during this study. A second study of VNS in treatment resistant RA was recently completed. Subjects were randomized to receive sham stimulation (n=4),VNS daily (n=4) or VNS for four times/day (n=6) for 12 weeks [27]. Although there was no statistically significant difference in clinical outcomes between the group of patients receiving VNS or sham stimulation in this small patient cohort, the authors noted improvement in several measurements of disease activity in individual patients receiving VNS (e.g. 5 patients achieved a clinically meaningful reduction in the DAI, 2 patients achieved an American College of Rheumatology Response Criteria 20 (ACR20), 1 patient achieved an ACR50 and 3 achieved a good EULAR response), compared to 0 patients achieving any of these outcomes in the group receiving sham stimulation. A 30% decrease in LPS-stimulated release of IL-1β, IL-6 and TNF in patients receiving VNS was also observed, with no change seen in patients receiving sham stimulation.

Figure 6. Disease Activity in Rheumatoid Arthritis Patients

Mean change in DAS28 CRP to Day 42 in Methotrexate resistant cohort (*), Biologic resistant cohort (n) and Combined Cohorts (▲). Modified from Koopman *et al.* J intern Med. 2017[25].



tcVNS in Human Disease

There have been no previous studies of tcVNS in children with JIA.

Studies performed using tcVNS applied to either the cymba concha or tragus have shown benefit in epilepsy, depression as well as in headache and pain [28-33]. Results from two trials employing tcVNS in RA have been reported. In one study, after 4 days of stimulation, small but significant decreases in DAS-CRP, CRP, and γIFN were seen in patients with high disease activity, but not in those with low disease activity [34] . In a second, larger study, 30 subjects who were predominantly biologic naïve with high disease activity, self-administered tcVNS for 12 weeks. After 1 week, DAS28-CRP was significantly reduced. This reduction was sustained through week 12 with 37% achieving DAS-28 low disease activity and 23% achieving DAS-28 remission. Serum CRP was unchanged, but both tender and swollen joint counts were significantly reduced [35].

The effectiveness of harnessing the CAP in another autoimmune disease, SLE, was assessed at the Feinstein Institutes, NY. A randomized, double-blind, sham-controlled pilot study of tcVNS was conducted in 18 SLE patients with musculoskeletal pain [36]. Patients with significant musculoskeletal pain (pain ≥4 on a 10 cm anchored VAS) were randomized 2:1 to receive tcVNS or sham stimulation (SS) for 5 minutes daily for 4 days. Patients were assessed at baseline, day 5 and day 12. Pain and fatigue on day 5 were significantly reduced from baseline; 10 of 12 patients receiving tcVNS and 1 of 6 who received SS experienced a minimally important reduction in pain. Subjects receiving tcVNS were additionally noted by a blinded assessor to have a significantly greater reduction in the percentage of tender and swollen joints. In most subjects, the effects of tcVNS persisted through day 12. Anecdotally, these effects have continued for 4-5 months in patients seen for routine care at the Feinstein Institute. Because the endpoint was reduction in pain, the investigators assayed for substance P and observed a significant decrease in plasma levels at day 5 from baseline in subjects receiving tcVNS compared to SS, p=0.008. Results of other biomarkers were not informative, serum levels of HGMB1 and CRP were low with no significant changes seen from baseline to day 5 in either arm. Similarly, there were no significant changes between groups in serum levels of IFNα, IL-1, IL-8, IL-10 or TNF, nor in metabolites of the tryptophan pathway. Using the Myriad TruCulture system, we also stimulated whole blood with TLR 4, 7 or 9 agonists and observed no significant change in stimulated cytokine release from baseline in either of the two study arms.

Using bulk RNA-sequencing, global gene expression in whole blood was measured (unpublished data from FIMR). Differential gene expression analysis was used to identify genes that significantly changed their expression from baseline to day 5 in the 10 subjects responding to tcVNS. Gene expression changes were analyzed using generalized linear modeling, to allow a pairwise comparison (i.e., the change in expression was computed per patient, then integrated across all patients). Forty-one genes were identified which demonstrated a statistically significant decrease from baseline to day 5 after tcVNS, and 33 genes showing a significant increase (FDR ≤ 0.1); none of these genes were affected by SS (using a less stringent FDR threshold of 0.2, to allow a broader definition of response in the case of SS). Of note, several of these genes included immune regulators, and some were previously implicated in SLE). For example, a significant decrease was found in the expression of AGER (RAGE), a receptor of

HMGB1, TNFRSF12A, a gene encoding the receptor of TWEAK and in IDO2, an immune regulator playing a role in autoimmunity but not involved in tryptophan metabolism (Figure 7).

Figure 7. Changes in Gene Expression Following tcVNS and Sham Stimulation in SLE Patients

Expression changes in selected genes induced following VNS and sham stimulation in SLE patients. Gene expression was measured using bulk RNA-seq at baseline and 5 days following the start of treatment. Lines show the change in gene expression per study subject. VNS- Vagus Nerve Stimulation; CPM- count per million reads (Unpublished data from FIMR).

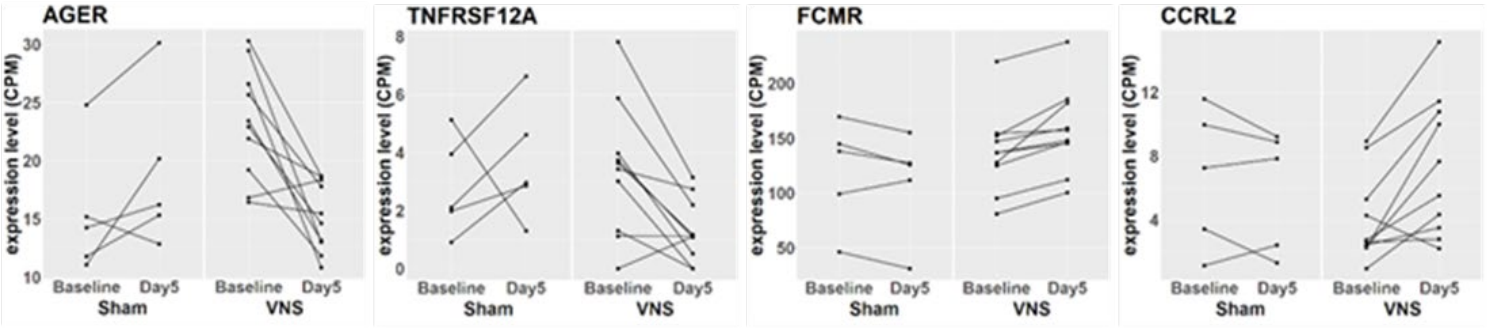
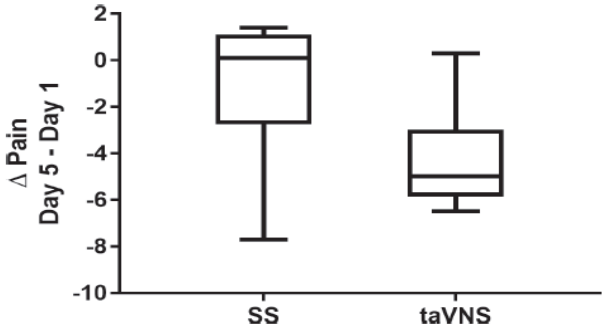


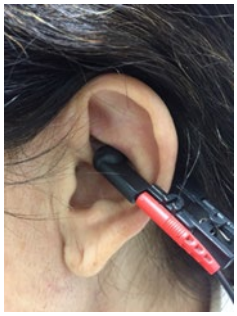
Figure 8. Change in Pain by Visual Analogue Scale in Patients Treated with Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) and Sham

Reduction in pain following short-term VNS compared to SS following 4 consecutive days of stimulation, as determined with a 10 cm visual analogue scale (VAS) (p = 0.049)[36].



The pulse generator for this study was a handheld Roscoe Medical TENS 7000 unit programmed to deliver stimulation pulses at 30 hz, 300msec. The electrodes, supplied by the Feinstein Bioelectronic Medicine Center were made of conductive material facilitating transcutaneous electrical stimulation and were configured as a clip. taVNS was administered by positioning the clip so that the anode was placed on the concha on the left ear (Figure 9; to avoid cardiac stimulation, a theoretical concern only, as the frequencies used were not those that would affect the heart); current was then increased to the maximum tolerated. SS was performed by placing the clip on the earlobe (an area without vagus innervation) and removing the batteries from the pulse generator. A dial on the unit was advanced so that subjects thought the stimulation was being progressively increased, and after 3 advances, they were told that the target stimulation had been reached. All subjects were told that they may or may not feel anything and all were told that the effects of stimulating different locations of the ear were evaluated. Voltage delivered was measured with a BK Precision oscilloscope. No subjects in this trial complained of pain or discomfort from the stimulation procedure.

Figure 9. Ear Clip Placed at the Cymba Concha



More recently the effect of tcVNS on fatigue in patients with Sjogren's Disease was evaluated by administering VNS via the gammaCore device [37]. This device stimulates the vagus nerve transcutaneously at the neck. In an uncontrolled 26 day open-label study, 15 patients received twice daily stimulation and reported a significant reduction of fatigue. Moreover, LPS-stimulated production of IL-6, IL-1β, IP-10, MIP1α, IL-1β, TNF-α, IL-6, and IP-10, was also significantly reduced.

2 Study Hypotheses/Objectives

2.1 Hypotheses

The tcVNS will result in an improved clinical status in children with JIA.

2.2 Primary Objective

The primary objective of this study is to determine the effect of tcVNS on disease activity in participants with active JIA as assessed by the JIA ACR 50 response.

2.3 Secondary Objectives

The secondary objectives of this study are to determine the effect of tcVNS on disease activity in participants with active JIA assessed by the JIA ACR 30 response, JIA ACR 70 response, and Juvenile Arthritis Disease Activity Score in 27 Joints (JADAS-27) response.

2.4 Exploratory Objectives

2.4.1 Clinical Exploratory Objectives

The clinical exploratory objectives of this study are to determine the effect of tcVNS on the following aspects of JIA in participants with active JIA:

- Pain
- Functional status
- Overall health and health-related quality of life
- Arthritis

2.4.2 Mechanistic Exploratory Objectives

The mechanistic objectives of this study seek to understand the mechanisms by which tcVNS may reduce inflammation and decrease musculoskeletal pain in participants with active JIA by examining serum, cellular and transcriptomic biomarkers of clinical response.

3 Study Design

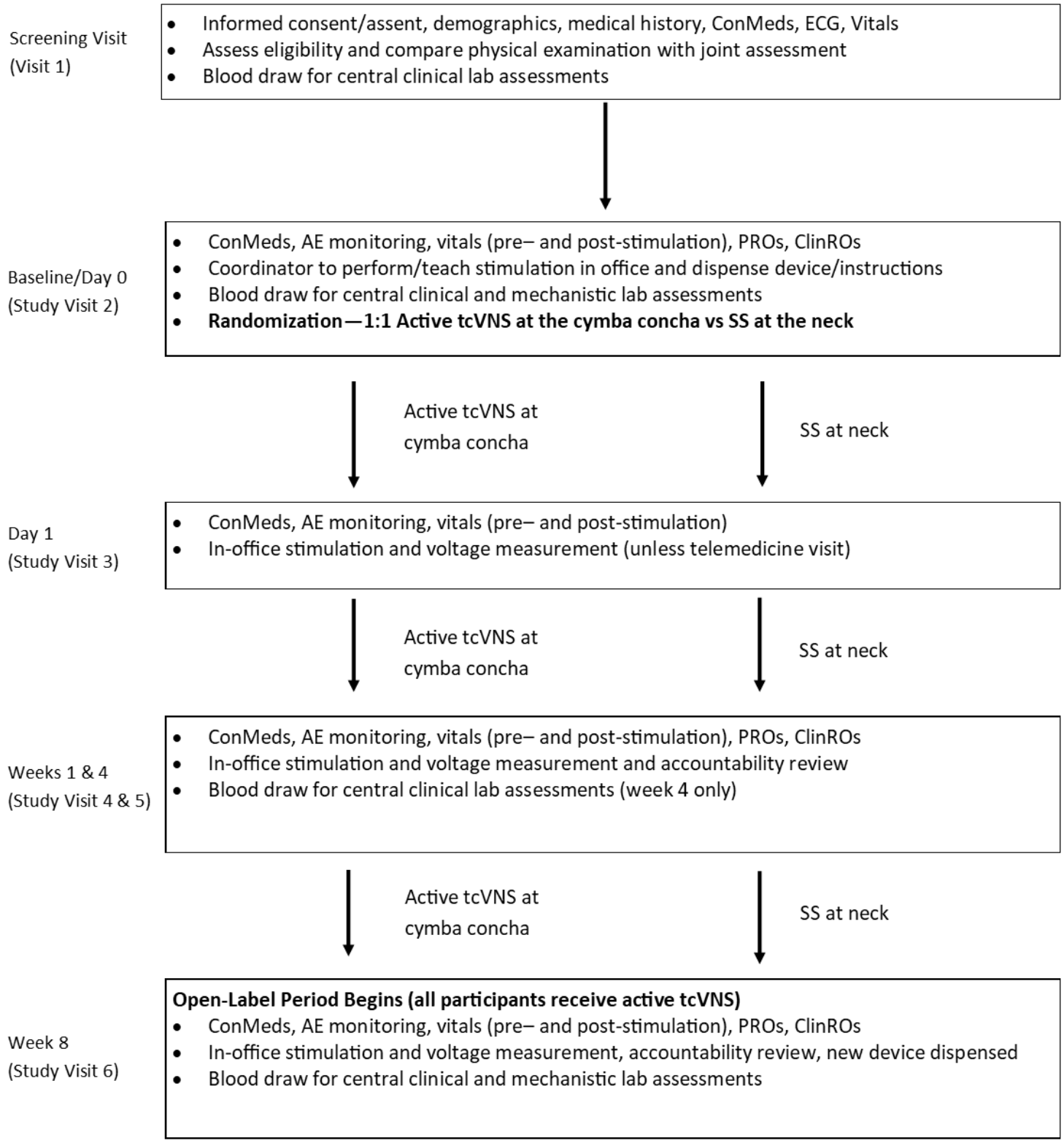
3.1 Description of Study Design

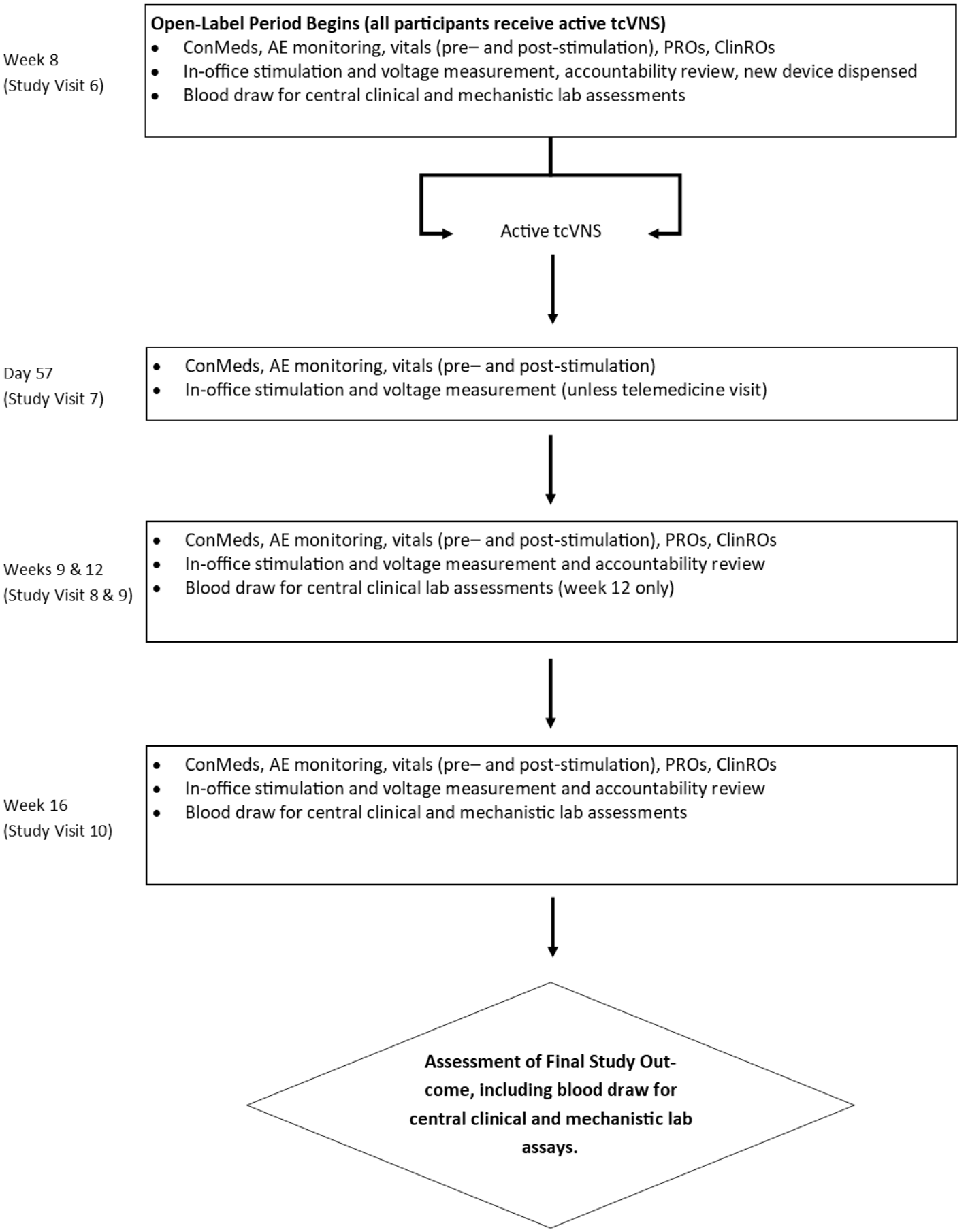
AJA01 is a multicenter, double-blind, sham-controlled, 16-week trial to evaluate the safety and effectiveness of tcVNS for the treatment of JIA. A total of 100 participants will be randomized 1:1 to treatment with active tcVNS at the cymba concha or SS at the neck for 5 minutes once a day for 8 weeks. During this time, participants, guardians, and participant assessors will be blinded to treatment assignment; treatments on clinic visit days will be conducted in the clinic under the supervision of a trained, unblinded staff member, and participants will only discuss the stimulation procedure with this staff member. Additional information on blinding can be found in Section 3.6, *Randomization, Stratification, and Blinding/Masking*.

The double-blind, sham-controlled 8-week period will be followed by an 8-week open-label period in which all participants will receive treatment with active tcVNS at the cymba concha once a day for 5 minutes. This will allow for an evaluation of response in responding participants at week 8, and sustainability after 8 weeks of tcVNS. Additionally, an open-label period will facilitate enrollment.

Consenting individuals meeting all entry criteria will have a Baseline/Day 0 Visit including vital signs, patient-reported outcomes (PROs), clinician-reported outcomes (ClinROs) including physical and joint exams conducted by blinded personnel and blood draws. All participants will be instructed not to discuss the study or their treatment experience with other participants, blinded site personnel, or people outside of the study. Participants, with guardian assistance as needed, will then administer 5 minutes of active tcVNS at the cymba concha or SS at the neck, followed by repeat vital signs, under the supervision of an unblinded coordinator/nurse. The treating site staff member will work with participants to ensure that they can properly administer future stimulations at home. Participants will then be sent home with the stimulation system for self-administration. Starting on Day 1, participants (with guardian assistance as needed) will self-administer stimulation at home once a day for 5 minutes, recording information in a diary, including any difficulties or side effects encountered. Follow-up visits will occur at Day 1 (telemedicine to confirm proper technique) and Weeks 1, 4, and 8. At the Week 8 Visit, all participants will receive an active tcVNS system with appropriate settings and other necessary items. Prior to receipt of the active tcVNS system at the Week 8 Visit, participants will be surveyed as to what treatment they believe that they received during the double-blind, sham controlled 8-week period. A similar schedule will be followed for the open-label extension period. Follow-up visits will occur at Day 57 (telemedicine to confirm proper technique) and Weeks 9, 12, and 16. At each follow-up visit, participants will perform transcutaneous stimulation in the clinic followed by vital signs readings, and the voltage of the device will be assessed by oscilloscope. PROs will be administered, ClinRos will be conducted, safety data will be collected, and central clinical and mechanistic labs will be assessed intermittently according to Table 8.1, *Schedule of Events*.

Figure 10. Flow Diagram of Study Visits




Week 8
(Study Visit 6)
Open-Label Period Begins (all participants receive active tcVNS)
ConMeds, AE monitoring, vitals (pre– and post-stimulation), PROs, ClinROs
In-office stimulation and voltage measurement, accountability review, new device dispensed
Blood draw for central clinical and mechanistic lab assessments
Active tcVNS
Day 57
(Study Visit 7)
ConMeds, AE monitoring, vitals (pre– and post-stimulation)
In-office stimulation and voltage measurement (unless telemedicine visit)
Weeks 9 & 12
(Study Visit 8 & 9)
ConMeds, AE monitoring, vitals (pre– and post-stimulation), PROs, ClinROs
In-office stimulation and voltage measurement and accountability review
Blood draw for central clinical lab assessments (week 12 only)
Week 16
(Study Visit 10)
ConMeds, AE monitoring, vitals (pre– and post-stimulation), PROs, ClinROs
In-office stimulation and voltage measurement and accountability review
Blood draw for central clinical and mechanistic lab assessments
Assessment of Final Study Out-come, including blood draw for central clinical and mechanistic lab assays.

3.2 Primary Efficacy Endpoint

The primary endpoint is the proportion of participants achieving ≥50% improvement in clinical status as defined by JIA ACR 50 at Week 8. This primary endpoint will be compared between the active tcVNS and SS treatment arms.

The JIA ACR 50 is a validated composite response consisting of 6 core criteria:

- number of joints with active arthritis
- number of joints with limited motion
- physician's assessment of disease activity (measured on a 10 cm (VAS)
- parent/patient assessment of overall well-being (measured on a 10 cm VAS)
- a validated measure of physical function CHAQ
- a laboratory measure of inflammation (CRP)

The JIA ACR 50 is achieved if 3 of any 6 core set variables improve by at least 50% from the baseline/Day 0 visit, and no more than 1 variable worsening by >30%. [1]

3.3 Secondary Efficacy Endpoints

The following secondary efficacy endpoints will be compared between the active tcVNS and SS treatment arms:

- Proportion of JIA ACR 50 responders at Weeks 4, 12, and 16 using Day 0 as Baseline.
- Proportion of JIA ACR 30 responders at Weeks 4, 8, 12, and 16 using Day 0 as Baseline.
- Proportion of JIA ACR 70 responders at Weeks 4, 8, 12, and 16 using Day 0 as Baseline.
- Proportion of JIA ACR 50 responders at Weeks 12, and 16 using Week 8 as Baseline.
- Proportion of JIA ACR 30 responders at Weeks 12, and 16 using Week 8 as Baseline.
- Proportion of JIA ACR 70 responders at Weeks 12, and 16 using Week 8 as Baseline.
- Change in JADAS-27 at Weeks 4, 8, 12, and 16 compared to Day 0.
- Change in JADAS-27 at Weeks 12, and 16 compared to Week 8.
- Longitudinal trends in JADAS-27 from Day 0 to Week 16.

3.4 Safety Endpoints

The following safety endpoints will be summarized in the active tcVNS and SS treatment arms:

- Incidence of specific grade 1 AEs (described in Section 12.3.1, *Grading Criteria*) plus all grade 2 or higher AEs.
- Incidence of SAEs.

3.5 Exploratory Endpoints

3.5.1 Clinical Exploratory Endpoints

The following exploratory efficacy endpoints will be compared between the active tcVNS and SS treatment arms:

- Change in each of the following endpoints at Weeks 1, 4, 8, 9, 12, and 16 compared to Day 0:
  - Pain Assessment by Wong-Baker FACES Scale
  - CHAQ
  - Patient-Reported Outcomes Measurement Information System (PROMIS) Mobility Questionnaire

- Patient-Reported Outcomes Measurement Information System (PROMIS) Upper Extremity Questionnaire
- Global Assessments by VAS
- PGA
- Joints with Active Arthritis
- Joints with Limited Motion

- Change in each of the following at Weeks 9, 12, and 16 compared to Week 8:
  - Pain Assessment by Wong-Baker FACES Scale
  - CHAQ
  - PROMIS Mobility Questionnaire
  - PROMIS Upper Extremity Questionnaire
  - Global Assessments by VAS
  - Physician Global Assessment of Disease Activity
  - Joints with Active Arthritis
  - Joints with Limited Motion

3.5.2 Mechanistic Exploratory Endpoints

The following endpoints will be compared between the active tcVNS and SS treatment arms:

1. Change in serum levels of soluble mediators regulated by the CAP at Weeks 8 and 16 compared to Day 0 and at Week 16 compared to Week 8.
2. Change in serum levels of substance P at Weeks 8 and 16 compared to Day 0 and at Week 16 compared to Week 8.
3. Peripheral blood cell production of soluble mediators after 24 hours of stimulation by LPS at Week 8 compared to Day 0.
4. Change in transcriptomics and of gene expression of select genes such as TNF, IL-6, and IL-1 at Week 8 compared to Day 0.
5. Change in composition of circulating blood cells at Week 8 compared to Day 0

3.6 Randomization, Stratification, and Blinding/Masking

3.6.1 Randomization and Stratification

The study plans to randomize 100 participants to active tcVNS at the cymba concha or SS at the neck, daily for 5 minutes, in a 1:1 ratio. Randomization will be stratified by 1) current or past use of a biologic and 2) number of active joints at the Baseline/Day 0 visit (3-4 joints vs 5 joints or more).

3.6.2 Blinding/Masking

Active tcVNS will be performed at the cymba concha, and SS will be performed on the neck. Participants will receive components and instructions for device use specific only to their treatment arm/location of treatment. At the time of the first treatment, an unblinded coordinator/nurse will provide instructions for placement of the electrodes and the stimulation procedure, and they will supervise the procedures to assure the participant (and guardian, if needed) is implementing them correctly. At each visit in the blinded period of the study, the participant will first be assessed by an unblinded nurse/coordinator who will conduct the initial evaluations for AEs and concomitant medication changes and observe that day's treatment; if AEs are reported that cause physical changes in the ear or neck, the unblinded investigator will be alerted to provide evaluation. Following these unblinded evaluations, the potential treatment sites on the left ear

and neck will be covered by a headband and scarf, respectively, so that blinded staff are unable to observe any potential visual indicators of which site is being treated. All components used for the device treatment will be packed in the provided bag before blinded personnel are invited into the room, and participants will be reminded that they cannot discuss any aspect of the device or treatment or any potentially unblinding AEs with the blinded personnel.

Information correlating individual device serial numbers to treatment arm will be accessible to unblinded personnel only.

3.6.3 Procedure for Unblinding/Unmasking

Unblinding before 8 weeks on the study must be approved by the study Medical Monitor unless an immediate life-threatening condition has developed, and the Medical Monitor is not accessible. The site investigator will notify the protocol chair(s) and the study Statistical and Clinical Coordinating Center (SCCC) team of the unblinding event on the next business day. The emergency unblinding will also be reported to the Data and Safety Monitoring Board (DSMB).

A full account of the event will be recorded, including the date and time of the unblinding, the reason for the decision to unblind, and the name of the individual who made the decision and the names of the Medical Monitor and others who were notified. The reasons for unblinding of a participant's treatment will be included in the final study report.

Unblinding the study due to an approved interim analysis, final analysis, or study termination will require written approval from the National Institute of Allergy and Infectious Diseases (NIAID).

4 Selection of Participants and Clinical Sites/Laboratories

4.1 Rationale for Study Population

Children from ages 5 – 18 were selected as most appropriate for this study as children below age 5 are not likely to be able to cooperate with the cutaneous stimulation for a duration of 5 minutes. At age 5 a child will be able to sit with the electrode clip in place. The pediatric population includes children through the age of 18 years; therefore, the study will enroll children up to the age of 18 years, inclusive.

The inclusion of JIA participants with 3 active joints will provide a unique treatment opportunity rarely afforded to arthritis patients since studies typically recruit polyarticular patients with at least 5 active joints. Children with one or two joints are frequently treated with NSAIDs or intra-articular steroid injections. Those with polyarticular involvement (> 5 joints) are treated with second-line medications. Children with 3-4 joints often present a treatment dilemma as they may not respond well to NSAID medications and guardians are frequently reluctant to commit their child to long-term medications with potentially significant serious side effects.

There will likely be more females than males enrolled in the study given an increased incidence and prevalence of JIA in girls than boys. No race or ethnicity is excluded but as JIA is less common in African American and Asian populations, it is likely that most participants in the study will be Caucasian.

4.2 Inclusion Criteria

Individuals who meet all the following criteria are eligible for enrollment as study participants:

1. Participant is 5 through 18 years of age (inclusive) at screening.
2. Regarding informed consent and compliance:
    a. If 5 through 6 years of age, the participant's guardian is willing and able to understand and provide informed consent and comply with study protocol.
    b. If 7 through 17 years of age, the participant is willing and able to sign assent and comply with study protocol, and the participant's guardian is willing and able to understand and provide informed consent and comply with study protocol.
    c. If 18 years of age, the participant is willing and able to understand and provide informed consent and comply with study protocol.
3. The participant has a JIA diagnosis meeting ILAR classification criteria with one of the following subtypes:
    - rheumatoid-factor negative polyarthritis.

- rheumatoid-factor positive polyarthritis.
- persistent oligoarthritis.
- extended oligoarthritis.
- psoriatic arthritis.
- enthesitis-related arthritis
- systemic arthritis.

4. The participant has ≥ 3 joints with active arthritis at screening.
5. If the participant is receiving therapy for JIA at screening, that therapy is stable for the time period outlined below and is expected to remain stable for the duration of the study:
   - Stable dose for at least 1 week prior to screening:
     - Oral steroids, ≤ 0.2 mg/kg/day with a maximum 10 mg/day dose
   - Stable dose for at least 2 weeks prior to screening:
     - NSAIDs
   - Stable dose for at least 8 weeks prior to screening
     - adalimumab
     - anakinra
     - canakinumab
     - certolizumab pegol
     - etanercept
     - golimumab
     - infliximab
     - leflunomide
     - methotrexate
     - tocilizumab
   - Stable dose for at least 12 weeks prior to screening
     - abatacept
6. If a female of child-bearing potential, the participant has a negative urine pregnancy test at screening.
7. If of reproductive potential, must agree to abstinence or effective methods of birth control for the duration of the study.

4.3 Exclusion Criteria

Individuals who meet any of these criteria are not eligible for enrollment as study participants:

1. Other than NSAIDs or intra-articular injections, participant has been treated for JIA with lack of efficacy with:
   a. More than 2 different classes of therapies or
   b. More than 3 medications in total
2. Participant has received high-dose steroids (≥0.2 mg/kg/day) within the 28 days prior to screening.
3. Participant has had active systemic disease (fever, systemic rash) within the 3 months prior to screening including any of the following lab manifestations at screening:

a. Ferritin >1000 ng/mL

b. WBC ≥15,000/mm^3

4. Participant has had an active acute systemic infection within 2 weeks of screening involving fever (100.4^oF or higher) for more than 24 hours, requirement for systemic antibiotics or antivirals, GI symptoms lasting 48 hours or more, or the need to hold second line medications for JIA (methotrexate or biologic).
5. Participant has a history of arrhythmia.
6. Participant has been diagnosed with postural orthostatic tachycardia syndrome (POTS).
7. Participant has received an intra-articular cortisone injection within the 28 days prior to screening.
8. Participant has received treatment with an investigational drug or device during the 28 days prior to screening or within five half-lives of the investigational drug prior to screening/baseline, whichever is the greater length of time.
9. Participant has received chronic treatment with an anti-cholinergic medication, including over the counter medications.
10. Participant has received treatment with rituximab:
    a. Within one year of screening
    b. At any time previously without documented B cell repletion
11. Participant has a comorbid disease that has required treatment with corticosteroids within the past year.
12. Participant has an implantable electronic device such as a pacemaker, defibrillator, hearing aid, cochlear implant, insulin pump or deep brain stimulator.
13. Participant has used cutaneous VNS within 12 weeks prior to screening.
14. Participant has received a live attenuated viral vaccine within 28 days prior to screening or is expected to receive one during the study.
15. Participant has any condition which, in the opinion of the investigator, would jeopardize the participant's safety following exposure to a study intervention.
16. Participant has any past or current medical problems or findings from a physical examination or laboratory testing that are not listed above but which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements, or may impact the quality or interpretation of the data obtained from the study.

4.4 Selection of Clinical Sites/Labs

Pediatric rheumatology centers were selected based upon their prior experience in participating in collaborative research. These investigators also have excellent working relationships and will easily be able to work towards the goal ensuring the success of enrollment for this study.

5 Known and Potential Risks and Benefits to Participants

tcVNS is a well-tolerated procedure and without the risks associated with surgical implantation of a stimulator placed around the vagus nerve in the neck. Changes in blood pressure and/or heart rate, though not clearly attributable to stimulation, have been inconsistently associated with cervical VNS. A 2012 study [38] showed no arrhythmic effects on cardiac function in patients receiving tcVNS for 24 months Stimulation with the investigational device may cause pain or intolerable discomfort if the level of treatment is too high. Participants will be instructed to dial the treatment knob until stimulation can be felt, but not beyond a level that is tolerable for 5 minutes and is not painful. For the sham tcVNS system, participants will be instructed not to dial the knob above the level of "3" to prevent overstimulation at this site. Some individuals may also experience transient erythema or skin irritation at the stimulation site. Potential cardiac effects and local irritation due to tcVNS are addressed in Sections 5.1.1 , *Risks of Investigational Device Cited in Medical Literature,* and 6.7 *Toxicity Prevention and Management.* There is also a risk of skin irritation or allergic reaction at the stimulation site due to the electrode gel [39] for participants in the active treatment group or the adhesive

electrodes [40] for participants in the sham treatment group. No additional risks of tcVNS beyond the ones noted here have been observed.

5.1 Risks of Investigational Device Cited in Medical Literature

5.1.1 Clinical Studies

A retrospective assessment of cardiac safety following transcutaneous electrical stimulation of the auricular branch of the vagus nerve revealed no indication of arrhythmic effects of tcVNS [38]. The authors of that study did note 2 adverse cardiac events but determined that these AEs were unlikely to have been caused by the tcVNS device [38]. A more recent study in patients with tinnitus also failed to detect changes in heart rate or blood pressure (BP) following tcVNS, while having a positive effect on tinnitus loudness and awareness [41]. Studies conducted at the FIMR over the last two decades have revealed that electrical vagus nerve stimulation may be an effective method to control inflammation [5, 9, 14, 42-49]. The Feinstein Institutes' Lupus Center of Excellence recently published a pilot study evaluating tcVNS compared with SS in patients with SLE and musculoskeletal pain [36]. Participants reported a marked reduction in pain and fatigue, and there were no adverse cardiac effects. This technology is now the basis of clinical trials being conducted in rheumatoid arthritis and inflammatory bowel disease [50].

Concerns regarding the cardiovascular and hemodynamic effects of tcVNS were surveyed across 54 clinical trials in epilepsy, depression, pain, tinnitus, and other clinical indications. Trials examined included those utilizing an intervention of tcVNS, percutaneous auricular electroacupuncture, and transcutaneous cervical vagus nerve stimulation. Most studies that looked at tcVNS utilized off-the-shelf transcutaneous electrical nerve stimulation (TENS units paired with electrodes designed to interface with the tragus or the cymba concha, both of which are innervated by the auricular branch of the vagus nerve). In general, the published clinical data suggest that tcVNS is well tolerated and safe [28, 29, 51-54]. Of the trials, few, if any, reported cardiovascular or hemodynamic effects of non-invasive vagus nerve stimulation. One study reported favorable effects on heart rate variability (HRV) in the context of left ventricular strain [55], while another study reported "increased parasympathetic activity and changed sympathovagal balance" in healthy and diabetic subjects but indicated that further studies were necessary [56]. A third study reported 2 adverse cardiac related events that were considered unlikely to have occurred as a result of the tcVNS treatment because the participants were ultimately found to have cardiac conditions present prior to study participation [38].

In a recent study by Badran [57], taVNS overall had no significant effect on the heart rate (HR) of healthy individuals compared to control. In that study, only two parameter combinations of taVNS (500 us pulse width at 10 Hz, and 500 us pulse width at 25 Hz) were associated with a minor decrease in HR during stimulation; that decrease in HR ranged between -2.17 and -3.13 bpm relative to pre-stimulation baseline HR. The stimulation settings to be used in our study are 30 µs pulse width at 30 Hz. In another recent study in healthy individuals [58], tcVNS had no effect on HR or BP. In another study [59] left or right tcVNS had minimal or no effects on heart rate variability, a measure of the effect of autonomic tone on the sinus node. In a clinical study in patients with paroxysmal atrial fibrillation [60] HR decreased by the same small amount in both the tcVNS and control groups (-2.6 and -2.7 bpm, respectively). The only clinical study that reported a clinically significant drop in HR and BP during tcVNS was that of Zamotrinsky et al [61]. In this study, tcVNS was delivered to patients with severe angina, during the preoperative period. During tcVNS, HR and BP decreased by about 10% relative to pre-stimulation levels. However, these findings (and others from that study) were not corroborated by subsequent clinical studies [62].

5.1.2 Safety of tcVNS

The TENS 7000 device is intended to be used in this study to administer a low amount of current and can be used for tcVNS. There are three categories of non-invasive VNS devices. The first category targets the cervical vagus nerve using external transcutaneous stimulators. The second and third categories target the auricular branch of the vagus nerve using either percutaneous (electroacupuncture) or transcutaneous methods. Devices belonging to the first and third category are the Gammacore (electroCore, indication: primary headache), and the Nemos (Cerbomed, indication: epilepsy), both of which have received CE marks for marketing in Europe.

As of November 2018, there are a total of 14 FDA cleared auricular VNS devices, all of which fall into the second category described above. Of these devices, 8 are currently marketed and are indicated for either substance abuse, opioid

withdrawal, or for use in electroacupuncture by qualified practitioners. These devices are Drug Relief (DyAnsys), NSS-2 Bridge (Innovative Health Solutions), ANSiStim (DyAnsys), Bridge Neurostimulation System (Innovative Health Solutions), Stivax (Biegler), e-Pulse (AMM Marketing), P-Stim (NeuroScience Therapy), and AcuStim (S.H.P International). These devices are all classified under FDA product codes BWK and PZR.

The FDA provides a searchable database of AEs reported related to device use, the Manufacturer and User Facility Device Experience (MAUDE) database. Within the MAUDE database there is only one reported AE for all the devices in product codes BWK and PZR which are currently marketed. The Bridge device had a reported adverse event described as a patient-initiated study withdrawal (cause unrelated to device use).

Based on the previous literature regarding transcutaneous VNS, tcVNS in healthy subjects and in select patient populations has not been associated with clinically significant effects on HR and BP. Therefore, the use of Roscoe Medical TENS 7000 unit to administer electrical stimulation to the auricular branch of the vagus nerve in this patient population does not present a potential for serious risk to the health, safety, or welfare of a subject; and should therefore meet the definition of a non-significant risk device.

5.1.3 Additional Safety Concerns

Beyond the safety considerations of the effects of tcVNS, there are concerns regarding the general safety of transcutaneous stimulation. The FDA provides guidance on the operation of powered muscle stimulators, which are also applicable to TENS stimulators. The requirements regarding power sources, leakage currents, and other aspects of device operation are all met by the TENS 7000 system, and do not change based on how the device is used or which electrodes are connected to it. The requirement of maximum power density does change based on the size of the electrode contacting the skin. The FDA recommends that the maximum power density remain below 0.25 W/cm^2 to reduce the risk of thermal burns[63]. Calculations of power density are provided below assuming the worst-case scenario of the maximum current and voltage specifications of the TENS 7000, applied at a frequency of 30 Hz. The calculations yield a minimum electrode area of 31.8 mm^2. The clip electrode has an area of 50 mm^2, and the adhesive electrode has an area of 110 mm^2. These calculations indicate that when used as directed, the TENS 7000 system is incapable of exceeding the maximum power density limit recommended by the FDA guidance.

$$I_{RMS} = \sqrt[2]{Freq * PulseWdith} * I_{max} = \sqrt[2]{30Hz * 300} * 80mA = 7.58\,mA\ [RMS]$$

$$V_{RMS} = \sqrt[2]{Freq * PulseWdith} * V_{max} = \sqrt[2]{30Hz * 300} * 110V = 10.43\,V\ [RMS]$$

$$Max\ Power\ Density = \frac{V_{RMS} * I_{RMS}}{Area} = \frac{10.43 * 7.58m}{1.1} = 26.1\ \frac{mW}{cm^2}$$ **<< 250 mW/cm²**

5.2 Risks of Other Protocol-specified Medications

Not applicable.

5.3 Risks of Other Study Procedures

- Venipuncture: The risks of having blood drawn include soreness and bruising at the puncture site, occasional lightheadedness or, rarely, fainting. There is a small risk of infection whenever blood is drawn.
- Loss of confidentiality: The clinical information collected in this study is related to JIA disease activity. Much information is the same as would be obtained as part of routine medical care; in addition, participants will also fill out questionnaires to assess various outcomes of the device treatment. The procedures followed to protect against a breach of confidentiality are more than sufficient to protect against the very slight possibility that the records would be accessed by non-study personnel. Data will be entered onto electronic case report forms (eCRFs) that are encrypted and password protected; source records are stored in locked file cabinets in locked areas with limited access.

5.4 Potential Benefits

A therapeutic approach that engages a homeostatic pathway is an attractive therapeutic approach. Participants and their guardians would welcome a “non-drug”. Engaging the CAP may be an efficacious, non-costly, easily accessible therapy without the immunosuppressive side effects.

6 Investigational Devices

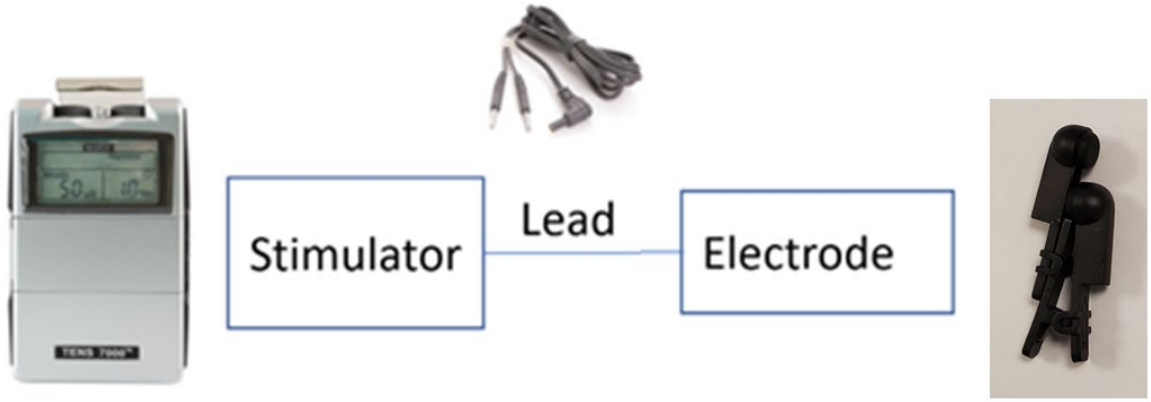
There will be 2 forms of the device utilized in this study: the active tcVNS system to administer active tcVNS at the cymba concha to participants in the active arm, and a sham tcVNS system to administer SS at the neck to participants in the sham arm. Both devices are assembled by FIMR from components derived from marketed products available without a prescription.

6.1 Device Descriptions

6.1.1 Active tcVNS System Description

The active tcVNS system consists of 3 major components: the stimulator, the lead, and an electrode. A diagram of the complete device is provided in Figure 6.1.

Figure 6.1. Components of Active tcVNS System



6.1.1.1 Stimulator

The stimulator is a TENS 7000 device (Roscoe Medical, Strongsville, OH; K110390) that is a handheld transcutaneous electrical nerve stimulator (TENS) marketed for pain relief and muscle stimulation (Figure 6.2). The stimulator provides 2 stimulation channels with independent amplitude settings and a control panel for selection of stimulation parameters. The system allows selection of waveform type, pulse duration, and frequency. The system also logs the total number of stimulation sessions and their durations. The specifications for the TENS 7000 device are provided in Table 6.1. The stimulator will be programmed to deliver stimulation pulses with specific characteristics. For the active tcVNS system, the unit will be set to normal mode with a 300-microsecond pulse at a frequency of 30 Hz. User access to other device functionality, including settings menus, will be prevented using tamper-evident sealing mechanisms, described in Section 6.3.1, *Tamper-evident Design*. FIMR will not make any other modifications to the stimulator beyond the specified stimulation programming and application of tamper-evident seals.

Figure 6.2: TENS 7000 Stimulator.

The left image shows the parameter control panel, and the right image shows the amplitude control knobs and leads connection points, and the right image shows the technical specifications.

Table 6.1 TENS 7000 Technical Specifications

	MECHANISM	TECHNICAL DESCRIPTION
01.	Channel	Dual, isolated between channels
02.	Pulse Amplitude	Adjustable, 0-100 mA at 500 ohm load each channel.
03.	Wave Form	Asymmetrical Bi-Phasic Square Pulse
04.	Voltage	0 - 50V (Load: 500 ohm)
05.	Power source	One 9 Volt Battery.
06.	Size	10.1cm(L) x 6.1cm(W) x 2.45cm(H)
07.	Weight	150 grams with battery.
08.	Pulse Rate	Adjustable, from 2 to 150 Hz, 1 Hz/step
09.	Pulse Width	Adjustable, from 50 to 300 µs microseconds,10µs/step

6.1.1.2 Lead

The lead utilized is the standard lead provided with the TENS 7000 stimulator. It consists of a plug that connects to the stimulator and 2 individual contacts, one for each of the 2 electrodes.

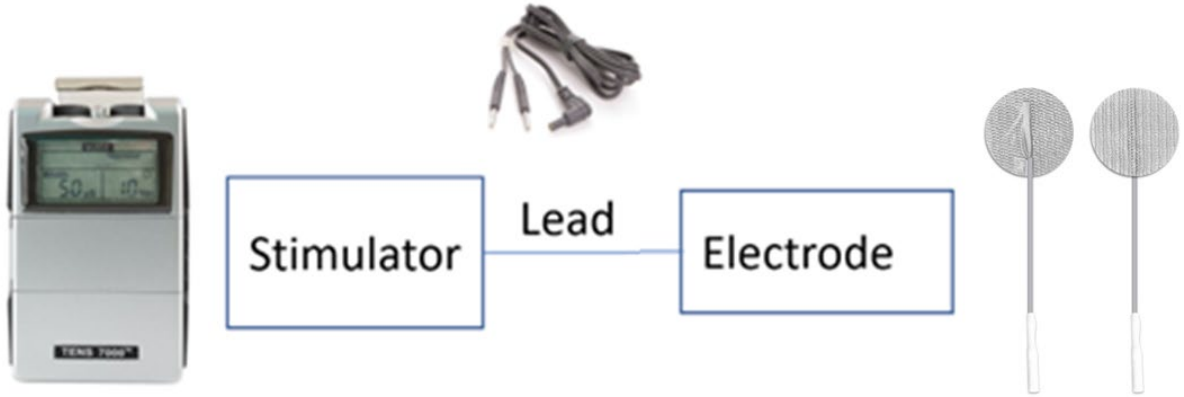
6.1.1.3 Electrode

The electrodes for the active tcVNS system will be mechanical clip electrodes (Lahsa Oms, Weymouth, MA; predicate 510k K062284) made of conductive carbon-loaded silicone. The pair of electrodes will be modified by FIMR from their original configuration to have 2 opposing stimulation sites to facilitate contact with the cymba concha and the back of the ear for application of tcVNS. Two sizes of electrode will be offered for optimal fit to participant ear size.

6.1.2 Sham tcVNS System Description

The sham tcVNS system consists of 3 major components: the stimulator, the lead, and a pair of electrodes. A diagram of the complete device is provided in Figure 6.3.

Figure 6.3. Components of Sham tcVNS System



6.1.2.1 Stimulator

The stimulator used in the sham tcVNS system is the same as that used in the active tcVNS system, which is described in Section 6.1.1.1, *Stimulator.* The unit will be set to normal mode with a 50-microsecond pulse at a frequency of 2 Hz. FIMR will not make any other modifications to the stimulator beyond the specified stimulation programming and application of tamper-evident seals.

6.1.2.2 Lead

The lead used in the sham tcVNS system is the same as that used in the active tcVNS system, which is described in Section 6.1.1.2, *Lead*.

6.1.2.3 Electrodes

The electrodes for the sham tcVNS system will be an adhesive hydrogel electrode designed for transcutaneous stimulation, PALS Platinum Neurostimulation Electrodes (Axelgaard, Fallbrook, CA; K132422). The adhesive hydrogel is conductive to assist in facilitating transcutaneous electrical stimulation. These electrodes are single use and will be used as supplied from the manufacturer. There will be no modifications made to the electrodes by FIMR.

6.2 Device Regimen

Participants will treat themselves for 5 minutes daily with stimulation using the provided investigational device per the procedure described in Section 6.4, *Preparation and Administration.* Participants randomized to the active arm will receive tcVNS at the cymba concha for 5 minutes daily for the entire period of their enrollment in the study, approximately 16 weeks. Participants randomized to the sham arm will receive SS at the neck for 5 minutes daily for 8 weeks followed by tcVNS at the cymba concha for 5 minutes daily for the following 8 weeks.

6.3 Device Packaging and Labeling

All stimulator devices will be provided to participating sites in a protective box and will be labeled with the following information:

1. Serial Number of the TENS stimulator unit
2. Manufacturer: Feinstein Institutes for Medical Research, Manhasset, NY 11030
3. Statement 1: CAUTION Investigational device. Limited by Federal law to investigational use
4. Statement 2: Use only as directed. Keep out of reach of children

All participants will be supplied with a stimulator with settings appropriate to their assigned treatment arm, lead, and spare batteries for replacement during the course of the study. All participants will also be provided with alcohol wipes and cotton swabs for cleaning the application site prior to placement of the electrodes. When a participant is to be treated with an active tcVNS system, they will be fitted with the appropriate size of clip electrode by the unblinded coordinator/nurse at the time of

their first treatment and will be provided with electrode gel. When a participant is to be treated with the sham tcVNS system, they will be provided with sufficient single-use adhesive electrodes. Participants will be provided with a bag containing all the provided components.

At the Week 8 visit, participants will be provided with an active tcVNS system and the appropriate electrodes and other items to administer active tcVNS at the cymba concha for the open-label period of the study.

6.3.1 Tamper-evident Design

Tamper-evident engineering practices have been employed to deter and make evident any device tampering. Seals will be placed across 2 locations: a) the seam of the device, and b) the panel that must be opened to adjust the device settings. Any attempt to open the device at these locations will require removal of the sticker which provides positive proof of the removal attempt.

Note that the battery can be accessed for replacement without disturbing the tamper-proof seals.

6.3.2 Storage

All devices will be stored at the central and satellite sites under lock and key.

6.3.3 Device Complaints

Sites will report any issues with the devices with regard to quality, durability, reliability, etc. to FIMR.

6.4 Preparation and Administration

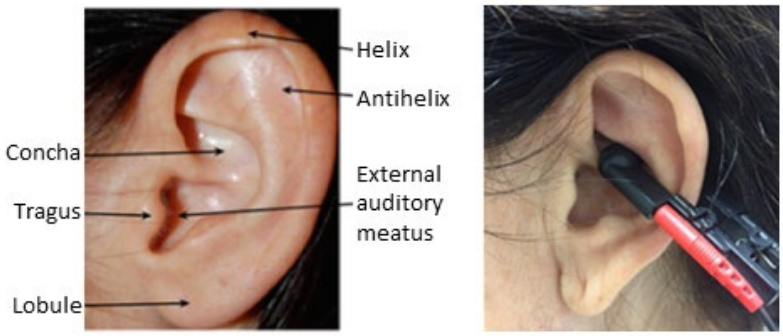
Participants will receive complete written instructions for preparation and administration of the device with which they are being treated. The first treatment will be conducted in the clinic under the oversight of an unblinded coordinator/nurse.

When the device is administered in the clinic during a study visit, vital signs will be taken before and after stimulation, as noted in Section 8, *Study Procedures*.

6.4.1 Preparation and Administration of the Active tcVNS System

Participants being treated with an active tcVNS system will attach the stimulator, lead, and clip electrode to assemble the complete device and coat the electrode surfaces with electrode gel. The electrode will be placed on the cymba concha of a left ear (shown in Figure 6.3) that is free of earrings or other objects, cleaned internally and externally, and coated with a thin layer of electrode gel. The participant will gradually advance the stimulator knob clockwise until they feel the electrical stimulation at a level that is easily tolerated for 5 minutes and is not painful. This treatment level may vary between treatments. The participant will apply the stimulation for 5 minutes, then the participant will turn off the stimulator. They will record the start and end times of the treatment in the supplied diary. The participant will remove the electrode from the ear and clean the ear and electrodes with gauze.

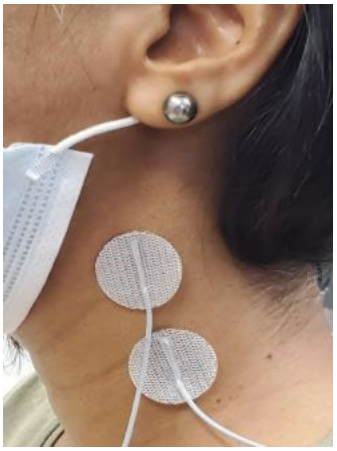
Figure 6.3: Placement of the clip electrode on the cymba concha.



6.4.2 Preparation and Administration of the Sham tcVNS System

For participants being treated with a sham tcVNS system, the adhesive electrodes will be placed on the left side of the neck, as shown in Figure 6.4, that has had neck jewelry removed and has been cleaned at the site of treatment. The participant will then connect the lead to the electrodes and the stimulator to assemble the complete sham tcVNS system. The participant will gradually advance the stimulator knob clockwise until they feel the electrical stimulation at a level that is easily tolerated for 5 minutes and is not painful. This treatment level may vary between treatments. Participants in this group will be instructed to not turn the knob above "3" on the dial as this is the maximum acceptable treatment level at the neck. The participant will apply the stimulation for 5 minutes, then the participant will turn off the stimulator. They will record the start and end times of the treatment in the supplied diary. The participant will remove the electrodes from the neck and dispose of the electrodes.

Figure 6.4: Placement of adhesive electrodes on the neck.



6.5 Device Accountability Log

Records for receipt, storage, use, and disposition will be maintained by the study site. A device log will be kept current for each site. The investigators will maintain adequate records of the disposition of each investigational device, including date of arrival at the site, date of dispensing to participant, and date of return to the investigator, as well as date of return to the central site. A record of the individual device(s) dispensed to each participant by serial number will be maintained by the study site.

All records regarding the disposition of the investigational product will be available for inspection.

6.6 Assessment of Participant Compliance with Investigational Device Treatment

Participants will maintain a daily diary of cutaneous nerve stimulation. They will record the date, treatment start and stop times, and if there are any side effects/difficulties associated with the treatment. The diary will be reviewed by the unblinded coordinator at each clinic visit.

6.7 Toxicity Prevention and Management

Participants will be counseled that the TENS unit should be turned on and the amount of stimulation increased only until the participant feels a sensation that is not uncomfortable or painful and can be easily tolerated for 5 minutes. If pain is felt, participants will be instructed to dial the knob back down slightly until the pain is no longer felt. Participants in the sham arm will be instructed not to dial the knob on the device above a "3".

Anticipated device effects include transient erythema or skin irritation at the stimulation site. Changes in blood pressure and/or heart rate, though not clearly attributable to stimulation, have been inconsistently associated with VNS. Participants will be instructed to be seated or lying down for administration of tcVNS to minimize occurrence of lightheadedness. For any unwanted effects participants/guardians will be instructed to stop the tcVNS administration session and notify the study site immediately.

Participants will be guided through their first treatment in the clinic under the supervision of an unblinded coordinator/nurse. A diagram of the ear or neck with the proper placement of electrodes and instructions on how to perform the procedure and care for the device will be provided to each participant. An unblinded coordinator/nurse will observe treatments at future visits to assure compliance with the procedures. Participants encouraged to make an on-site visit (versus virtual check-in) for the Days 2 and 57 timepoints or an unscheduled visit if at all uncomfortable with administration of tcVNS.

6.8 Premature Discontinuation of tcVNS

Study participants who prematurely discontinue tcVNS but are willing and able to remain in the study and undergo evaluation related to safety and durability of response per protocol will be encouraged to do so.

Study therapy may be prematurely discontinued for any participant for any of the following reasons:

1. At any time during the study at the request of the participant or their guardian.
2. Participant develops a severe flare of arthritis and must discontinue the study to add a new medication.
3. Investigator believes that the study treatment is no longer in the best interests of the participant.
4. Greater than 2 episodes of fainting associated with device use.
5. The occurrence of Grade 2 palpitations associated with device use.
6. Persistent discomfort at the stimulation site.
7. Pregnancy

7 Other Medications

7.1 Concomitant Medications

7.1.1 Protocol-mandated Medications

Not applicable.

7.1.2 Prophylactic Medications

Not applicable.

7.1.3 Other permitted concomitant medications

Participants will continue prior medications that were prescribed for their arthritis prior to entry, at stable doses throughout the duration of this 16-week study. These medications may include NSAIDs, methotrexate or other DMARDs, and biologics at standard doses for pediatrics. Please see protocol Section 4.2, *Inclusion Criteria* for detailed information. While the goal is to maintain stable background therapy for JIA during the study, changes should be made if clinically indicated in order to provide the participant with the best possible medical care. If this occurs, tcVNS will be discontinued at that time.

7.2 Prohibited Medications

Participants may not take chronic anti-cholinergic medications while participating in this trial. Please see Section 4.3, *Exclusion Criteria* for additional information. Intra-articular steroid injections, new immunosuppressants for any indication, new medications to treat JIA, changes in doses of any JIA medications (except those required for management of toxicity), and corticosteroids in addition to a participant's dose at baseline, if applicable, are not permitted. No live virus vaccine should be given during this study.

8 Study Procedures

8.1 Study Visits

8.1.1 Screening and Enrollment: Visit 1 (-28 Days Prior to Baseline/Day 0)

The study will be explained in lay terms to potential participants and their guardian(s) who have been identified by investigators at participating sites. Informed consent will be obtained from a guardian or legal guardian and when applicable, based on age, an assent will be signed by the child. After consent is signed, participants will be assigned a unique study identification number.

The purpose of the screening period is to confirm eligibility to participate in the study.

General Assessments:

a. Informed Consent/Assent when applicable based on participant age

b. Demographics

c. Vital signs, including height and weight, BP and HR

d. Complete medical history, including prior/concomitant medications

e. Electrocardiogram (ECG) per site clinical practice

f. Eligibility Review

    i. For eligibility, the participant must meet ILAR criteria for diagnosis of JIA and have at least 3 joints with active arthritis in addition to remainder of inclusion criteria.

ClinROs to be conducted:

a. Complete physical exam, including joint count for active arthritis

Laboratory Assessments:

a. Complete Blood Count (CBC), Comprehensive Metabolic Panel (CMP) and ferritin

b. Urine pregnancy for females of childbearing potential

If a participant fails screening, they may be rescreened at any time based on the discretion of the investigator.

8.1.2 Baseline/Day 0: Visit 2

At the baseline visit, participants will be randomized in a 1:1 ratio to be treated with active tcVNS at the cymba concha or SS at the neck. Randomization will take place at the baseline visit after confirmation of eligibility by the site investigator.The appropriate device, items to administer tcVNS, written instructions, and the participant diary will be dispensed at the visit.

PROs to be administered:

a. CHAQ

b. Pain assessment by Wong-Baker FACES Scale

c. PROMIS Measures

d. Parent/patient global assessment of disease activity by VAS

ClinROs to be conducted:

a. Complete physical exam, including joint count for active arthritis

b. PGA using a VAS

General Assessments:

a. Prior/concomitant medications

b. AE monitoring

Laboratory Assessments:

a. Urine pregnancy for females of childbearing potential.

b. Serology (RF (Rheumatoid Factor), anti-CCP)

c. CRP

d. Mechanistic Assessments.

Study Procedure:

a. Vital signs, including height and weight, BP and HR

b. Receipt of appropriate device, head and neck coverings, and participant diary

c. Stimulation will be performed for 5 minutes by guardians with measurement of voltage, while being monitored for accuracy of technique by the unblinded coordinator. Guardians, participants, and the examining physician/provider will be blinded as to which arm of the trial they are enrolled into, to minimize bias. Note: BP and HR will be recorded prior to and immediately following device stimulation.

8.1.3 Day 1/Day 57: Visit 3/7 (+1 Day)

Participants will complete a telemedicine visit on Day 1 and Day 57 (+1 Day). This visit will be conducted by the unblinded coordinator at the site. Participants have the option to attend this visit in-person at the site if desired.

General Assessments:

a. Prior/concomitant medications

b. AE monitoring

Study Procedure (via telemedicine or on-site):

a. Stimulation will be performed for 5 minutes by guardians while being monitored for accuracy of technique.

8.1.4 Week 1/Week 9: Visit 4/8 (+/-3 Days)

PROs to be administered:

a. CHAQ

b. Pain assessment by Wong-Baker FACES Scale

c. PROMIS Measures

d. Parent/patient global assessment of disease activity by VAS

ClinROs to be conducted:

a. Complete physical exam, including joint count for active arthritis

b. PGA using a VAS

General Assessments:

a. Prior/concomitant medications

b. AE monitoring

c. Study Procedure

d. Vital signs including height and weight, BP and HR

e. Stimulation will be performed for 5 minutes by guardians with measurement of voltage, while being monitored for accuracy of technique. Note: BP and HR will be recorded prior to and immediately following device stimulation.

f. Review of Participant Diary

Study Procedure:

a. Vital signs including height and weight, BP and HR

b. Stimulation will be performed for 5 minutes by guardians with measurement of voltage, while being monitored for accuracy of technique. Note: BP and HR will be recorded prior to and immediately following device stimulation.

c. Review of Participant Diary

8.1.5 Week 4/12: Visit 5/9 (+/- 3 Days)

PROs to be administered:

a. CHAQ

b. Pain assessment by Wong-Baker FACES Scale

c. PROMIS Measures

d. Parent/patient global assessment of disease activity by VAS

ClinROs to be conducted:

a. Complete physical exam, including joint count for active arthritis

b. PGA using a VAS

General Assessments:

a. Prior/concomitant medications

b. AE monitoring

Laboratory Assessments:

a. CBC, CMP

b. CRP

Study Procedure:

a. Vital signs including height and weight, BP and HR

b. Stimulation will be performed for 5 minutes by guardians with measurement of voltage, while being monitored for accuracy of technique. Note: BP and HR will be recorded prior to and immediately following device stimulation.

c. Review of Participant Diary

8.1.6 Week 8: Visit 6 (+/- 3 Days)

At Week 8 (Visit 6), all participants receiving SS at the neck will begin active tcVNS at the cymba concha. Participants will be provided with an active tcVNS system , items to administer tcVNS at the cymba concha, written instructions, and the participant diary will be dispensed. Participants already receiving active tcVNS treatment will continue to receive that treatment. Prior to receipt of the active tcVNS system, participants will be surveyed as to what treatment they believe that they received during the double-blind, sham controlled 8-week period.

PROs to be administered:

a. CHAQ

b. Pain assessment by Wong-Baker FACES Scale

c. PROMIS Measures

d. Parent/patient global assessment of disease activity by VAS

ClinROs to be conducted:

a. Complete physical exam, including joint count for active arthritis

b. PGA using a VAS

General Assessments:

a. Prior/concomitant medications

b. AE monitoring

Laboratory Assessments:

a. CBC, CMP

b. Serology (RF, anti-CCP)

c. Urine pregnancy for females of childbearing potential.

d. CRP

e. Mechanistic Assessments

Study Procedure:

a. Vital signs including height and weight, BP and HR

b. Treatment Survey

c. Collection of device and receipt of an active tcVNS systemdevice

d. Stimulation will be performed for 5 minutes by guardians with measurement of voltage, while being monitored for accuracy of technique. Note: BP and HR will be recorded prior to and immediately following device stimulation.

e. Review of Participant Diary

8.1.7 Week 16: Visit 10 (+/-3 Days)

PROs to be administered:

a. CHAQ

b. Pain assessment by Wong-Baker FACES Scale

c. PROMIS Measures

d. Parent/patient global assessment of disease activity by VAS

ClinROs to be conducted:

a. Complete physical exam, including joint count for active arthritis

b. PGA using a VAS

General Assessments:

a. Prior/concomitant medications

b. AE monitoring

Laboratory Assessments:

a. CBC, CMP

b. Serology (RF, anti-CCP)

c. Urine pregnancy for females of childbearing potential.

d. CRP

e. Mechanistic Assessments

Study Procedure:

a. Vital signs including height and weight, BP and HR

b. Stimulation will be performed for 5 minutes by guardians with measurement of voltage, while being monitored for accuracy of technique. Note: BP and HR will be recorded prior to and immediately following device stimulation.

c. Review of Participant Diary

d. Collection of Device

8.1.8 Unscheduled Visits

Unscheduled visits may be performed to evaluate concerns, including potential disease flares, side effects of the device, or AEs. Assessments obtained during an unscheduled visit are outlined in Table 8.1, Schedule of Events. Some assessments for an unscheduled visit may be omitted at the discretion of the investigator if not clinically indicated. Additional assessments may be performed based on the medical indication and judgment of the site investigator.

8.1.9 Early Termination Visits

An Early Termination Visit should be requested for participants who withdraw from the study prior to study completion. Assessments to be conducted at an early termination visit can be found in Table 8.1, *Schedule of Events*.

8.2 Disease Activity Assessments

8.2.1 JIA ACR 50

The JIA ACR 50 is a validated composite response consisting of 6 core criteria as outlined in Section 3.2, *Primary Efficacy Endpoint*. JIA ACR 50 will be determined at Baseline/Day 0 and Weeks 4, 8, 12, and 16.

8.2.2 JIA ACR 30

The JIA ACR 30 response criteria utilize the same 6 core criteria as the JIA ACR 50 but requires a 30% improvement in any 3 of the 6 core criteria with no worsening by 30% in any one core criteria. JIA ACR 30 will be determined at Baseline/Day 0 and Weeks 4, 8, 12, and 16.

8.2.3 JIA ACR 70

The JIA ACR 70 response criteria utilize the same 6 core criteria as the JIA ACR 50 but requires a 70% improvement in any 3 of the 6 core criteria with no worsening by 30% in any one core criteria. JIA ACR 70 will be determined at Baseline/Day 0 and Weeks 4, 8, 12, and 16.

8.2.4 JADAS-27

JADAS-27 is a measurement of disease activity and is determined by adding the scores of its 4 components[64, 65]:

1. PGA
2. parent/patient global assessment of well-being
3. count of joints with active disease; and
4. CRP (CRP is “normalized” to a value between 0-10).

The JADAS-27 includes the following joints: cervical spine, elbows, wrists, metacarpophalangeal joints (from first to third), proximal inter-phalangeal joints, hips, knees, and ankles. JADAS-27 will be determined at Baseline/Day 0, and Weeks 4, 8, 12, and 16, [66].

8.2.5 Pain Assessment by Wong-Baker FACES Scale

Guardians/participants will be asked to assess average pain in the past week utilizing the Wong-Baker FACES Scale for Pain. This measure is validated for children 3 years of age and older. It depicts faces with varying degrees of sadness due to pain, beginning on the far left with a happy face having no pain with the last picture on the far right showing a very sad face depicting a great deal of pain. There is also a description under each face explaining its meaning. Each face has a numeric score. The Wong-Baker FACES Scale will be assessed at Baseline/Day 0 and Weeks 1, 4, 8, 9 12 and 16.

8.2.6 Childhood Health Assessment Questionnaire (CHAQ)

Function will be assessed using the CHAQ, which is a self-administered, disease-specific measure adapted for use in children with juvenile arthritis between the ages of 1-19 years [67]. Questions assess function in each of the following eight categories of function: dressing and grooming, arising, eating, walking, hygiene, reach, grip and activities. The CHAQ also includes questions about the need for any assistive devices and any activities that require assistance from another person. Scores on the CHAQ may range from 0 (no functional limitation) to 3 (the most severe functional disability). A minimum decrease of 0.13 in CHAQ has demonstrated to indicate functional improvement in children with arthritis [68]. Functional Assessment will be measured at Baseline/Day 0 and Weeks 1, 4, 8, 9 12 and 16.

8.2.7 Patient-Reported Outcomes Measurement Information System (PROMIS) Modules

PROMIS is a set of publicly available patient-reported health status tools developed by the National Institutes of Health (NIH) to evaluate many domains of physical, mental, and social health in both adults and children. Several PROMIS measures, including the fatigue domain, have been validated in children with JIA [69]. The pediatric v2.1 and parent proxy v2.0 fatigue short forms are 8 and 10 item questionnaires respectively that will be used to assess fatigue in JIA participants for this study [69]. In addition, the PROMIS Mobility questionnaire v2.0, and Upper Extremity questionnaire v2.0 has versions for the patient to complete (ages 8 – 17 years old) or a parent proxy version (ages 5 – 17 years). The PROMIS questionnaires will be measured at Baseline/Day 0 and Weeks 1, 4, 8, 9, 12 and 16.

8.2.8 Global Assessment by Visual Analogue Scale (VAS)

Parent/Patient Global Assessment will be measured using a VAS. Guardians/participants will be asked to assess how they are doing overall in the past 1 week related to their arthritis by placing a mark on a 10 cm line which is anchored at the left with a “0” and the words “very well” and on the right with a “10” and “very poor”. Parent/Patient Global Assessment will be measured at Baseline/Day 0 and Weeks 1, 4, 8, 9, 12 and 16.

8.2.9 Physician Global Assessment (PGA) of Disease Activity

Physicians will assess disease activity by marking a 10 cm VAS that is anchored on the left with a “0” and the words “no activity” and on the right with a “10” and “extreme activity”. PGA will be measured at Baseline/Day 0 and Weeks 1, 4, 8, 9, 12 and 16.

8.2.10 Joints with Active Arthritis

Number of joints with active arthritis will be determined on physical examination at Screening, Baseline and Weeks 1, 4, 8, 9, 12 and 16.

Active arthritis is non-boney swelling (joint effusion) or limitation of motion accompanied by warmth, pain on motion, or tenderness to palpation.

i. Warmth will not be collected because it is subjective, difficult to assess, and not consistent with industry standards for JIA trials.

ii. If swelling, limitation of motion, and pain on motion/tenderness can all be assessed for a joint, a joint is considered active if it has either a) swelling and/**or** b) limitation of motion **and** pain on motion/tenderness.

iii. For sternoclavicular and acro clavicular, since the limitation of motion is not assessable, these joints would be considered active if they have either a) swelling **or** b) pain on motion/tenderness.

iv. For hip, cervical spine, thoracic spine, and lumbosacral spine, since swelling is not assessable, these joints would be considered active if they have both limitation of motion **and** pain on motion/tenderness.

v. For sacroiliac, this joint is considered active if it has pain on motion/tenderness since we are not assessing limitation of motion and swelling is not assessable.

8.2.11 Joints with Limited Motion

Number of joints with limited motion will be determined by physical examination at Screening, Baseline and Weeks 1, 4, 8, 9, 12 and 16.

Table 8.1 Schedule of Events

Visit Name	Visit 1	Visit 2	Visit 3[E]	Visit 4	Visit 5	Visit 6	Visit 7[E]	Visit 8	Visit 9	Visit 10	Unscheduled Visit	Early Termination
Timepoint	Screening	Baseline	Day 1	Week 1	Week 4	Week 8	Day 57	Week 9	Week 12	Week 16		
Visit Windows (Days)	-28 Days	Day 0	+ 1 Day	+/- 3 Days	+/- 3 Days	+/- 3 Days	+ 1 Day	+/- 3 Days	+/- 3 Days	+/- 3 Days		
Central Clinical Blood Draw (mL)	8	8.5	0	0	8	8	0	0	8	11.5	8	11.5
Research Blood Draw (mL)	0	17	0	0	0	11	0	0	0	3	0	3
Visit Draw Total (mL)	8	25.5	0	0	8	19	0	0	8	14.5	8	14.5
General Assessments												
Informed Consent	X											
Assent	X											
Demographics	X											
Medical History	X											
Prior/Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Event Monitoring		X	X	X	X	X	X	X	X	X	X	X
ECG	X											
Eligibility Review	X	X										
Randomization		X										
Vital Signs[AB]	X	X	X[F]	X	X	X	X[F]	X	X	X	X[L]	X
Patient Reported Outcomes												
CHAQ		X		X	X	X		X	X	X	X[L]	X
Wong-Baker FACES Scale		X		X	X	X		X	X	X	X[L]	X
PROMIS Modules (Fatigue Short Form, Mobility Measure and Upper Extremity Measure)		X		X	X	X		X	X	X	X[L]	X
Parent/Patient Global Assessment		X		X	X	X		X	X	X	X[L]	X

Visit Name	Visit 1	Visit 2	Visit 3[E]	Visit 4	Visit 5	Visit 6	Visit 7[E]	Visit 8	Visit 9	Visit 10	Unscheduled Visit	Early Termination
Timepoint	Screening	Baseline	Day 1	Week 1	Week 4	Week 8	Day 57	Week 9	Week 12	Week 16		
Visit Windows (Days)	-28 Days	Day 0	+ 1 Day	+/- 3 Days	+/- 3 Days	+/- 3 Days	+ 1 Day	+/- 3 Days	+/- 3 Days	+/- 3 Days		
Disease-Specific Assessments												
Physician global assessment of Disease Activity (PGA)		X		X	X	X		X	X	X	X[L]	X
Physical Examination with joint assessment	X	X		X	X	X		X	X	X	X[L]	X
Study Procedure												
Administer Stimulation		X	X[F]	X	X	X	X[F]	X	X	X	X[L]	X
Measure Voltage		X	X[F]	X	X	X	X[F]	X	X	X	X[L]	X
Review Participant Diary				X	X	X		X	X	X	X	X
Dispense Device (instruction) and Participant Diary		X[C]				X					X[L]	
Treatment Survey						X						
Central Clinical Laboratory Assessments[D]												
Complete Blood Count (CBC)	X				X	X			X	X	X[L]	X
Comprehensive Metabolic Panel (CMP)	X				X	X			X	X	X[L]	X
CRP		X			X	X			X	X	X[L]	X
Ferritin	X											
Urine Pregnancy [K]	X	X				X				X	X[L]	X
Serology (RF, anti-CCP)		X								X		X
Mechanistic Laboratory Assessments[D]												
Serum[G]		X				X				X	X[L]	X
Whole Blood[H]		X				X						

Visit Name	Visit 1	Visit 2	Visit 3[E]	Visit 4	Visit 5	Visit 6	Visit 7[E]	Visit 8	Visit 9	Visit 10	Unscheduled Visit	Early Termination
Timepoint	Screening	Baseline	Day 1	Week 1	Week 4	Week 8	Day 57	Week 9	Week 12	Week 16		
Visit Windows (Days)	-28 Days	Day 0	+ 1 Day	+/- 3 Days	+/- 3 Days	+/- 3 Days	+ 1 Day	+/- 3 Days	+/- 3 Days	+/- 3 Days		
Mechanistic Laboratory Assessments[D] Continued												
PBMCs/Anti-coagulated whole blood[I]		X				X						
Plasma for DNA[J]		X										

A. Vitals before and after stimulation (HR and BP)
B. Height and weight
C. Ensure guardian demonstration for use of device, make sure any treatment information goes to unblinded coordinator, not blinded investigator
D. Blood must be obtained prior to stimulation
E. May be performed via telemedicine
F. If in clinic
G. 3 mL SST (Red/Gray Top Tube)
H. (2) 1mL TruCulture™ Tubes
I. 5 mL into 10 mL Green Top Tube
J. 6 mL Lavender Top Tube
K. Females of childbearing potential only. The pregnancy test should be performed locally using kits provided by the central lab.
L. May be omitted at the discretion of the investigator if not clinically indicated

9 Mechanistic Assays

a. Serum levels CRP, 1 and soluble mediators such as: HMGB1, TNF, IL-1β, IL-1 RA, TGFβ, IL-10, IL-18, IL-6, IL-8, IL-17, RANKL, OPG, G-CSF, RANTES and MIP1α.

b. Serum levels of substance P by enzyme-linked immunosorbent assay (ELISA)

c. Peripheral blood cell cytokine monocyte production stimulated by: TLR 4 (triggered via LPS). Peripheral blood cell cytokine production will be determined on whole blood stimulated by 100ng/ml LPS (ie stimulation of TLR 4) or unstimulated.

d. CITE-Seq on whole blood for blood cell composition

e. CITE-Seq on whole blood for single cell transcriptomics

A study in schizophrenia of an α7nAChR agonist evaluated genetic variations in the α7nAChR CHRNA7 gene and demonstrated a pharmacogenetic effect of a minor allele variant, rs3087454 A/C [70]. However, more extensive studies of this single nucleotide polymorphism (SNP) at the FIMR have found that both copy number, and the allelic variants are present. DNA will be collected on each participant for "future studies" which would be pursued if additional funds are made available from the ACE, or can be obtained from outside sources to evaluate rs3087454 A/C.

10 Biospecimen Storage

Mechanistic specimens will be batched at sites and sent every 4 months to the Boaz Biorepository at the FIMR until the completion of the completion of all planned analyses.

11 Criteria for Participant and Study Completion and Premature Study Termination

11.1 Participant Completion

This study will be completed at 16 weeks after the end of study visit.

11.2 Participant Stopping Rules and Withdrawal Criteria

Participants may be prematurely terminated from the study for the following reasons:

1. The guardian or participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant dies.
3. The Investigator no longer believes participation is in the best interest of the participant.
4. The study is stopped by the sponsor.

Premature discontinuation of study treatment without study withdrawal is discussed in Section 6.8, *Premature Discontinuation of tcVNS*.

11.3 Participant Replacement

Participants who withdraw or are withdrawn will not be replaced once they have begun treatment with the investigational device.

11.4 Follow-up after Early Study Withdrawal

If study treatment is prematurely withdrawn for any reason, the participant will be asked to complete the remaining study visits for safety and durability of response assessments. If a participant elects to withdraw from the study, the participant will be asked to return for an Early Termination Visit.

11.5 Study Stopping Guidance

See Section 12.8.2.2, *Ad Hoc DSMB Reviews* for more information on study stopping guidance.

12 Safety Monitoring and Reporting

12.1 Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. AEs that are classified as serious according to the definition of health authorities must be reported promptly (per Section 12.5, *Reporting of Serious Adverse Events and Adverse Events*) to DAIT/NIAID as applicable. Appropriate notifications will also be made to site principal investigators, Institutional Review Boards (IRBs), and health authorities (as applicable).

Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice*, 21CFR Parts 312 and 320, 812, and 812.2(b), and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_60.

12.2 Definitions

12.2.1 Adverse Event (AE)

Any untoward or unfavorable medical occurrence associated with the participant's participation in the research, whether or not considered related to the participant's participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice) (from OHRP "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2). For the AJA01 study, JIA flares will also be collected as AEs.

12.2.2 Unexpected Adverse Event

An AE or suspected adverse reaction (SAR) is considered "unexpected" if it is not listed in protocol, (Protocol Section 5, *Known and Potential Risks and Benefits to Participants*, Protocol Section 5.1*: Risks of the Investigational Device cited in the Medical Literature;* Protocol Section 5.3*: Risks of Other Study Procedures)* or is not listed at the specificity, severity, or rate of occurrence that has been observed.

12.2.3 Serious Adverse Event (SAE)

An AE or SAR is considered "serious" if, in the view of either the investigator or DAIT/NIAID it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death.
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or DAIT/NIAID, its occurrence places the participant at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Elective hospitalizations or hospital admissions for the purpose of conduct of protocol mandated procedures are not to be reported as an SAE unless hospitalization is prolonged due to complications.

12.2.4 Adverse Device Effect (ADE)

An adverse device effect (ADE) is an AE that is suspected to be related to the device. ADEs can be either anticipated, which means that they have been previously identified in this protocol (Section 12.2.2, *Unexpected Adverse Event*), or unanticipated.

12.2.5 Unanticipated Adverse Device Effect (UADE)

The investigational device exemption (IDE) regulations define an unanticipated adverse device effect (UADE) 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" (21 CFR 812.3(s)). UADEs will be reported as SAEs by site investigators to DAIT/NIAID (Section 12.2.3, *Serious Adverse Event*, and 12.5.1, *Reporting of Serious Adverse Events to DAIT/NIAID.*)

12.3 Grading and Attribution of Adverse Events

12.3.1 Grading Criteria

The study site will grade the severity of AEs experienced by the study participants according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0, other than the exceptions listed below. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs. The NCI-CTCAE has been reviewed by the Protocol Chairs and has been deemed appropriate for the participant population to be studied in this protocol.

AEs will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Grade 1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 = Moderate; minimal, local or noninvasive intervention indicated; limiting ageappropriate instrumental ADL*.

Grade 3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**.

Grade 4 = Life-threatening consequences; urgent intervention indicated.

Grade 5 = Death related to AE.

Activities of Daily Living (ADL) *Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc. **Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Grade 1 AEs will be recorded on the appropriate AE case report form for any of the following:

- Mild palpitations, documented arrhythmia, or any unpleasant sensations caused by palpitations (grade 1 = mild symptoms for which no intervention is indicated)
- Stimulation site reactions such as cutaneous irritation, pain, or skin burns (grade 1 = minimal symptoms for which no intervention is indicated)
- Ear and labyrinth disorders as listed in the CTCAE V5.0, except those of the middle ear.
- JIA flares (fulfills criteria outlined below under JIA Flares but does not interfere with function)

All other AEs Grade 2 and higher will be recorded on the appropriate AE case report form for this study.

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment-emergent AE is defined as an increase in grade from baseline or from the last post-baseline value that does not meet grading criteria. Changes in grade from screening to baseline will also be recorded as AE but are not treatment emergent. If a specific event or result from a given clinical or laboratory evaluation is not included in the NCI-CTCAE manual, then an abnormal result would be considered an AE if changes in therapy or monitoring are implemented as a result of the event/result.

JIA Flares

JIA ACR flare is defined as a worsening of 30% or more in 3 or more of the 6 variables of the JIA core set listed above in Section 3.2, *Primary Efficacy Endpoint,* with no more than one variable improving by 30% or more.[71] In addition, the following minimum worsening contingencies apply: if either the number of active joints or the number of joints with limited range of motion are included in the calculation of "flare" then there must be a worsening of at least two joints. If the Physician's or parent/patient global assessment scores are used in the definition of "flare" then there must be a worsening of at least 2 units on the 10-unit scales. If the CRP is used in the definition of "flare" then the second values for the CRP used in the calculation must be above the upper limit of normal for the CRP. JIA flares will be graded as follows:.

Grade 1 = Fulfills criteria for JIA flare but does not interfere with function

Grade 2 = Fulfills criteria for JIA flare and interferes with function, but does not interfere with ADL

Grade 3 = Fulfills criteria for JIA flare and interferes with ADL

Grade 4 = Fulfills criteria for JIA flare and is Disabling

Grade 5 = Fulfills criteria for JIA flare and resulted in Death

Increases in joint activity in a participant not meeting a definition of flare, should be captured using an Adverse Event of "Arthritis".

Liver Function Abnormalities

Liver function abnormalities will be graded using alternative criteria which are based on CTCAE version 4.0, and are defined relative to the ULN as follows:

- Aspartate aminotransferase [AST] increased
  - Grade 1: >ULN – 3.0x ULN
  - Grade 2: >3.0x ULN – 5.0x ULN
  - Grade 3: >5.0x ULN – 20.0x ULN
  - Grade 4: >20.0x ULN
- Alanine aminotransferase [ALT] increased
  - Grade 1: >ULN – 3.0x ULN
  - Grade 2: >3.0x ULN – 5.0x ULN
  - Grade 3: >5.0x ULN – 20.0x ULN
  - Grade 4: >20.0x ULN
- Alkaline phosphatase [ALP] increased
  - Grade 1: >ULN -2.5x ULN
  - Grade 2: >2.5x ULN – 5.0x ULN
  - Grade 3: >5.0x ULN – 20.0x ULN
  - Grade 4: >20.0x ULN
- Blood bilirubin increased
  - Grade 1: >ULN – 1.5x ULN
  - Grade 2: >1.5x ULN – 3.0x ULN
  - Grade 3: > 3.0x ULN – 10.0x ULN
  - Grade 4: > 10.0x ULN

12.3.2 Attribution Definitions

The relationship, or attribution, of an AE to the study therapy regimen or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE eCRF. Final determination of attribution for safety reporting will be determined by DAIT/NIAID. The relationship of an AE to the investigational medical device or procedures will be determined using the descriptors and definitions provided in Table 12.3.2.

For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site: Common Terminology Criteria for Adverse Events (CTCAE) (cancer.gov).

Table 12.3.2 Attribution of Adverse Events

Code	Descriptor	Relationship (to investigational medical device or study procedure)
UNRELATED CATEGORY		
1	Not Related	The adverse event is clearly not related: there is insufficient evidence to suggest a causal relationship.
RELATED CATEGORIES		
2	Possibly Related	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Related	The adverse event is clearly related.

12.4 Collection and Recording of Adverse Events

12.4.1 Collection Period

AEs related to study procedures will be collected from the time of screening until a participant completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study. All AEs will be collected from the time of the administration of the first stimulation until a participant completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

12.4.2 Collecting Adverse Events

AEs (including SAEs) may be discovered through any of these methods:

- Observing the participant.
- Interviewing the participant [e.g., using a checklist, structured questioning, diary, etc.].
- Receiving an unsolicited complaint from the participant.
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event, as defined in Section 12.3, *Grading and Attribution of Adverse Events*.

12.4.3 Recording Adverse Events

Throughout the study, the investigator will record AEs and SAEs as described previously (Section 12.2, *Definitions*) on the appropriate AE eCRF regardless of the relationship to the investigational medical device.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the participant prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

12.5 Reporting of Serious Adverse Events and Adverse Events

12.5.1 Reporting of Serious Adverse Events to DAIT/NIAID

This section describes the responsibilities of the site investigator to report SAEs to the sponsor. Timely reporting of AEs is required by 21 CFR and ICH E6 guidelines.

Site investigators will report all SAEs (see Section 12.2.3, *Serious Adverse Event*), regardless of relationship or expectedness within 24 hours of discovering the event. In addition, Unanticipated Adverse Device Effects (UADEs) (Section 12.2.5, *Unanticipated Adverse Device Effect (UADE))* will be reported by the site investigators as SAEs.

For SAEs, all requested information on the AE/SAE eCRF will be provided. However, unavailable details of the event will not delay submission of the known information. As additional details become available, the AE/SAE eCRF will be updated and submitted.

12.5.2 Reporting to Health Authority

As stated in the FDA Information Sheet Guidance For IRBs, Clinical Investigators, and Sponsors: Frequently Asked Questions About Medical Devices (January 2006), an NSR device is an investigational device that does not meet the definition of a significant risk device. If an IRB finds that an investigational medical device study poses a NSR, the sponsor does not need to submit an IDE to FDA before starting the study. If the IRB determines that the proposed study is an NSR study, the IRB may proceed to review the study under 21 CFR 56.109 and 21 CFR 56.111. FDA considers an NSR device study to have an approved IDE after IRB approval and when sponsors meet the abbreviated requirements at 21 CFR 812.2(b). Consequently, in most cases, FDA is not aware of non-significant risk device studies.

Since this study has been determined to be a nonsignificant risk (NSR) study, it is not being conducted under an IDE.

12.5.2.1 Unanticipated Adverse Device Effects (UADEs)

If a UADE is identified by a site investigator then it will be submitted by the unblinded investigator to the sponsor, DAIT/NIAID, per the same mechanism as reporting of an SAE (*Section 12.5.1: Reporting of Serious Adverse Events to DAIT/NIAID*). The study sponsor will review the report, to be prepared on FDA Form 3500 with associated narrative, and when the report is deemed complete and accurate, the report will be returned to the unblinded investigator at the site of occurrence to submit to the FDA under 21 CFR 812.46(b). A maximum of 10 working days are allotted for this reporting to the FDA from the time of discovery of the event by the site of occurrence. Additionally, the IRB and participating investigators are to be informed within the same timeframe. DAIT/NIAID will facilitate information compilation for submission of any additional reports by the investigator of origin concerning the effect as requested by FDA.

12.5.3 Reporting of Adverse Events to the IRB

All investigators shall report AEs, including expedited reports, in a timely fashion to the single IRB (sIRB) of record in accordance with applicable regulations and guidelines. As described in Section 12.5.2.1, *Unanticipated Adverse Device Effects (UADEs),* per 21 CFR 812.150(b), UADEs will be reported to the IRB and participating investigators within 10 working days of DAIT/NIAID receiving notice of the UADE. SAEs that are determined by DAIT/NIAID to have potential impact on the safety of all trial participants will be distributed by the DAIT/NIAID (or designee) to all participating site investigators and the sIRB of record.

12.6 Pregnancy Reporting

The investigator shall be informed immediately of any pregnancy in a study participant. A pregnant participant shall be instructed to stop using the investigational device. The investigator shall counsel the participant and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant participant shall continue until the conclusion of the pregnancy. Partner pregnancies will not be collected on AJA01.

The investigator shall report to the DAIT/NIAID all pregnancies within 1 business day of becoming aware of the event using the Pregnancy eCRF. All pregnancies identified during the study shall be followed to conclusion and the outcome of each must be reported. The Pregnancy eCRF shall be updated and submitted to the SACCC when details about the outcome are available.

Information requested about the delivery shall include:

- Gestational age at delivery
- Birth weight, length, and head circumference, Gender
- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities.

All pregnancy complications that result in a congenital abnormality, birth defect, miscarriage meeting criteria of SAE, or medically indicated abortion will be submitted to DAIT/NIAID using the SAE reporting procedures described above.

12.7 Reporting of Other Safety Information

An investigator shall promptly notify the site IRB as well as DAIT/NIAID when an "unanticipated problem involving risks to participants or others" is identified, which is not otherwise reportable as an AE.

12.8 Review of Safety Information

12.8.1 Medical Monitor Review

The DAIT/NIAID Medical Monitor shall receive monthly reports as specified by DAIT/NIAID from the SACCC, compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the study sites on appropriate eCRFs.

In addition, the Medical Monitor shall review and make decisions on the disposition of the SAE and pregnancy reports received by the SACCC (See Sections 12.5.1, *Reporting of Serious Adverse Events to DAIT/NIAID,* and 12.6, *Pregnancy Reporting*).

12.8.2 DSMB Review

12.8.2.1 Planned DSMB Review

The DSMB shall review safety data at least yearly during planned DSMB Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs.

The DSMB will be informed of an Expedited Safety Report in a timely manner.

12.8.2.2 *Ad hoc* DSMB Reviews

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for ad hoc reviews. The DSMB will review any event that potentially impacts safety at the request of the protocol chair or DAIT/NIAID. In addition, the following events will trigger an ad hoc comprehensive DSMB Safety Review:

1. Any death (regardless of relationship to investigational device),
2. Any NCI-CTCAE Grade 4 or higher adverse event in a single participant.
3. The occurrence of NCI-CTCAE Grade 3 or higher AEs (including SAEs) that are related to investigational device in 2 of the first 10 participants or 20% of all participants at any time.
4. At the request of the DSMB/NIAID.

The DSMB may recommend stopping of the trial only for safety concerns.

After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

12.8.2.2.1 Temporary Suspension of Randomization for ad hoc DSMB Safety Review

If any of the events listed in Section 12.8.2.2, *Ad hoc DSMB Reviews*, occur, the chair of the DSMB will be notified and a review of the safety data will be performed. The DSMB will have the discretion to recommend actions regarding study conduct and will determine if enrollment in the study should be stopped and/or administration of study intervention should be halted.

If a death or NCI-CTCAE Grade 4 or higher AE in a single participant occurs during the study, a temporary halt in consenting and screening of new participants as well as randomizing participants will be implemented until after DSMB completes review of the safety data. Participants on study will be permitted to continue their assigned treatment unless they are the subject of the review.

If an ad hoc DSMB is called for excessive Grade 3 or higher AEs (including SAEs) that are related to investigational device, the study will proceed as planned pending DSMB review of the data. However, if 2 weeks have elapsed and the DSMB has not met, then no new participants will be consented, screened, or randomized until after the DSMB completes review of the safety data. Participants on study will be permitted to continue their assigned treatment unless they are the subject of the review.

13 Statistical Considerations and Analytical Plan

13.1 Overview

This is a multicenter, double-blind, sham-controlled clinical trial of participants aged 5-18 years (inclusive) with JIA who have at least 3 joints with active arthritis. All enrolled participants will be randomized to active tcVNS at the cymba concha or SS at the neck for 8 weeks, followed by an 8-week open-label extension period where all participants will receive active tcVNS at the cymba concha. The primary objective is to investigate the effectiveness of tcVNS on JIA ACR 50 in participants with JIA. This study also aims to evaluate the safety of tcVNS.

13.2 Endpoints

Endpoints are listed in Sections 3.2, *Primary Efficacy Endpoint*, 3.3, *Secondary Efficacy Endpoints*, 3.4. *Safety Endpoints,* and 3.5 *Exploratory Endpoints*.

13.3 Measures to Minimize Bias

This study has a randomized, double-blind, sham-controlled design with well-defined entry criteria. An unblinded coordinator or nurse will be identified at each site to provide instructions and assistance with the placement of the electrodes and the stimulation procedure, and the unblinded investigator will assess potential physical adverse effects from the stimulation, while all other site personnel who manage participants will be kept blinded to treatment assignment through the first 8 weeks of the trial. Additionally, participants will cover their neck with a scarf and their ears with a headband during clinic visits. Central laboratories will be used for consistency in assay procedures and measurements for clinical and mechanistic endpoints. To minimize the potential threat to study integrity by loss of participants during the first 8 weeks of the study, assessments are kept to a minimum, attention will be paid to participant well-being during and after procedures, and all participants will continue to an 8-week open-label extension period in which they will be treated with active tcVNS at the cymba concha.

13.4 Analysis Plan

13.4.1 Analysis Populations

Safety sample: All participants who receive at least one active tcVNS stimulation or SS. The safety analysis will be based on the actual tcVNS stimulation/SS the participants receive during the double-blind period.

Modified Intent to treat (mITT) sample: All randomized participants who receive any active tcVNS stimulation or SS. The efficacy analyses will be based on the mITT sample according to the group to which the participants are assigned.

Per protocol (PP) sample: All participants in the mITT sample with no major protocol deviations that impact efficacy assessments, who receive at least 45 of 56 active tcVNS stimulation or SS, and who have the Week 8 JIA ACR 50 assessment. The reported major deviations will be reviewed during a masked data review after the last participant's primary endpoint visit to determine which participants should be excluded from the per protocol population.

13.4.2 Timing of Analysis

Analysis of the primary and select secondary endpoints through Week 8 will be conducted when all study participants have completed the Week 8 assessments, and data collected through Week 8 is frozen. Analysis of the other secondary and

exploratory endpoints will be conducted after participants have completed study participation through Week 16 and all data collected is locked.

13.4.3 Primary Analysis of Primary Endpoint

The primary endpoint is the proportion of participants achieving a JIA ACR 50 response at Week 8 and will be calculated as a binary response (Yes or No) indicating whether the participant met the criteria for JIA ACR 50 at Week 8, per the primary endpoint definition in Section 3.2, *Primary Efficacy Endpoint*. The primary analysis of the primary endpoint will be performed on the mITT sample and is designed to test the following hypotheses:

- Null hypothesis: The proportion achieving a JIA ACR 50 response at Week 8 does not differ between the active tcVNS and SS arms.
- Alternate hypothesis: The proportion achieving a JIA ACR 50 response at Week 8 differs between the active tcVNS and SS arms.

The proportion of participants achieving JIA ACR 50 response will be estimated for the active tcVNS and SS arms. The two treatment arms will be compared using a two-sided Fisher's Exact test using a Type 1 error rate of α=0.05.

The primary estimand is defined to be the difference in the proportion of participants with JIA in the active tcVNS arm versus the SS arm who achieve a JIA ACR 50 response at Week 8. The target population for the estimand will be the participants who meet criteria for the mITT analysis population. Intercurrent events will be analyzed by methods to best inform the estimand. For the primary analysis of the primary endpoint, we assume all participants for whom the JIA ACR 50 cannot be evaluated at Week 8 would have failed the primary endpoint.

13.4.4 Supportive Analyses of the Primary Endpoint

At a minimum, the following sensitivity analyses for the primary analysis will be performed:

- An analysis comparing treatment group estimates from an exact logistic regression. The exact logistic regression model will use the binary response variable for JIA ACR 50 response status as the dependent variable, the treatment arm as the independent variable, and the stratification factors defined in Section 3.6, *Randomization, Stratification and Blinding/Masking* as a covariate.
- An analysis analogous to the primary endpoint analysis using only mITT participants with observed JIA ACR 50 data at Week 8 (i.e., dropping participants with missing Week 8 JIA ACR 50 rather than assuming non-response).
- An analysis analogous to the primary endpoint analysis using the PP participants.
- A stratified analysis of JIA ACR 50 response using the Cochran-Mantel-Haenszel statistic to compare treatment groups after controlling for the stratification factors defined in Section 3.6 *Randomization, Stratification and Blinding/Masking* at baseline.

13.4.5 Analyses of Secondary Efficacy Endpoints

All secondary inferential analyses are considered supportive; p-values for tests of differences among groups will be presented without adjustment for multiple comparisons. The mITT and PP populations will be used for all secondary efficacy analyses. The null hypothesis proposes that there are no differences in secondary endpoints between the two arms, active tcVNS and SS through Week 8 or active tcVNS and SS + active tcVNS after Week 8. Unless specified otherwise, data will not be imputed for analyses of secondary endpoints.

For secondary endpoints that are defined as proportions achieving ACR response, analyses will be analogous to that described for the analysis of the primary endpoint. Participants for whom the endpoint cannot be evaluated at the timepoint of interest will be deemed response failures. For supportive analyses, only mITT participants for whom the endpoint can be evaluated at the timepoint of interest will be used (i.e., dropping participants with missing ACR data rather than assuming non-response). Otherwise, data will not be imputed for analyses of secondary endpoints. For the open-label portion of the study, the Week 8 visit will be used as the Baseline visit to estimate ACR responders in each arm.

The change in JADAS-27 scores from Day 0 will be analyzed using an analysis of covariance (ANCOVA) model with the JADAS-27 score at the timepoint as the independent variable, and the JADAS-27 score at Day 0 and treatment as covariates.

For the open-label portion of the study, the change in JADAS-27 scores from Week 8 will be analyzed using an analysis of covariance (ANCOVA) model with the JADAS-27 score at the timepoint as the independent variable, and the JADAS-27 score at Week 8 and treatment as covariates.

Longitudinal trends for JADAS-27 scores will be explored using repeated measures or mixed models that account for within-participant correlation, under the assumption that data are missing at random. Covariates will include treatment, time, treatment*time, and the stratification factors defined in Section 3.6, *Randomization, Stratification and Blinding/Masking*.

13.4.6 Analyses of Secondary Safety Endpoints

AEs and SAEs will be tabulated by MedDRA Preferred Term and Organ Class for both treatment arms. Comparisons of incidence and incidence rates of serious infections between arms will be descriptive and include estimates of differences (or ratios) along with confidence intervals at the 95% confidence level.

13.4.7 Analyses of Exploratory Endpoints

All exploratory inferential analyses are considered supportive; p-values for tests of differences among groups will be presented without adjustment for multiple comparisons. The PP population will be used for all exploratory efficacy analyses. Unless specified otherwise, data will not be imputed for analyses of exploratory endpoints.

In general, descriptive statistics and/or data plots will be used to gain an understanding of the nature of exploratory endpoints. Inferential analysis will be considered to evaluate the hypotheses generated by the descriptive analyses. Additional exploratory analyses may also be considered based on results of planned analyses.

13.4.8 Descriptive Analyses

Descriptive statistics will be provided by treatment group for participant disposition, baseline and demographic characteristics, and use of concomitant medications. Continuous measures will be summarized using n, mean, standard deviation (SD), median, minimum, and maximum. Categorical variables will be summarized using counts and percentages. Confidence intervals at the 95% confidence level will be calculated using the exact method.

13.5 Interim Analysis

13.5.1 Futility

One interim analysis for futility is planned for the study once the Week 8 JIA ACR 50 assessment is completed for the first 50 participants. Enrollment will not be suspended for the interim analysis. Based on a comparison of the proportion of participants in the active tcVNS and SS arms who have achieved JIA ACR 50 at Week 8, the study may be stopped early for futility. An exact conditional power calculation will be performed using the observed frequency of participants achieving JIA ACR 50 response in the active tcVNS and SS arms and assuming the pattern of the remaining participants follows the original design assumptions, that the active tcVNS response rate is 50% and the SS response rate is 20%. If the conditional power is less than 20%, the DSMB may recommend the study be terminated. This futility analysis is designed to be non-binding, so the DSMB may elect not to recommend terminating the trial after considering all the data, even if the conditional power is less than 20%, and DAIT/NAIAD may or may not elect to accept the DSMB recommendation.

If the null hypothesis specified in Section 13.4.3, *Primary Analysis of Primary Endpoint* is true, and the active tcVNS response rate and SS response rate are both 20%, the probability of crossing the futility boundary and stopping the trial early is 42.3%. Additionally, if the null hypothesis is true, but the tcVNS and SS response rates are both 35%, an increase of 75%, the probability of stopping the trail for futility is 53.0%. If the alternate hypothesis specified in Section 13.4.3, *Primary Analysis of Primary Endpoint* is true, and the tcVNS response rate is 50% and the SS response rate is 20%, the probability of stopping the trial early for futility is only 1.3%.

In addition to the conditional power analysis based on Week 8 JIA ACR 50 response for participants in the active tcVNS and SS arms, all safety assessments for planned DSMB reviews outlined in Section 12.8.2.1 *Planned DSMB Review* will be included in the report.

13.6 Statistical Hypotheses

The statistical hypothesis for the primary analysis is provided in Section 13.4.3, *Primary Analysis of Primary Endpoint*.

13.7 Sample Size Considerations

For the primary analysis, the proportions of participants achieving ≥50% improvement in clinical status based on JIA ACR 50 at Week 8 in the active tcVNS and SS arms will be compared. The response rate for JIA ACR 50 in the SS arm could not be estimated directly from published JIA trial results. Most pediatric studies are withdrawal studies where all enrolled subjects receive the active intervention at the start of the study with responders subsequently being randomized to placebo or continuation. However, one 52-week study of 122 participants reported a JIA ACR 50 placebo response rate of 18% at week 6 and 33.9% at week 14 in 59 participants receiving placebo on background methotrexate [72]. Additionally, a seven-month study of 69 participants evaluating the safety and efficacy of etanercept that included a three-month open label phase followed by a four-month double-blind placebo-controlled study had a 50% improvement response of 23% of participants in the placebo arm [73]. Based on these two studies, the SS response rate was estimated to be 20%. If the SS response is 20% and the active tcVNS response is at least 50%, then a study of 100 participants randomized 1:1 to tcVNS and SS, respectively, will provide at least 84.9% power using a 2-sided Fisher's Exact test at α=0.05. For the secondary per-protocol analysis, if the sample size drops to 90 (10% loss), the power is still >80% for a SS JIA ACR 50 rate of 20% and an active tcVNS JIA ACR 50 rate of 50%.

14 Identification and Access to Source Data

14.1 Source Data

Source documents and source data are considered to be the original documentation where participant information, visits consultations, examinations and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations and other activities during a clinical trial.

14.2 Access to Source Data

The site investigators and site staff will make all source data available to the DAIT/NIAID, NIAID representatives, agents, employees, contractors, and other persons assisting in conducting, monitoring, or analyzing the study, as well as to relevant health authorities. Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

All documentation pertaining to the trial should be retained at the investigator site for a period of at least 2 years after the last marketing application approval or, if no application will be filed or if the application is not approved, 2 years following the discontinuation of the investigation. Sites should follow any local requirements if this requirement differs from any local regulations unless the local retention policy is less than 2 years. The local storage location of the study records or subsequent relocation of the original study storage must be informed to DAIT/NIAID by the investigator. The site investigator must obtain prior authorization before destroying any study documents. Any loss of documents due to any natural or manmade disasters or theft must be immediately notified to DAIT/NIAID.

15 Quality Assurance and Quality Control

A Quality management system (QMS) focuses on activities essential to ensuring human subject protection and reliability of research results. Quality Management is the responsibility of the site principal investigator and the sponsor.

The principal investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. The principal investigator is required to ensure that all eCRFs are completed for every participant as specified for the study. Additional investigator responsibilities include but are not limited to (a) supervision of individuals or parties to whom study-related duties and functions are delegated and (b) ensuring individuals and parties are qualified for such duties. In addition to required protocol training, key study personnel must have completed HSP and GCP training in accordance with the NIH policy (insert link). Quality management procedures at the site level mut be in place to ensure integrity of study implementation and data collection and protection of human subjects. Expectations will be communicated to each site regarding study conduct. The investigational site will provide direct access to all physical locations where the research is conducted, and to source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

The Sponsor is responsible for regular inspection of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data. The Sponsor will develop a monitoring plan focused on risk assessment of human protection and data integrity. This risk-based monitoring (RBM) will be conducted with a combination of on-site and centralized monitoring. Sponsor representative monitors will follow written Standard Operating Procedures (SOPs) to

verify that the clinical study is conducted, data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)). Quality control (QC) procedures will be implemented for data collection beginning with the data entry system and data QC checks that will be run on a regular basis. Univariate data validation tests are performed as the data are keyed. The clinical database is backed up nightly; backup tapes are saved in a secure, off-site location. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution. All elements of data entry (i.e., time, date, verbatim text, and the person performing the data entry) will be recorded in an electronic audit trail to allow all data changes in the database to be monitored and maintained in accordance with federal regulations.

16 Protocol Deviations

16.1 Protocol Deviation Definitions

16.1.1 Protocol Deviation

Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. Protocol deviations occur for a variety of reasons, such as an investigator's intentional or unintentional departure from the protocol, the participant's lack of adherence to the protocol, or external/environmental factors (e.g., severe weather or holidays) that change the performance of a protocol. Some protocol deviations are anticipated and/or intentional; others are not. Some protocol deviations are known or identified before they occur; others are only discovered to have occurred after the fact.

The investigators and site staff will conduct the study in accordance to the protocol; no deviations from the protocol are permitted. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

16.1.2 Major Protocol Deviation (Protocol Violation)

A Protocol Violation is a deviation from the IRB approved protocol that may affect the participant's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human subject protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures.

16.1.3 Non-Major Protocol Deviation

A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the participant's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

16.2 Reporting and Managing Protocol Deviations

The study site principal investigator has the responsibility to identify, document and report protocol deviations as directed by the study Sponsor. However, protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

When a deviation occurs, corrective actions may be necessary depending on the nature of the deviation. The PI and NIAID/DAIT conduct a risk assessment. The PI is responsible for the timely correction and documentation of problems identified by study personnel, outside monitors or auditors, or other parties involved in the conduct of a study. The depth of Corrective Action/Preventive Action (CAPA) required should match the risk and impact on safety of participants and/or the quality of the data.

Upon determination that a protocol deviation has occurred, the PI/designated study staff will report the deviation according to the processes outlined for the study. A major deviation is to be reported within 3 business days and reported by the PI to the IRB per IRB reporting requirements. The study sponsor, NIAID/DAIT, will determine if the deviation is reportable to the DSMB and FDA, as applicable.

17 Ethical Considerations and Compliance with Good Clinical Practice

17.1 Statement of Compliance

This clinical study will be conducted using good clinical practice (GCP), as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by an IRB. Any amendments to the protocol or to the consent/assent materials will also be approved by the IRB *before* they are implemented.

17.2 Informed Consent Process

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The principal investigator or designee will review the consent and/or assent and answer questions. The prospective participant and if applicable, the participant's guardian will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. All participants (and/or their legally guardian) will read, sign, and date a consent and/or assent form before undergoing any study procedures. Consent/assent materials will be presented in participants' primary language. A copy of the signed consent/assent form will be given to the participant.

The consent process will be ongoing. The consent/assent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

17.3 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. Site personnel will not transmit documents containing personal health identifiers (PHI) to the study sponsor or their representatives.

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of NIAID/DAIT.

The study Monitor or other authorized representatives of the NIAID/DAIT may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

18 Publication Policy

The ACE policy on the publication of study results will apply to this trial. Authorized study personnel may find details regarding the policy on the ACE study portal. Site investigators are encouraged to communicate and publish study results with prior notification of and review by DAIT/NIAID.

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20 Appendices

20.1 Patient Reported Outcomes and Disease Specific Assessments

20.1.1 CHAQ

CHILDHOOD HEALTH ASSESSMENT QUESTIONNAIRE

Participant ID _____
Person Completing: ___Mother ___Father ___Patient ___Other_____
Date of assessment(mm/dd/yyyy _____
Visit number _____

In this section we are interested in learning how your child's illness affects his/her ability to function in daily life. Please feel free to add a comments on the back of this page. In the following questions, please check the one response which best describes your child's usual activiti (average over an entire day) **OVER THE PAST WEEK. ONLY NOTE THOSE DIFFICULTIES OR LIMITATIONS WHICH ARE DUE T ILLNESS.** If most children at your child's age are not expected to do a certain activity, please mark "Not Applicable". For example, if your chi has difficulty in doing a certain activity or is unable to do it because he/she is too young but **NOT** because he/she is **RESTRICTED BY ILLNES** please mark "Not Applicable".

	Without ANY Difficulty	With SOME Difficulty	With MUCH Difficulty	UNABLE To do	NOT Applicable
DRESSING & GROOMING					
Is your child able to: -Dress, including tying shoelaces and doing buttons?	○	○	○	○	○
-Shampoo his/her hair?	○	○	○	○	○
-Remove socks?	○	○	○	○	○
-Cut fingernails?	○	○	○	○	○
ARISING					
Is your child able to: -Stand up from a low chair or floor?	○	○	○	○	○
-Get in and out of bed or stand up in crib?	○	○	○	○	○
EATING					
Is your child able to: -Cut his /her own meat?	○	○	○	○	○
-Lift a cup or glass to mouth?	○	○	○	○	○
-Open a new cereal box?	○	○	○	○	○
WALKING					
Is your child able to: -Walk outdoors on flat ground?	○	○	○	○	○
-Climb up five steps?	○	○	○	○	○

*** Please check any AIDS or DEVICES that your child usually uses for any of the above activities:**

☐ Cane
☐ Walker
☐ Crutches
☐ Wheelchair
☐ Devices used for dressing (button hook, zipper pull, long-handled shoe horn, etc)
☐ Built up pencil or special utensils
☐ Special or built up chair
☐ Other (Specify:_____)

*** Please check any category for which your child usually needs help from another person BECAUSE OF ILLNESS:**

☐ Dressing and Grooming
☐ Arising
☐ Eating
☐ Walking

CHILDHOOD HEALTH ASSESSMENT QUESTIONNAIRE

	Without ANY Difficulty	With SOME Difficulty	With MUCH Difficulty	UNABLE To do	Not Applicabl
HYGIENE					
Is your child able to: -Wash and dry entire body?	○	○	○	○	○
-Take a tub bath (get in & out of tub)?	○	○	○	○	○
-Get on and off the toilet or potty chair?	○	○	○	○	○
-Brush teeth?	○	○	○	○	○
-Comb/brush hair?	○	○	○	○	○
REACH					
Is your child able to: -Reach and get down a heavy object such as a large game or books from just above his/her head?	○	○	○	○	○
-Bend down to pick up clothing or a piece of paper from the floor?	○	○	○	○	○
-Pull on a sweater over his/her head? head?	○	○	○	○	○
-Turn neck to look back over shoulder?	○	○	○	○	○
GRIP					
Is your child able to: -Write or scribble with pen or pencil?	○	○	○	○	○
-Open car doors?	○	○	○	○	○
-Open jars which have been previously opened?	○	○	○	○	○
-Turn faucets on and off?	○	○	○	○	○
-Push open a door when he/she to turn a door knob?	○	○	○	○	○
ACTIVITIES					
Is your child able to: -Run errands and shop?	○	○	○	○	○
-Get in and out of car or toy car or school?	○	○	○	○	○
-Ride bike or tricycle?	○	○	○	○	○
-Do household chores (eg, wash dishes, take out trash, vacuuming, yard work, make bed, clean room)?	○	○	○	○	○
-Run and play?	○	○	○	○	○

Please check any AIDS or DEVICES that your child usually uses for any of the above activities:

- ☐ Raised toilet seat
- ☐ Bathtub seat
- ☐ Jar opener (for jars previously opened)
- ☐ Bathtub bar
- ☐ Long-handled appliances for Reach
- ☐ Long-handled appliances in bathroom

Please check any categories for which your child usually needs help from another person BECAUSE OF ILLNESS?

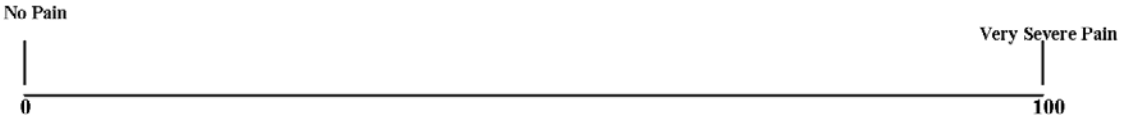
- ☐ Hygiene
- ☐ Reach
- ☐ Gripping and opening things
- ☐ Errands and chores

CHILDHOOD HEALTH ASSESSMENT QUESTIONNAIRE

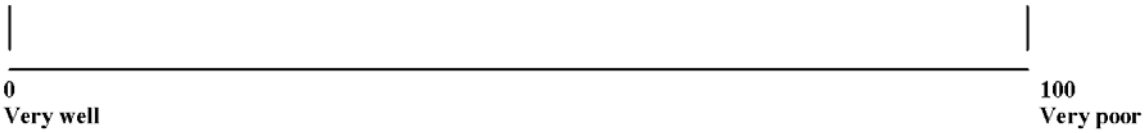
We are also interested in learning whether or not your child has been affected by pain because of his or her illness.

How much pain do you think your child has had because of his or her illness IN THE PAST WEEK?

Place a mark on the line below to indicate the severity of pain.



Considering all the ways that myositis affects your child, rate how your child is doing on the following scale by placing a mark on the line.



CHILDHOOD HEALTH ASSESSMENT QUESTIONNAIRE

20.1.2 Wong-Baker FACES Scale

Instructions for Usage

Explain to the person that each face represents a person who has no pain (hurt), or some, or a lot of pain.

Face 0 doesn't hurt at all. Face 2 hurts just a little bit. Face 4 hurts a little bit more. Face 6 hurts even more. Face 8 hurt a whole lot. Face 10 hurts as much as you can imagine, although you don't have to be crying to have this worst pain.

Ask the person to choose the face that best depicts the pain they are experiencing.

20.1.3 PROMIS Modules

20.1.3.1 Neuro-QoL Short Form v2.1 – Pediatric Fatigue

Neuro-QOL Item Bank 2.1 –Pediatric Fatigue – Short Form

Pediatric Fatigue –Short Form

Please respond to each question or statement by marking one box per row.

	In the past 7 days…	None of the time	A little bit of time	Some of the time	Most of the time	All of the time
NQFTGped01	I felt tired……..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQFTGped04	I had trouble starting things because I was too tired……..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQFTGped05	I had trouble finishing things because I was too tired……..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQFTGped06	I needed to sleep during the day……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQFTGped08	Being tired made it hard to play or go out with my friends as much as I would like...	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQFTGped11r1	I was too tired to eat……..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQFTGped12	Being tired makes me sad……..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQFTGped13	Being tired makes me mad……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

English
July 11, 2014

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20.1.3.2 PROMIS Parent Proxy Short Form v2.0 – Fatigue 10a

PROMIS® Parent Proxy Item Bank v2.0 – Fatigue – Short Form 10a

Parent Proxy Fatigue – Short Form 10a

Please respond to each question or statement by marking one box per row.

	In the past 7 days…	Never	Almost Never	Sometimes	Often	Almost Always
Pf4fatigue12r	Being tired made it hard for my child to play or go out with friends as much as he/she would like.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf4fatigue8r	My child felt weak.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf4fatigue3r	My child got tired easily.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf2fatigue8r	Being tired made it hard for my child to keep up with schoolwork.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf2fatigue4r	My child had trouble finishing things because he/she was too tired.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf3fatigue7r	My child had trouble starting things because he/she was too tired.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf3fatigue12r	My child was so tired it was hard for him/her to pay attention.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf3fatigue8r	My child was too tired to do sports or exercise	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf3fatigue4r	My child was too tired to do things outside.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf4fatigue4r	My child was too tired to enjoy the things he/she likes to do.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

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20.1.3.3 PROMIS Parent Proxy Short Form v2.0 – Mobility 8a

PROMIS Parent Proxy Item Bank v2.0 – Mobility– Short Form 8a

Parent Proxy Mobility – Short Form 8a

Please respond to each question or statement by marking one box per row.

	In the past 7 days…	With no trouble	With a little trouble	With some trouble	With a lot of trouble	Not able to do
Pf1mobil3r	My child could do sports and exercise that other kids his/her age could do	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pf3mobil9r	My child could get up from the floor.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pf4mobil4r	My child could keep up when he/she played with other kids	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pf3mobil8r	My child could move his/her legs	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pf3mobil3r	My child could stand up without help.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pf2mobil7r	My child could stand up on his/her tiptoes	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pf2mobil4r	My child could walk up stairs without holding on to anything	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pf1mobil1r	My child has been physically able to do the activities he/she enjoys most	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

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20.1.3.4 PROMIS Parent Proxy Short Form v2.0 – Upper Extremity 8a

PROMIS® Parent Proxy Item Bank v2.0 – Upper Extremity– Short Form 8a

Parent Proxy Upper Extremity – Short Form 8a

Please respond to each question or statement by marking one box per row.

	In the past 7 days…	With no trouble	With a little trouble	With some trouble	With a lot of trouble	Not able to do
Pf2uprext3r	My child could button his/her shirt or pants	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pf4uprext1r	My child could open a jar by himself/herself	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pf3uprext11r	My child could open the rings in school binders	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pf4uprext10r	My child could pour a drink from a full pitcher	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pf3uprext4r	My child could pull a shirt on over his/her head without help	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pf3uprext9r	My child could pull open heavy doors	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pf2uprext2r	My child could put on his/her shoes without help	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Pf3uprext7r	My child could use a key to unlock a door	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

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20.1.3.5 PROMIS Pediatric Form v2.0 – Mobility 8a

PROMIS Pediatric Item Bank v2.0 – Mobility– Short Form 8a

Pediatric Mobility – Short Form 8a

Please respond to each question or statement by marking one box per row.

	In the past 7 days…	With no trouble	With a little trouble	With some trouble	With a lot of trouble	Not able to do
235R1r	I could do sports and exercise that other kids my age could do	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
4124R1r	I could get up from the floor	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
236R1r	I could keep up when I played with other kids	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
3892R1r	I could move my legs	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
2646R1r	I could stand up by myself	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
4185R1r	I could stand up on my tiptoes	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
2707R2r	I could walk up stairs without holding on to anything	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
5023R1r	I have been physically able to do the activities I enjoy most	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

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20.1.3.6 PROMIS Pediatric Form v2.0 – Upper Extremity Short Form 8a

PROMIS® Pediatric Item Bank v2.0 – Upper Extremity – Short Form 8a

Pediatric Upper Extremity – Short Form 8a

Please respond to each question or statement by marking one box per row.

	In the past 7 days…	With no trouble	With a little trouble	With some trouble	With a lot of trouble	Not able to do
3880R2r	I could button my shirt or pants.....	☐ 4	☐ 3	☐ 2	☐ 1	☐ 1
2671R1r	I could open a jar by myself.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
4143R1r	I could open the rings in school binders...	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
4112R1r	I could pour a drink from a full pitcher....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
3881R1r	I could pull a shirt on over my head by myself.....	☐ 4	☐ 3	☐ 2	☐ 1	☐ 1
4130R1r	I could pull open heavy doors.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
2657bR1r	I could put on my shoes by myself.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
4109R1r	I could use a key to unlock a door.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

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20.1.4 Parent/Patient Global Assessment

PARENT/PATIENT GLOBAL ASSESSMENT:
(100 mm)

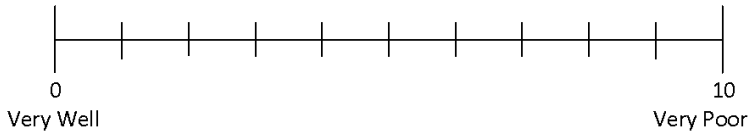
Participant ID #: _____ **Assessment Date:** _____ / _____ / _____
(dd/mon/yyyy)

Parent Instructions: 'You' will refer to the participant enrolled in the study. Please answer the question by placing a vertical (|) mark to indicate your response. Please sign and date this form.

Participant Instructions: Please answer the question by placing a vertical (|) mark to indicate your response. Please sign and date this form.

Considering all the ways Juvenile Idiopathic Arthritis affects you, mark a vertical line at the spot on the horizontal line to describe how you are doing overall in the past one week from 0 (very well) to 10 (very poor).

YOUR RESPONSE:



Parent or Participant's Initials: _____ **Date:** _____

---------- **For Site Coordinator Use Only** ----------

Site Coordinator Directions: Using the markings provided measure from the "0" to the vertical line placed by the patient. Enter the distance in millimeters as indicated below, then transfer the pertinent information onto the appropriate eCRF page, and place this document in the participant's research record.

Length of line
(from 0 to vertical assessment line) _____ mm

Initials of site personnel measuring the line: _____ Date: _____
(dd/mon/yyyy)

20.1.5 PGA

PHYSICIAN GLOBAL ASSESSMENT:

(100mm)

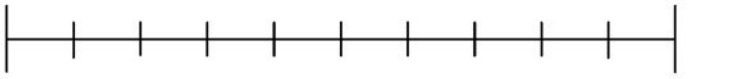
Participant ID #: _____ **Assessment Date:** _____ / _____ / _____
(dd/mon/yyyy)

EXAMPLE

0 Not Active — 10 Extremely Active

YOUR RESPONSE:

Please rate the patient's current Juvenile Idiopathic Arthritis Related Disease activity on the scale below, with 0 being no disease activity and 10 being extremely active disease activity.



0 No Activity — 10 Extreme Activity

Signature of Physician marking the line: _____ Date: _____
(dd/mon/yyyy)

---------- **For Site Coordinator Use Only** ----------

Site Coordinator Directions: Using the markings provided, measure from the "0" to the vertical line placed by the physician. Enter the distance in millimeters as indicated below, then transfer the pertinent information onto the appropriate eCRF page, and place this document in the participant's research record.

Length of line
(from 0 to vertical assessment line) _____ mm

Initials of site personnel measuring the line: _____ Date: _____
(dd/mon/yyyy)

20.1.6 Joint Assessment

AJA01 PHYSICIAN JOINT ASSESSMENT LOWER REQUIRED WORKSHEET

Participant ID #: _____ **Assessment Date:** _____ / _____ / _____
(dd/mon/yyyy)

RIGHT SIDE				LOWER	LEFT SIDE			
Swelling	Pain on Motion and/or Tenderness	Limitation of Motion	Not Applicable	Joint	Swelling	Pain on Motion and/or Tenderness	Limitation of Motion	Not Applicable
				Hip				
				Knee				
				Ankle				
				Subtalar				
				Tarsi				
				MTP I				
				MTP II				
				MTP III				
				MTP IV				
				MTP V				
				Toes (PIP) I				
				Toes (PIP) II				
				Toes (PIP) III				
				Toes (PIP) IV				
				Toes (PIP) V				
				Sacroiliac				
				Cervical Spine				
				Thoracic Spine				
				Lumbosacral Spine				

Number of active joints* lower: _____
**Active joint is defined as non-boney swelling (joint effusion) or limitation of motion accompanied by warmth, pain on motion or tenderness to palpation.*

_____ _____
Signature of assessor **Date** *(dd/mon/yyyy)*

AJA01 PHYSICIAN JOINT ASSESSMENT UPPER REQUIRED WORKSHEET

Participant ID #: _____ **Assessment Date:** _____ / _____ / _____
(dd/mon/yyyy)

RIGHT SIDE				UPPER	LEFT SIDE			
Swelling	Pain on Motion/ Tenderness	Limitation of Motion	Not Applicable	Joint	Swelling	Pain on Motion/ Tenderness	Limitation of Motion	Not Applicable
				Temp. Mand.				
				Sterno. Clav.				
				Acro. Clav.				
				Shoulder				
				Elbow				
				Wrist				
				MCP I				
				MCP II				
				MCP III				
				MCP IV				
				MCP V				
				IP I				
				PIP II				
				PIP III				
				PIP IV				
				PIP V				
				DIP II				
				DIP III				
				DIP IV				
				DIP V				

Number of active joints* upper: _____

**Active joint is defined as non-boney swelling (joint effusion) or limitation of motion accompanied by warmth, pain on motion or tenderness to palpation.*

_____ _____

Signature of assessor **Date** *(dd/mon/yyyy)*