PROSPECTIVE EVALUATION OF COMPUTED TOMOGRAPHY GUIDED ABLATION OF CARDIAC GANGLIONATED PLEXI IN PATIENTS WITH ATRIAL FIBRILLATION

Principal Investigator *Saman Nazarian MD, PhD*
Department of Medicine, Division of Cardiology, Section for Cardiac Electrophysiology
3400 Spruce Street, Founders 9124
215-615-5219
Saman.nazarian@pennmedicine.upenn.edu

Co-Investigator *Timothy Markman MD*
Department of Medicine, Division of Cardiology, Section for Cardiac Electrophysiology
3400 Civic Center Blvd
267-593-0103
Timothy.markman@pennmedicine.upenn.edu

Co-Investigator *Pasquale Santangeli MD, PhD*
Department of Medicine, Division of Cardiology, Section for Cardiac Electrophysiology
3400 Civic Center Blvd
Pasquale.Santangeli@pennmedicine.upenn.edu

NCT: 04642976
Date: 6/24/21

Table of Contents

Abbreviations

AE	Adverse Event
AF	Atrial Fibrillation
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CT	Computed Tomography
MP	Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GP	Ganglionated Plexus
GWAS	Genome-Wide Association Studies
HFS	High Frequency Stimulation
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption

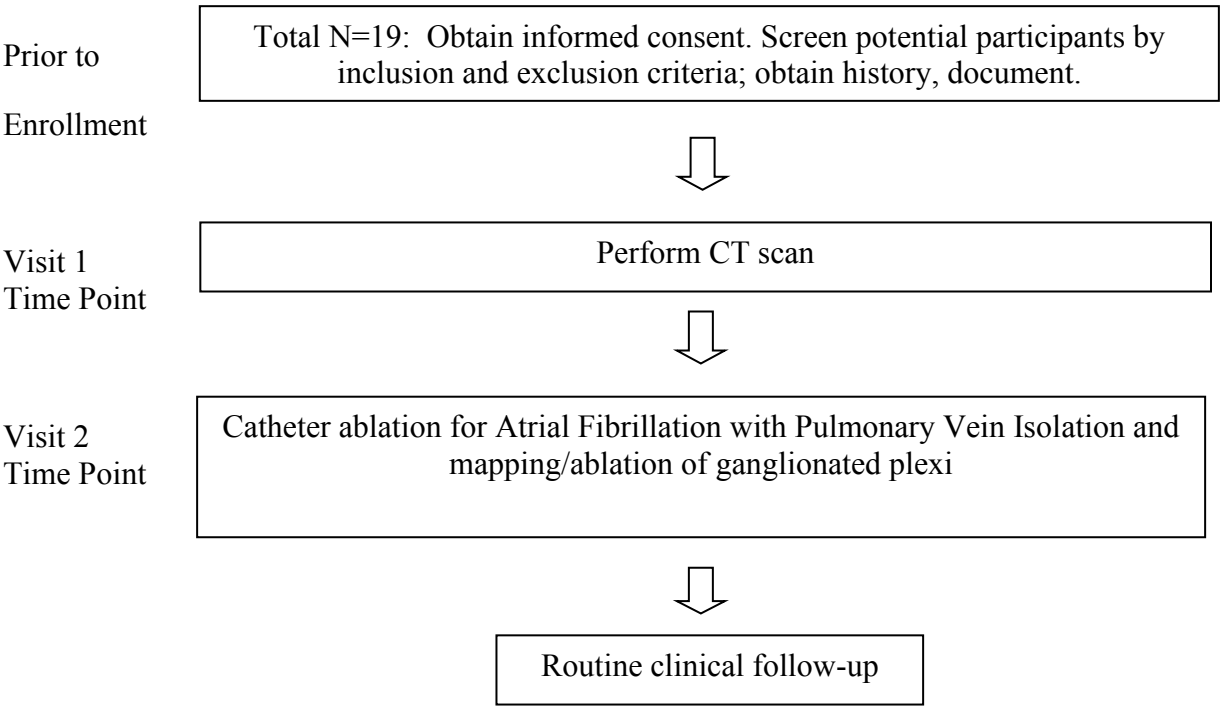
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-Squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
DSMC	Data Safety Monitoring Committee
SoA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UADE	Unanticipated Adverse Device Effect
UP	Unanticipated Problem
US	United States

1 STUDY SUMMARY

1.1 Synopsis

Title:	PROSPECTIVE EVALUATION OF COMPUTED TOMOGRAPHY GUIDED MAPPING AND ABLATION OF CARDIAC GANGLIONATED PLEXI IN PATIENTS WITH ATRIAL FIBRILLATION
Short Title:	CT Guided Ablation of Ganglionated Plexi
Study Description:	Single-arm prospective study to evaluate the role of high frequency stimulation (HFS) and computed tomography (CT) guided mapping and ablation of ganglionated plexi (GP) in patients with atrial fibrillation.
Objectives:	Primary: Secondary: To assess the effects of GP ablation on local HFS response and global vagal stimulation in order to characterize the neural pathways connecting GPs
Primary Endpoint:	The primary outcome of interest will be the successful identification of GP signals by high frequency stimulation and anatomic correlation between these points and CT identified epicardial adipose tissue within 3 pixels (0.625x3=1.875mm).
Secondary Endpoints:	• Elimination of local of HFS response following GP ablation • Elimination of global vagal response to non-invasive ear stimulation • Characterization of amplitude, duration, and fractionation of signals at sites with HFS responses and successful HFS elimination
Study Population:	19 patients with atrial fibrillation undergoing catheter ablation of atrial fibrillation
Description of Sites/Facilities	All ablation procedures will be performed at the Hospital of the University of Pennsylvania.

1.3 Schema


Prior to Enrollment
Total N=19: Obtain informed consent. Screen potential participants by inclusion and exclusion criteria; obtain history, document.
Visit 1 Time Point
Perform CT scan
Visit 2 Time Point
Catheter ablation for Atrial Fibrillation with Pulmonary Vein Isolation and mapping/ablation of ganglionated plexi
Routine clinical follow-up

2 INTRODUCTION AND RATIONALE

The heart has a complex network of autonomic innervation with the majority of input occurring at a series of ganglionated plexi. These ganglionated plexi are found in the epicardial adipose tissue around the base of the heart and interruption of these regions, most commonly through endocardial ablation, has been a potential treatment for multiple cardiac arrhythmias. This approach has become an accepted strategy for treating atrial fibrillation but may also be of value for vasovagal syncope, sinus node dysfunction, and heart block. Identification of the ganglionated plexi is challenging and multiple techniques have been utilized. We anticipate that using standard cardiac computed tomography (CT) to identify epicardial adipose tissue can provide a safe, non-invasive option to locate these regions and guide appropriate intervention. We also anticipate that a consistent network of innervation between the GPs will be identifiable with distinct innervation of the sinus and AV nodes.

2.1 Study Rationale

Despite progress made in treating atrial fibrillation with catheter ablation, outcomes remain suboptimal with pulmonary vein isolation alone. Ablation of ganglionated plexi has been shown to be beneficial in patients with atrial fibrillation although optimal strategies of localization of these regions have yet to be determined. We aim to develop a novel strategy of CT guided ganglionated plexi localization to guide ablation in patients with atrial fibrillation. We also aim to clarify the network of innervation between GP to clarify the optimal ablation strategy.

2.2 Background

Autonomic cardiac innervation arises from the brainstem and travels to the heart via the cervical and thoracic stellate ganglion (sympathetic system) and the vagus nerve (parasympathetic system). The two systems combine to innervate the heart in a dense network of post-ganglionic axons called the ganglionated plexi (Hou Y, et al). These ganglionated plexi are located at the base of the heart in the epicardial adipose tissue (Goudis CA, et al; Armour JA, et al).

With direct visualization of the epicardial adipose tissue at the time of cardiac surgery, autonomic modulation with injection of botulinum toxin has been shown to reduce short and long term burden of atrial fibrillation (Romanov A et al; Waldron et al). Several techniques have been proposed to identify the ganglionated plexi at the time of endocardial procedures. Anatomic approaches have been utilized targeting classical regions of autonomic innervation, although this procedure has several limitations including individual anatomic

variation. In addition, high frequency stimulation mapping to identify local vagal responses has been used (Kim MY, et al; Hu F, et al). This technique is limited by the need for general anesthesia and the duration of time required to map the entire atrium, which is especially relevant for non-atrial fibrillation ablation procedures where such mapping would not otherwise be required. More recently, techniques to evaluate local electrograms have been developed based on the principle that fractionated electrical activity may be noted in the endocardium beneath ganglionated plexi due to the inter-myocardial nerve fibers that interrupt normal myocardial-to-myocardial conduction (Pachon M JC, et al; Lellouche N, et al).

We have recently shown that there is an association between left atrial epicardial adipose tissue identified by CT imaging and electrogram fractionation (Zghaib T, et al). This association is consistent with other descriptions of local electrograms at the site of successful ablation of ganglionated plexi. Given the routine nature of pre-procedure imaging, which is frequently performed prior to atrial fibrillation ablation to clarify relevant anatomy, and the ability to identify this epicardial adipose tissue, we anticipate CT can provide a safe, non-invasive option to locate these regions and guide appropriate intervention. Ablation targeting ganglionated plexi have been used to treat atrial fibrillation as well as vasovagal syncope, sinus node dysfunction, and heart block. A simple and reliable method of identifying ablation target could improve the safety and efficacy of these procedures.

Update to 1/23/24 version: Although tragus stimulation has been frequently utilized, there is evidence to suggest more robust vagus nerve innervation in the region of the cymba concha (Frangos et al 2015; Rong et al 2012; Courties et al 2022). For this reason, electrodes positioned in this area may have more consistent physiologic effects related to vagus nerve stimulation.

2.2.1 Clinical Adverse Event Profile

Potential complications of catheter ablation of atrial fibrillation include bleeding, vascular injury, cardiac perforation, atrioesophageal fistula formation, phrenic nerve injury, bradycardia, arrhythmia induction, myocardial infarction, and stroke. These complications are related to the insertion of catheters from the femoral veins into the heart and across the intra-atrial septum to the left atrium as well as the delivery of radiofrequency energy in the left atrium. We do not anticipate the mapping portion of the procedure, which will be performed with a standard ablation catheter, to confer significant additional risk. The most likely increased risk from high frequency stimulation is the induction of atrial fibrillation, which is common as part of routine ablation procedures and does not pose significant risk. We do not anticipate these risks to be significant modified by the additional ablation targeting ganglionated plexi, which are located in regions frequently targeted in ablation of atrial fibrillation and atypical atrial flutters.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

As discussed above, potential risks of ablation of ganglionated plexi include the risk factors associated with catheter ablation for atrial fibrillation as discussed above. An additional theoretical risk includes increased sinus rates following vagal denervation, which has been described as transient in the literature and not seen in our own experience. Potential risks of ear stimulation include local discomfort or skin irritation/burns. Potential risks of high frequency stimulation include pain, which will not be perceived given the use of general anesthesia, and the induction of atrial fibrillation, which routinely occurs during atrial fibrillation ablations.

2.3.2 Known Potential Benefits

Potential benefits include the reduction in burden of atrial fibrillation by eliminating vagal triggers for atrial fibrillation. In addition, societal benefits include the development of procedural techniques that may improve treatment of atrial fibrillation, vasovagal syncope, and other conditions.

2.3.3 Assessment of Potential Risks and Benefits

The risks of participating in the study are not significant beyond the risks associated with routine clinical care (including pre-procedural CT imaging, atrial mapping and ablation). Therefore, the potential benefits to the individual subject and to society outweigh the risks of participating in the study.

3 STUDY OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
	• Successful identification of GP signals by high frequency stimulation and anatomic correlation between these points and CT identified epicardial adipose tissue within 3 pixels (0.625x3=1.875mm).	This correlation with allow us to determine the ability of pre-procedural CT imaging to predict GP location.
Secondary		
To assess the effects of GP ablation on local HFS response and global vagal stimulation in order to characterize the neural pathways connecting GPs	• Elimination of local of HFS response following GP ablation • Elimination of global vagal response to non-invasive ear stimulation	
	• Amplitude, duration, and fractionation of signals at sites with HFS responses and successful HFS elimination	

4 STUDY PLAN

4.1 Study Design

This is an observational, single-center study evaluating feasibility and efficacy of CT and high frequency stimulation guided GP mapping and ablation in patients with AF undergoing catheter ablation with pulmonary vein isolation.

4.2 Scientific Rationale for Study Design

This study is designed to characterize the association between CT identified epicardial adipose tissue and HFS mapping identified GP sites in the right and left atrium. Given the evidence that GP ablation can be beneficial for patients undergoing catheter ablation for atrial fibrillation, all patients will undergo GP ablation following mapping in addition to standard pulmonary vein isolation. The hierarchical relationship within the network of cardiac GPs is unknown as is the optimal order of GP ablation. Given equipoise in the order of GP ablation, we will equally allocate the site of initial ablation between the 8 major GP ablation locations.

4.3 End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit.

STUDY POPULATION

4.4 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:
1. Provision of signed and dated informed consent form
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Male or female, aged >18 years
4. History of AF with plan to undergo catheter ablation

4.5 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:
1. Contraindication or unwillingness to undergo CT imaging or catheter ablation.
2. Pregnancy or lactation

4.6 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

4.7 Strategies for Recruitment and Retention

Participants will be recruited from the investigator's clinical practice. We anticipate an accrual rate of approximately 4 subjects per month. Long term follow-up for the study will include the standard of care monitoring of patients following catheter ablation.

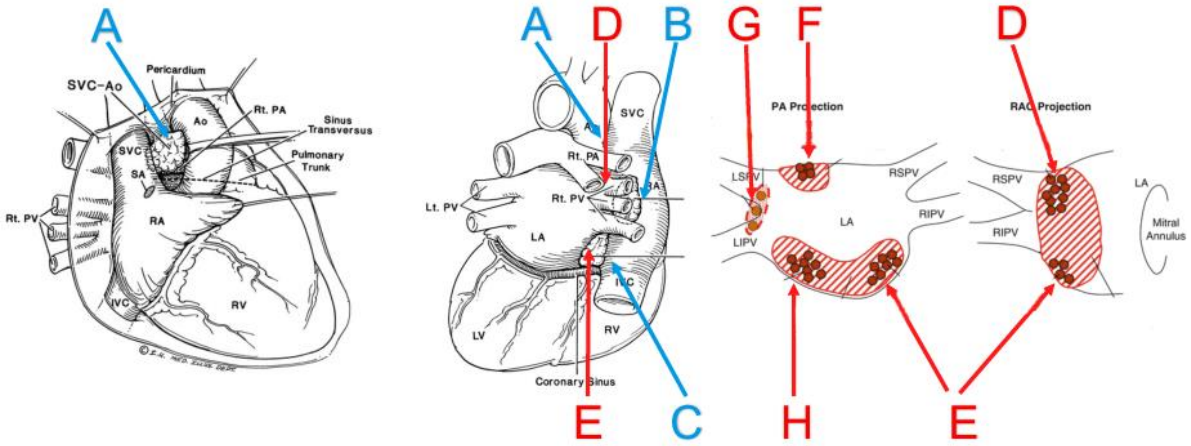
STUDY INTERVENTION

4.8 Study Intervention(s) Administration

4.8.1 Study Intervention Description

All patients will have GP mapping performed using the Nicolet (Natus) Cortical Stimulator (see attached) with 12V output at 20Hz frequency with 10ms pulse duration with at least 3grams of contact force. Stimulation will be provided for up to 10 seconds or until vagal response is noted (>2s ventricular asystole, AV block, or R-R interval increase by at least 50%). Natus stimulator had FDA clearance (K072964) for cortical stimulation. Prior to ablation, baseline ear vagus nerve stimulation will be performed via stimulation of bilateral cymba concha to document response using Parasym tragal stimulator (IDE information attached). Ablation of mapped GPs will be performed using standard ablation catheter with routine approaches to ablation energy delivery and monitoring (see below flowchart). Following ablation of each GP, local HFS will be repeated in all GP regions and ear vagus nerve stimulation will be repeated via bilateral cymba concha. After all GP are ablated, all patients be given IV atropine (0.04mg/kg max: 4mg) to assess global vagal tone. Following this, patients will undergo pulmonary vein isolation using standard equipment and techniques.

Devices will be obtained from the manufacturer (Natus and Parasym respectively) and will be stored in a secured laboratory space. No particular temperature or climate requirements are present for either device. All devices will be labeled according to the manufacturer guidance and will be returned to the manufacturer in the event of any device failure. Devices will be stored and managed in compliance with OCR guidelines.



4.9 Study Intervention Compliance

Following the ablation procedure, compliance will only be necessary for follow-up visits, which will be performed per clinical routine.

5 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

5.1 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant non-compliance with follow-up visits
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form and are randomized but do not undergo ablation may be replaced. Subjects who sign the informed consent form and are randomized and undergo ablation, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.]

5.2 Lost To Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for two scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 3 months and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

6 STUDY ASSESSMENT AND PROCEDURES

6.1 Efficacy Assessments

- Elimination of local HFS response and ear vagus nerve stimulation will determine acute procedural efficacy
- Loop recorder or ambulatory monitors interrogations to evaluate presence and burden of atrial fibrillation
- History to identify symptomatic atrial fibrillation and to identify episodes of AF that may have been vagally mediated

6.2 Safety and Other Assessments

- Physical examination will be performed following the ablation procedure to identify any procedural complications including vascular or neurological injury.
- Repeat examination and history will be performed at follow-up visit to identify any potential procedural complications.

6.3 Adverse Events and Serious Adverse Events

6.3.1 Definition of Adverse Events (AE)

An adverse event (AE) is any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention related. Intercurrent illnesses or injuries should be regarded as adverse events.

A pre-existing condition should be recorded as an adverse event if the frequency, intensity or the character of the condition changes.

6.3.2 Definition of Serious Adverse Events (SAE)

Serious Adverse Events (SAE)

Adverse events are classified as serious or non-serious. A serious adverse event is any AE that, in the view of either the investigator or the sponsor, is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event when the event does not fit the other outcomes, but the event may jeopardize the patient and may require medical or surgical intervention (treatment) to prevent one of the other outcomes.
- required intervention to prevent permanent impairment or damage (for devices only)

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

6.3.3 Classification of an Adverse Event

6.3.3.1 Severity of Event

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.

- Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

- Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

6.3.3.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to procedure assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be considered.

- Related – The AE is known to occur with the GP mapping or ablation, there is a reasonable possibility that the procedure caused the AE, or there is a temporal relationship between the procedure and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the procedure and the AE.

- Not Related – There is not a reasonable possibility that the administration of the procedure caused the event, there is no temporal relationship between the procedure and event onset, or an alternate etiology has been established.

6.3.3.3 Expectedness

The primary investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the procedure.

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

Safety will be assessed by monitoring and recording potential adverse effects using the CTCAE grading system at each study visit. Participants will be monitored by medical histories, physical examinations, and loop recorder interrogations. If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, life-threatening, and death, corresponding to Grades 1-5, will be used whenever possible.

At each contact with the subject, the investigator will seek information on adverse events by non-directive questioning and, as appropriate, by examination. Adverse events may also be detected when they are volunteered by the subject during the screening process or between visits, or through physical examination, laboratory test, or other assessments. Information on all adverse events will be recorded in the source documentation. To the extent possible, adverse events will be recorded as a diagnosis and symptoms used to make the diagnosis recorded within the diagnosis event.

As much as possible, each adverse event or follow-up information will be evaluated to determine:

1. Severity grade (CTCAE Grade 1-5)

2. Duration (start and end dates)

3. Relationship to the study treatment or process – [Reasonable possibility that AE is related: No (unrelated/ not suspected) or Yes (a suspected adverse reaction)]. If yes (suspected) - is the event possibly, probably or definitely related to the investigational treatment?

4. Expectedness to study treatment or process – [Unexpected – if the event severity and/or frequency is not described in the investigator brochure (if applicable) or protocol].

5. Action taken with respect to study or investigational treatment or process (none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable)

6. Whether medication or therapy taken (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)

7. Whether the event is serious

Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome.

6.3.5 Adverse Event Reporting

Reporting Period

Adverse events will be reported from the time of informed consent until study completion.

Investigator Reporting: Notifying the Study Sponsor

Every SAE, regardless of suspected causality (e.g., relationship to study product(s) or study procedure(s) or disease progression) must be reported to the sponsor within **24 hours** of learning of its occurrence.

Recurrent episodes, complications, or progression of the initial SAE must be reported to the Sponsor as a follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. A SAE considered completely unrelated to a previously reported one should be reported separately as a new event.

New information regarding the SAE will be reported as it becomes available and in the same manner that the initial SAE (i.e. SAE form). The investigator must follow the event to resolution or until the event is deemed and documented irreversible, whichever is longer.

Investigator Reporting: Local Reporting Requirements

The investigator will report AEs and SAEs to the IRB/EC of record and other local regulatory groups per the local requirements.

6.3.6 Serious Adverse Event Reporting

The study investigator shall complete an Unanticipated Adverse Device Effect Form and submit to the study sponsor and to the reviewing Institutional Review Board (IRB) . The study sponsor is responsible for conducting an evaluation of an unanticipated adverse device effect and shall report the results of such evaluation to the Food and Drug Administration (FDA) and to all reviewing IRBs and participating investigators per the applicable regulation.

6.4 Unanticipated Problems

6.4.1 Definition of Unanticipated Problems (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

6.4.2 Unanticipated Problem Reporting

Unanticipated problems (UPs) such as:

- Post-marketing withdrawal of a drug, device, or biologic used in a research protocol due to safety concerns.
- FDA ban of a drug, device, or biologic used in a research protocol due to safety concerns.
- Complaint of a participant when the complaint indicates unexpected risks, or the complaint cannot be resolved by the research team
- Breach of confidentiality
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study
- Premature closure of a study (e.g., due safety, lack of efficacy, feasibility, financial reasons, etc.)

should be reported by the investigator to the Institutional Review Board (IRB). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported as any other SAE.
- Any other UP will be reported to the IRB 10 business days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 10 business days of the IRB's receipt of the report of the problem from the investigator.

7 STATISTICAL CONSIDERATIONS

7.1 Statistical Hypotheses

- Primary Efficacy Endpoint(s): We anticipate anatomic correlation between CT identified epicardial adipose tissue within 3 pixels (0.625x3=1.875mm) of an endocardial mapping point where HFS confirmed a vagal response consistent with ganglionated plexus innervation.

- Secondary Efficacy Endpoint(s): Acute procedural success defined by elimination of ear vagus nerve stimulation response will be achievable in all patients.

7.2 Statistical Analyses

7.2.1 General Approach

- For descriptive statistics, categorical data will be presented as percentages and continuous data will be presented as medians with intraquartile ranges.

- All statistical tests will be 2-sided, with $p<0.05$ indicating statistical significance.

7.2.2 Analysis of the Primary Efficacy Endpoint(s)

- Anatomic correlation between CT and GP mapping will be defined based on review of electroanatomic maps from the ablation procedure merged with segmented pre-procedural CT images.

7.2.3 Analysis of the Secondary Endpoint(s)

- AF burden will be defined based on loop recorder interrogation and patient history of requiring cardioversion

7.2.4 Safety Analyses

A summary statistic for all adverse events will be reported. All adverse events will be presented based on severity, frequency, and relationship to study intervention. Adverse events leading to premature discontinuation from the study intervention and serious treatment-emergent AEs will be separately listed.

7.2.5 Baseline Descriptive Statistics

Patients will be compared based on baseline characteristics, including demographics and laboratory measurements, using descriptive statistics including Chi-squared tests for categorical variables, and analysis of variance for continuous variables. All statistical tests will be 2-sided, with $p<0.5$ indicating statistical significance.

7.2.6 Sub-Group Analyses

Primary and secondary endpoints will be analyzed based on age, sex, race/ethnicity or other demographic characteristic(s).

7.2.7 Tabulation of Individual Participant Data

Individual participant data will not be listed by measure and time point.

8 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

8.1 Regulatory, Ethical, and Study Oversight Considerations

8.1.1 Informed Consent Process

8.1.1.1 Consent/Assent and Other Informational Documents Provided To Participants

Consent forms describing in detail the GP mapping and ablation study procedures, and risks are given to the participant and written documentation of informed consent is required prior to enrollment. The following consent materials are submitted with this protocol: Consent and HIPAA authorization form.

8.1.1.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

8.1.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated by the Sponsor or the PI at any site if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

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

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

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

In terminating the study, the Sponsor and the Principal Investigator will assure that adequate consideration is given to the protection of the subjects' interests.

8.1.3 Confidentiality and Privacy

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

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

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

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored in our Data Coordinating Center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived.

8.1.4 Future Use of Stored Specimens and Data

Data collected for this study will be analyzed and stored in an encrypted, password protected file After the study is completed, the de-identified, archived data will be transmitted to and stored in an encrypted, password protected file for use by other researchers including those outside of the study.

8.1.5 Safety Oversight

Safety oversight will be under the direction of a primary investigator and co-investigators, who will review safety data for each patient following enrollment and randomization.

8.1.6 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s). The primary investigator and co-investigator will be responsible for clinical site monitoring.

8.1.7 Quality Assurance and Quality Control

All monitoring and audits are to be performed according to ICH GCP E6(R2).

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

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

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated, and specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

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

8.1.8 Data Handling and Record Keeping

8.1.8.1 Data Collection and Management Responsibilities

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

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into our data collection system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

8.1.8.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the trial. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

8.1.9 Protocol Deviations

The PI and the study team should document all scenarios where the protocol is not followed and provide, in particular:

- Who deviated from the protocol
- What was the deviation
- When did the deviation occur
- How did the deviation happen
- What is the impact of the deviation
- A root cause analysis of why the deviation occurred

If the assessment results in a determination that any of the following are potentially affected, the deviation would be considered of significant impact:

- having the potential to adversely affect subject safety; OR
- increases risks to participants; OR
- adversely affects the integrity of the data; OR
- violates the rights and welfare of participants, OR
- affects the subject's willingness to participate in research.
- there is a potential for an overall impact on the research that should be shared with the IRB for consideration and development of next best steps to address it

8.1.10 Publication and Data Sharing Policy

This study will comply with the data sharing agreement.

8.1.11 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial.

8.2 Additional Considerations

Not-applicable.

8.3 Protocol Amendment History

Version	Date	Description of Change	Brief Rationale

Version	Date	Description of Change	Brief Rationale

9 REFERENCES

Pachon JC et al. Cardioneuroablation: Catheter Vagal Denervation as a New Therapy for Cardioinhibitory Syncope. J Cardiac Arrhythmia. 2019:32(3)182-196

Armour JA et al. Gross and Microscopic Anatomy of the Human Intrinsic Cardiac Nervous System. The Anatomical Record 1997;247:289-298

Nakagawa H, Scherlag BJ, Patterson E, Ikeda A, Lockwood D, Jackman WM. Pathophysiologic basis of autonomic ganglionated plexus ablation in patients with atrial fibrillation. Heart Rhythm. 2009:6(12):S26-34.

Drissen AHG, Berger WR, Krul SPJ, et al. Ganglionated plexus ablation in advanced atrial fibrillation: The AFACT Stidy. J Am Coll Cardiol. 2016;68(11):1155-1165

Brignole M, Deharo JC, Menozzi C, Moya A, Sutton R, Tomaino M, Ungar A. The benefit of pacemaker therapy in patients with neurally mediated syncope and documented asystole: a meta-analysis of implantable loop recorder studies. Europace. 2018;20(8):1362.

Romanov A, Pokushalov E, et al. Long-term supression of atrial fibrillation by botulinum toxin injection into epicardial fat pads in patients undergoing cariac surgery: three year follow up of a randomized study, Heart Rhythm 2019;16(2):172-177

Herring N, Kalla M, Paterson DJ. The Autonomic nervous system and cardiac arrhythmias: current concepts and emerging therapies. Nature Reviews Cardiology. 2019(16):707-726

Zghaib T, et al. Association of left atrial epicardial adipose tissue with electrogram bipolar voltage and fractionation: Electrophysiologic substrates for atrial fibrillation. Heart Rhythm. 2016 (8) 232-239

Frangos, Et, Ellrich, J, et al. Non-invasive Access to the Vagus Nerve Central Projections via Electrical Stimulation of the External Ear. Brain Stimul. 2015 (8)3:624-36

Rong P, Fang JL, et al. Transcutaneous vagus nerve stimulation for the treatment of depression: a study protocol for a double blinded randomized clinical trial. BMC Complement Altern Med. 2012 (12)255

Courties A; Deprouw C, et al. Effects of Transcutanous Vagaus Nerve Stimulation in Erosive Hand Osteoarthritis. J Clin Med. 2022(11)4

10 APPENDIX

END OF DOCUMENT