Utility of Esophageal Cooling Therapy for the Prevention of Thermal Injury During Atrial Fibrillation Ablation:
an investigator initiated, randomized, blinded, single center pilot study

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List of Abbreviations

AE: Adverse event

AEF: Atrio-esophageal fistula

AF: Atrial Fibrillation

EGD: Oesophagogastroduodenoscopy

EMR: Electronic Medical Record

LET: luminal esophageal temperature

RF: Radiofrequency

SAE: Serious adverse event

SOC: Standard of care

UP: Unanticipated Problem

Study Summary

Title	Utility of Esophageal Cooling Therapy for the Prevention of Thermal Injury During Atrial Fibrillation Ablation
Short Title	eCoolAF
IRB Number	*831401*
Phase	Pilot Study
Methodology	Randomized, blinded single center pilot study
Study Duration	Approximately 2- 3 years
Study Center	University of Pennsylvania

Objectives	Primary:
	• To determine the occurrence rate of esophageal thermal injury between groups
	• To determine the severity and size of esophageal injury between groups
	Secondary:
	• Obtain posterior wall ablation parameters:
	  ○ Initial temperature
	  ○ Max temperature
	  ○ Impedance drop
	• Determine the occurrence and location of acute PV reconnection
	• Determine freedom from AF at 3 months, 6 months, and 12 months

Number of Participants	About 70 enrolled to allow for 60 completed subjects
Length of Participation	Up to 3 days intervention phase; 1 month follow up, 1 year data collection
Inclusion Criteria	1. Patients above the age of 18 years old. 2. Patients with the diagnosis of atrial fibrillation undergoing clinically indicated de-novo AF ablation procedure. 3. Patients must be able to understand and critically review the informed consent form.

Exclusion Criteria	1. Patients whom are unable to provide informed consent. 2. Patients with contraindication to EGD. 3. History of prior AF ablation procedures. 4. Significant co-morbidities that preclude standard ablation procedure 5. Patients with <40 kg of body mass
Investigational Device	Attune Medical Esophageal Heat Transfer Device- EnsoETM Used during standard of care AF ablation
Duration of administration	During AF ablation (7-8 hours)
Reference therapy	EnsoETM will replace standard of care temperature probe
Statistical Methodology	Based on prior studies, esophageal injury following ablation is anticipated to be approximately 35% in the control group. A total of 30 patients for each group will be required to yield the necessary pilot data to make an appropriate conclusion regarding the effect of esophageal thermal cooling on prevention of esophageal thermal injury.
Safety Evaluations	All patients will undergo oesophagogastroduodenoscopy (EGD) 0-2 days following the ablation procedure.
Data and Safety Monitoring Plan	The PI and research team will be responsible for data and safety monitoring.

This study will be conducted in full accordance all applicable University of Pennsylvania Research Policies and Procedures and all applicable Federal and state laws and regulations.

1.0 Background and Study Rationale

1.1 Introduction

Radiofrequency (RF) catheter ablation of atrial fibrillation (AF) has become a common ablation procedure performed worldwide. The cornerstone of this procedure is pulmonary vein isolation (PVI). Energy delivery may extend beyond the atrial myocardium and result in damage to adjacent structures, including the esophagus. Atrio-esophageal fistula (AEF) is a rare, but a well-recognized complication of percutaneous AF ablation with an estimated incidence of 0.02–0.11%[1]. Although the pathophysiology is not entirely understood, it is clear that thermal injury to the esophagus during ablation of the posterior left atrial wall plays a crucial role in triggering a cascade of events that eventually results in the development of AEF. Common clinical strategies to prevent esophageal injury include delivery of lower ablation energy along the posterior left atrium as well as the use of a luminal esophageal temperature (LET) probe aimed to alert and guide energy delivery. Nonetheless, esophageal injury is still common, and AEF continues to be reported despite adherence to these guidelines.

1.2 Background and Relevant Literature

Due to the low occurrence rate of AEF, prior studies aimed to develop strategies to prevent it have used the occurrence of esophageal injury as assessed with endoscopy as a surrogate endpoint. The occurrence rate of esophageal injury has varied depending on the reporting center, timing of endoscopy, and the ablation technique utilized. Esophageal ulceration is likely the initial injury that leads to AEF formation and is probably present within hours to days of the ablation procedure. Deneke et al. have assessed the outcomes of >800 patients who underwent first-time catheter ablation of AF followed by EGD within 4 days of the procedure. They found that 82% of patients had EGD without esophageal lesions and no subsequent esophageal events. Of the patients with esophageal abnormalities, two-thirds had erythema with no further complications. The 5 documented esophageal perforations occurred in patients with esophageal ulceration (ratio of esophageal perforation to ulcer, 1:10)[2]. In a study performed by one of the study investigators at a previous institution that utilizes a similar technique as Penn, Tschabrunn, et al. found evidence of esophageal thermal injury in 9/20 (45%) on EGD the day after the ablation procedure[3].

1.3 Name and Description of the Investigational Product

The EnsoETM is an FDA cleared device. The EnsoETM is a non-sterile multi-lumen silicone tube placed in the esophagus for the purpose of cooling or warming a patient while simultaneously allowing gastric decompression and drainage. Modulation and control of the patient's temperature is achieved by connecting the EnsoETM to an external heat exchanger (Medi-Therm Gaymar or similar device). Two lumens connect to the external heat exchanger, while a third central lumen provides stomach access for connection to a fluid collection device with low intermittent suction for gastric decompression. Distilled water circulates within the EnsoETM just like a water blanket. The EnsoETM is made of standard medical-grade silicone. The versatile device can be placed by most providers in the operating room, emergency room, or intensive care unit. Providers placing devices will be appropriately trained. The EnsoETM is a single-use, disposable, non-implantable device with an intended duration of use of 72 hours or less. For this study, the device will be used during cardiac ablation procedures for the intended indication of patient temperature management using approved settings/parameters as detailed in the product instructions for use document.

1.3.1 Nonclinical Data

Montoya et al. found in pre-clinical investigation that internal esophageal cooling provided significant protective effect from thermal injury, with transmurality of lesions decreasing as circulating water temperature decreased[4].An in-vivo animal study using a different esophageal cooling balloon device has been performed. Using an animal model, Arruda, et al. demonstrated that the use of a compliant balloon device that circulates cold fluid between 5-10°C can prevent collateral thermal injury to the esophagus[5]. In this study, the authors demonstrate that they were able to safely perform esophageal cooling to prevent esophageal thermal injury without adversely affecting the surrounding structures or the biophysical parameters of the radiofrequency ablation lesions[5].

1.3.2 Clinical Data to Date

One clinical observational study has indicated that esophageal cooling can reduce thermal injury during AF ablation, but this strategy is not routinely used in clinical practice, in part due to the absence of randomized clinical trial data to support its use. Sohara, et al. reported in an observational study that included 318 consecutive patients that esophageal cooling can decrease the incidence and severity of esophageal thermal injury[6]. Leung et al. performed a meta-analysis of 3 clinical studies and found that esophageal cooling reduces the severity of lesions resulting from RF ablation, with an odds ratio of 0.39 (95% CI 0.17 to 0.89)[7]. Based on these animal and clinical prior studies, it is our hypothesis that the use of the EnsoETM device may reduce the occurrence and/or severity of esophageal thermal injury as it is a compliant balloon technology that can reach a cooling temperature of 4°C. In addition, it is FDA cleared and has been used safely in thousands of patients for temperature control making it the ideal device to use for this study.

2 Study Objectives

The purpose of the proposed pilot study is to determine if esophageal cooling using the Attune Medical Esophageal Heat Transfer Device (EnsoETM) limits the frequency or severity of thermal injury during catheter ablation of atrial fibrillation.

2.1 Primary Objectives

1. To determine the occurrence rate of esophageal thermal injury between groups
2. To determine the severity and size of esophageal injury between groups

2.2 Secondary Objectives

1. Evaluation of posterior wall ablation parameters:
    - Initial temperature
    - Max temperature
    - Impedance drop
2. Determine the occurrence and location of any acute PV reconnection between groups
3. Determine freedom from AF at 3 months, 6 months, and 12 months

3 Investigational Plan

3.1 General Design

This is a small scale pilot study to evaluate if esophageal cooling using the Attune Medical Esophageal Heat Transfer Device (EnsoETM) limits the frequency or severity of thermal injury during catheter ablation of atrial fibrillation. This prospective, randomized study will include 70 patients to allow for 60 completed patients with symptomatic AF undergoing index PVI at the Hospital of the University of Pennsylvania. Patients will be randomized in a 1:1 fashion with 30 patients (Group A) randomized to undergo the ablation procedure with esophageal cooling and the other 30 patients (Group B) will serve as the control group and will not have the EnsoETM device used. Patients randomized to Group A will have the EnsoETM device placed in the electrophysiology laboratory following intubation and prior to the ablation procedure. All patients will undergo oesophagogastroduodenoscopy (EGD) 0-2 days following the ablation procedure.

3.1.1 Screening Phase

Subjects will be recruited from the University of Pennsylvania's Electrophysiology department. Patients will first be pre-screened using the procedure schedule to identify those that are undergoing an initial AF ablation procedure and therefore may be potentially eligible for enrollment. Patients who have met guideline recommendations for first-time AF ablation procedure will be approached by an EP physician (co-investigators) or a member of the research team prior to the AF ablation procedure. All potentially eligible patients will first have signed an informed consent for the clinical procedure by the appropriate clinical staff. We will follow applicable University of Pennsylvania regulations and Pennsylvania law pertaining to consent process and obtaining signatures. Written informed consent

for the research study (use of EnsoETM + EGDs) will be obtained the morning of the clinical procedure prior to any research procedures taking place.

3.1.2 Study Intervention Phase

Participants who have signed the research study consent form will be randomized in a 1:1 fashion to the study device (EnsoETM) or to standard of care (standard temperature probe monitoring). Insertion of the study device will be in place of the standard temperature probe and will not add any additional time to the standard AF ablation procedure. The device will be used as indicated (for cooling). Temperature measures will be collected for both the device and standard of care (SOC) arms during the clinical ablation procedure.

Ablation Procedure (Day 0)
All procedures will be performed using Penn's standard approach under general anesthesia with high-frequency JET ventilation. Using a double transseptal approach, wide circumferential isolation of the pulmonary veins will be performed with a point-by-point ablation approach. No additional left atrial ablation lines will be placed unless clinically indicated. All procedures will be performed with the use of CARTO 3 (Biosense Webster, Inc.) electroanatomical mapping system and open-irrigated catheters (ST-SF Thermocool). Ablation in the anterior and septal aspects of the pulmonary veins will be performed using a power of up to 40W for a duration of up to 40s unless, under the discretion of the attending physician, it is clinically necessary to use a higher index. Ablation on the posterior wall will be performed with a power of up to 30W for up to 20s. Lesions will be delivered using a minimum target contact force of 10g. Successful PVI will be defined as achieving entrance and exit block of each PV with assessment for acute reconnection using adenosine and/or isoproterenol. Following PV isolation, high-dose isoproterenol challenge will be performed to guide additional extra-PV ablation. Patients randomized to the device arm (Group A) will undergo posterior wall ablation when the EnsoETM device has reached a temperature of 4-6°C for at least 10 minutes. Temperatures on the device will then be adjusted to return to nominal approximately 20 minutes(at least) after completion of the posterior wall ablation.

Endoscopic Evaluation (Days 0-2)
All patients will undergo EGD 0-2 days after the ablation procedure. Studies will be interpreted for research purposes by an experienced gastroenterologist who is blinded to the patient's study group. Esophageal injury will be attributed to ablation if located on the anterior wall of the mid-esophagus (20 to 30 cm from the incisors). EGD findings of thermal injury will be classified into four injury grades: (1) erythema – mild injury; (2) superficial ulceration – moderate injury; (3) deep ulceration – significant injury; and (4) fistula/perforation. Clinical interpretation of the EGD study will still be performed and reported in PennChart by a Penn gastroenterologist according to standard clinical guidelines.

3.1.3 *Follow Up Visit (Day 30 -14/+30 days)*

Follow up data will be collected at approximately 1 month following the ablation procedure. This is a standard of care follow up visits that typically occurs following an atrial fibrillation ablation procedure. The standard of care procedures that will be done at these visits include:

- Physical Exam
- Interim History and concomitant medication use
- Vital signs

In addition to the standard of care procedures, the research team will also collect the following:

- Adverse event information
- Atrial fibrillation recurrence

If a participant does not attend their routine 1 month clinical follow up, the research staff will attempt to contact the participant by phone to review their interim history, medication use and collect adverse event and atrial fibrillation recurrence information.

3.1.4 *Follow Up Data Collection Phase*

The standard of care follow up visits that typically occur following an atrial fibrillation ablation procedure will be documented by the clinical care team in the participant's electronic medical record (EMR) and will be reviewed by research staff for follow up data collection. This will occur at approximately 3 months, 6 months and 12 months. The research team will collect the following data if available:

- Physical Exam

- Interim History and concomitant medication use
- Vital signs
- Atrial fibrillation recurrence

If a participant does not attend their clinical follow up visits, the research staff will attempt to contact the participant by phone to review their interim history, medication use and collect atrial fibrillation recurrence information.

3.1.5 Allocation to Interventional Group

The randomization algorithm will be built into the electronic data capture system, Redcap, which will be used to collect data in the study. Once the randomization form is entered into the system and saved, the back end algorithm will run and a participant will be assigned an arm corresponding to either study device (Group A – Esophageal Cooling) or standard of care (Group B – Control). Patients will undergo randomization after being enrolled into the study and prior to the start of the clinically indicated AF ablation procedure. Randomization will be performed in a 1:1 fashion and maintained on the limited access, encrypted, Redcap database.

3.2 Study Endpoints

The purpose of the proposed pilot study is to determine if esophageal cooling using the Attune Medical Esophageal Heat Transfer Device (EnsoETM) limits the frequency or severity of thermal injury during catheter ablation of atrial fibrillation.

3.2.1 Primary Study Endpoints

The primary endpoints of this study will be:

1. Occurrence rate of esophageal thermal injury between groups
2. Severity (Grade 1-4) and number of esophageal thermal injuries

3.2.2 Secondary Study Endpoints

1. Evaluation of posterior wall ablation parameters:
   - Number of posterior wall ablations
   - Total posterior wall ablation time
   - Biophysical parameters (power, time, temperature, impedance changes, contact force)
2. Occurrence and location of any acute PV reconnection
3. Freedom from AF at 3 months, 6 months, and 12 months

3.2.3 Primary Safety Endpoints

All patients will undergo EGD following the procedure to evaluate the primary endpoint of esophageal thermal injury. We will also collect adverse events for all participants for 1 month following the ablation procedure.

4 Study Population and Duration of Participation

All patients will have met guideline recommendations for the AF ablation procedure and will have signed an informed consent for the clinical procedure.

4.1 Inclusion Criteria

1. Patients above the age of 18 years old.
2. Patients with the diagnosis of atrial fibrillation undergoing clinically indicated de-novo AF ablation procedure.
3. Patients must be able to understand and critically review the informed consent form.

4.2 Exclusion Criteria

1. Patients whom are unable to provide informed consent.
2. Patients with contraindication to EGD.
3. History of prior AF ablation procedures.

4. Significant co-morbidities that preclude standard ablation procedure.
5. Patient is ineligible for EnsoETM placement due to:
    - Known esophageal deformity or evidence of esophageal trauma (for example history of esophagectomy, previous swallowing disorders, achalasia).
    - Known ingestion of acidic or caustic poisons within the prior 24 hours.
    - Patients with <40 kg of body mass.

4.3 Subject Recruitment

Subjects will be recruited for the study from investigator or sub-investigator clinical practices who have a diagnosis of atrial fibrillation and will undergo a clinically indicated de-novo AF ablation procedure at the Hospital of the University of Pennsylvania. We may develop recruitment materials to be placed in clinic rooms and patient waiting rooms. All recruitment materials, which will be seen by potential participants, will be approved by the IRB.

4.4 Duration of Study Participation

Participants will be involved for approximately 13 months including screening and follow-up. After consent, their active participation in the intervention phase will last up to 3 days to complete the ablation procedure and EGD testing. The follow up for safety evaluation will occur approximately 1-month post-ablation during the routine clinical follow up visit. Additional data will be collected via chart review for 1-year post-ablation from routine clinical follow up care post ablation which occurs at approximately 3, 6 and 12 months.

4.5 Total Number of Subjects and Sites

Recruitment will end when approximately 60 participants are randomized. It is expected that approximately 70 subjects will be consented in order to produce 60 randomized & evaluable subjects.

5 Study Intervention

5.1 Description

The EnsoETM is a non-sterile multi-lumen silicone tube placed in the esophagus for the purpose of cooling or warming a patient while simultaneously allowing gastric decompression and drainage. Modulation and control of the patient's temperature is achieved by connecting the EnsoETM to an external heat exchanger. Two lumens connect to the external heat exchanger, while a third central lumen provides stomach access for connection to a fluid collection device with low intermittent suction for gastric decompression. The EnsoETM is made of standard medical-grade silicone. It is a single-use, disposable, non-implantable device with an intended duration of use of 72 hours or less. Distilled water circulates within the EnsoETM just like a water blanket.

5.2 Intervention Regimen

Patients who are randomized to the EnsoETM device will have the device placed in the electrophysiology laboratory following intubation and prior to the ablation procedure. The device will remain in place until the ablation procedure is completed. These patients will undergo posterior wall ablation when the EnsoETM device has reached a temperature of 4-6°C for at least 2 minutes. The device will be set to nominal temperature (no cooling) during other aspects of the procedure.

5.3 Storage

The EnsoETM device should be stored in a dry and clean place in the EP laboratory storage closet. This device is for single use only. Reuse may expose patient to infection risks. Investigators should not use the device if the packaging is compromised.

5.4 Preparation and Packaging & Supply

The EnsoETM device will be packaged and supplied by Attune Medical.

5.5 Blinding

Due to the nature of this study, the physicians will not be blinded to the randomization assignment, however participants will be blinded. Once a subject is randomized, the research team will receive the randomization assignment (EnsoETM or SOC) and proceed with the procedures per the assignment. In addition, the gastroenterologist interpreting the EGD study for the primary endpoint assessment will be blinded to the participants' randomization.

5.6 Device Accountability

Device accountability logs will be kept on file, will be completed on a regular basis, and will include product number, date of use, participant ID code, device damaged/destroyed and date of damage/destruction (including return date to Attune).

6 Schedule of Study Visits, Procedures, and Data Extraction

Study Phase	**Randomization/ Intervention Phase**		**Follow up Visit**
Visit Number	**1**	**2**	**3**
Study Days	**Day 0**	**Day 0-2**	**Day 30 -14/+30 days**
Research & Clinical Procedures			
Informed Consent	X		
Review Inclusion/Exclusion Criteria	X		
Randomization	X		
Ablation procedure (with temperature monitoring or EnsoETM device)	X		
Overnight monitoring post ablation	X		
EGD (Esophagogastroduodenoscopy)		X	
Discharge (1-2 days post ablation)		X	
Adverse Event / Unanticipated Problems Assessment	X	X	X
Data collection			
Demographics	X		
Medical History/Interim History*	X	X	X
Physical Examination*	X	X	X
NYHA	X		
Vital Signs: BP, HR, RR*	X	X	X
Height and Weight	X		
Pregnancy Test	X		
Clinical Laboratory Evaluation	X	X	
Clinical Imaging	X		
Prior/Concomitant Medications	X		X

*** Interim medical history, physical exam, and vitals will be collected via chart review from routine clinical care follow up post-ablation visits that occur at approximately 3, 6 and 12 months. If a participant does not attend their follow up visits, the research staff will attempt to contact the participant by phone to review their interim history, medication use and collect atrial fibrillation recurrence information.**

6.1 Pre-Screening

Patients will be pre-screened by the study team via EMR using data/testing collected for clinical purposes prior to the Randomization visit to determine potential eligibility. Patients identified as potentially eligible will be approached for participation in the research study.

6.2 Study Visits & Data Collection

6.2.1 Visit 1/Day 0 (Screening, Enrollment, Randomization, and Ablation)

Study personnel will assess each subject against each inclusion and each exclusion criterion and the Investigator will determine the subject's eligibility for study participation. Appropriately delegated study staff will review the consent form with subjects. The principal investigator or appropriately delegated members of the research team will also discuss the underlying rationale for the study, the procedures to be followed, the potential benefits and risks, and other issues mandated by the consent process. Delegated study physicians, will discuss the device/risks/benefits and obtain documented consent. Before or after the discussion of risks and benefits of the experimental product by the study physician, a study nurse or clinical coordinator may consent the research subject with respect to all other aspects of the research. The participant will be given the consent form for review and will be given the time to ask questions and have them answered. The participant will be asked if they would like to participate, and if so, this will be documented using the study ICF. Determining the eligibility for each participant will require information generated during the course of routine clinical care as dictated by their attending physicians. Selected testing data will be collected in order to characterize the type, cause and severity of the patient's AF and the treatments received prior to enrollment. The data will include information from the evaluations listed below.

Once the Investigator has reviewed and signed the eligibility checklist, the research staff will enter the participant's information into the RedCap system to obtain the randomization assignment.

The following procedures will be performed during Visit 1:

<u>Research and Clinical Procedures:</u>

- Clinical consent for ablation procedure
- Study Informed Consent
- Documentation of Inclusion/Exclusion criteria review
- Randomization
- Ablation procedure (with standard temperature monitoring or with EnsoETM cooling device)
- Overnight post-op monitoring
- Adverse Event/Unanticipated Problem assessment and recording

<u>Data collection from Clinical procedures:</u>

- Collect Demographics (including sex/gender, race, ethnicity, and age via date of birth)
- Medical Record Review (including any history of disease, social history, physical exam findings and physicians notes)
- Review of Concurrent Medications
- Physical Exam
- Vital Signs: blood pressure, heart rate, respiration rate, height and weight
- Routine Clinical Labs/Phlebotomy: such as a complete blood count (CBC) and chemistry profiles
- Pregnancy test for women of childbearing potential
- New York Heart Association Assessment – classification of heart failure based on symptoms and functional limitations

6.2.2 Visit 2 (Post-op)

As part of routine post-op monitoring, participants will stay overnight in the hospital for monitoring. They may be discharged the following day or up to 3 days later depending upon their clinical status. Prior to discharge, we will conduct Visit 2 assessment.

The following procedures will be collected at Visit 2:

- Interim medical history review
- Physical Exam
- Vital Signs
- Routine Clinical Labs/Phlebotomy: such as a complete blood count (CBC) and chemistry profiles
- Research EGD (Esophagogastroduodenoscopy)
- Adverse Event/Unanticipated Problem assessment and recording

The Research EGD may be conducted immediately following the ablation or up to 2 days post ablation procedure.

6.2.3 Visit 3 (Post ablation Month 1)

Participants will be scheduled for a routine follow up visit post-ablation by the clinical staff at approximately 1 month after having had the ablation procedure.

The following procedures will be collected at Visit 3:

- Interim medical history review /concomitant medications
- Physical Exam & Vital Signs
- Adverse Event/Unanticipated Problem assessment and recording

Participants that do not return for their clinical follow up will be contacted by the research staff at approximately 1 month to collect interim history, medication use, and adverse event information.

6.2.4 Follow up data collection (Post ablation Month 3,6,12)

Participants will be scheduled for routine follow up visits post-ablation by the clinical staff at approximately 3 months, 6 months, and 12 months after having had the ablation procedure.

The following data will be collected from the EMR if available:

- Interim medical history/concomitant medications
- Physical Exam & Vital Signs

6.3 Rescue Therapy

The EnsoETM device will be removed and replaced if at any time there is evidence of a potential device malfunction, such as the inability to adequately reach target cooling temperature. The device can be easily removed and replaced during the ablation procedure if needed. Should evidence of continued malfunction occur after replacement of the study device, the EnsoETM will be removed and replaced with the standard temperature probe allowing the patient to undergo the standard ablation procedure.

6.4 Unscheduled Visits

The research team will assess for the occurrence of any adverse event(s) / unanticipated problem (s) should a patient present for any unscheduled visit to University of Pennsylvania Cardiology or GI clinical practice up through 30 days following the ablation procedure.

6.5 Subject Withdrawal

Subjects may withdraw from the study at any time without impact to their care. They may also be discontinued from the study at the discretion of the Investigator for lack of adherence to study procedures or visit schedules, AEs, or should it become clinically necessary to deviate from the study protocol during the course of the ablation procedure. The Investigator may also withdraw subjects who violate the study plan, or to protect the subject for reasons of safety or for administrative reasons. It will be documented whether or not each subject completes the clinical study. We will attempt to have one final visit or contact at approximately 30 days to follow up regarding adverse events for participants who withdraw prior to the 30 day follow up visit.

6.5.1 Data Collection and Follow-up for Withdrawn Subjects

We will attempt to have one final visit or contact at approximately 30 days to follow up regarding adverse events for participants who withdraw prior to the 30 day follow up visit. During this contact they will be asked for permission to have the study team look into their survival status via their electronic medical records and publicly available means.

7 Statistical Plan

Primary Endpoint

The primary endpoint of this study will be the occurrence rate of esophageal thermal injury, the severity (Grade 1-4) of thermal injuries, and number of distinct injuries present. This will be assessed by blinded gastroenterologist using images captured during the EGD procedure.

7.1 Secondary Endpoints

Secondary endpoints include:

1. Evaluation of posterior wall ablation parameters:
   - Number of posterior wall ablations
   - Total posterior wall ablation time
   - Biophysical parameters (power, time, temperature, impedance changes, contact force)

2. Occurrence and location of any acute PV reconnection
3. Freedom from AF at 3 months, 6 months, and 12 months

7.2 Sample Size and Power Determination

Based on prior studies, esophageal injury following ablation is anticipated to be approximately 35% in the control group. However, as we do not know the occurrence rate of the primary outcome of esophageal injury using the Penn ablation technique that would represent the control group nor in the device arm we are unable to accurately perform a power calculation for this pilot study. We believe that a total of 30 patients for each group will be required to yield the necessary pilot data to make an appropriate conclusion regarding the potential utility of esophageal thermal cooling using this device on the reduction of esophageal thermal injury. It is also anticipated that data from this pilot study can be used for planning future larger studies.

7.3 Statistical Methods

We will utilize a calculated esophageal lesion score to account for both lesion severity and the number of distinct lesions identified per patient for comparison between patient groups. The score will be a four-digit number WXYZ where each digit represents the number of lesions at each severity level per patient defined as: Z: Grade 1; Y: Grade 2; X Grade 3; W: Grade 4. This will be inputted into the following formula in order to calculate the thermal injury score for each patient: Score = 1000*W + 100*X + 10*Y + Z.

7.3.1 Baseline Data

Baseline and demographic characteristics will be summarized by standard descriptive statistics (including mean and standard deviation for continuous variables such as age and standard percentages for categorical variables such as gender).

7.3.2 Efficacy Analysis

This is a pilot study to determine the potential role of the EnsoETM device for the prevention and/or reduction of esophageal thermal injury during atrial fibrillation ablation procedures. This analysis will be performed using the calculated esophageal lesions score as described above based on the primary outcome data of thermal injury occurrence rate, severity (Grade 1-4) of injuries, and number of distinct injuries present between study groups.

7.3.3 Interim Safety Analysis

All subjects entered into the study and randomized at the baseline visit will have detailed information collected on adverse events for the overall study safety analysis. An interim safety analysis will be performed after the first 10

subjects are enrolled in the trial. At this time the safety and tolerability of the study device and research EGD procedure will be assessed and if, deemed safe and appropriate, enrollment will continue up to 70 subjects.

7.4 Subject Population(s) for Analysis

All patients enrolled, randomized to a study arm, and complete the initial phase of the study (ablation procedure and EGD) will be included for analysis.

8 Safety and Adverse Events

8.1 Definitions

8.1.1 Unanticipated Adverse Device Effect

An Unanticipated Device Effect is 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.

Serious injury:
Any injury or illness that is any one of the following:

- life-threatening
- results in permanent impairment of a body function or permanent damage to body structure
- necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure

8.1.2 Adverse Event

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

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

8.1.3 Serious Adverse Event

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

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

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, 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.

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

8.1.4 Unanticipated Problems(UP) Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets all of the following criteria:

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

8.1.5 Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

8.1.6 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

8.1.7 Post-study Adverse Event

All unresolved adverse events considered probably or definitely related should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

8.1.8 Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for additional surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event case report form (CRF). All clearly related signs, symptoms, and clinically significant abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period (consent through Visit 3 – 1 month follow up) will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study intervention or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study intervention or study participation will be recorded and reported immediately.

8.3 Classification of Adverse Events

Severity

- **Grade 1**: **mild**; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention is not indicated.
- **Grade 2**: **moderate**; minimal, local, or noninvasive intervention is indicated; limiting to age-appropriate instrumental activities of daily living (ADL; instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc).
- **Grade 3**: **severe** or medically significant but not immediately life threatening; hospitalization or prolongation of hospitalization is indicated; disabling; limiting to self-care ADL (self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).
- **Grade 4**: **life-threatening** consequences; urgent intervention is indicated.
- **Grade 5**: **death** due to an AE.

Relatedness

1) Definite: the AE is clearly related to the research procedures
2) Probably: the AE is likely related to the research procedures
3) Possible: the AE may be related to the research procedures
4) Unlikely: the AE is doubtfully related to the research procedures
5) Unrelated: the AE is clearly not related to the research procedures

Expectedness
AEs must be assessed as to whether they were expected to occur or were unexpected, meaning not anticipated based on current knowledge found in the protocol, investigator brochure, product insert, or label.

Expected: an AE known to be associated with the intervention or condition under study.

OHRP defines an **unexpected AE** as any AE occurring in one or more subjects participating in a research protocol, the nature, severity, or frequency of which is **not** consistent with either:

1) the known or foreseeable risk of AEs associated with the procedures involved in the research that are described in a) the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document, and b) other relevant sources of information, such as product labeling and package inserts; or

2) the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the AE and the subject's predisposing risk factor profile for the AE.

8.4 Adverse Event Reporting Period

For this study period during which adverse events must be reported is defined as the period from the initiation of any study procedures (consent) to the end of the study follow-up (1-Month follow up visit/Visit3). Adverse events that do not require expedited reporting (see section 9.5 below) will be reported in summary to the IRB at continuing review.

8.5 Expedited Reporting of Events

Any study-related unanticipated problem posing risk to subjects or others, and any type of serious adverse event or unanticipated device reaction, will be reported to the IRB and funding sponsor within 24 hours of knowledge of the event. Investigators will use the appropriate SAE/UP CRF to record events and a line item will also be added to the AE log CRF.

The minimum necessary information to be provided at the time of the initial expedited event report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study intervention was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study intervention

8.5.1 Follow-up report

If an SAE, UP or Unanticipated Adverse Device Effect has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the IRB. The investigator is responsible for ensuring that all events are followed until either resolved or stable.

8.5.2 Sponsor reporting: Notifying the Funding Sponsor

Electronic notification of any adverse events related to the use of the EnsoETM study device will be sent to the funding sponsor as determined by the principal investigator and study team.

8.6 Unblinding Procedures

The study team will not be blinded to the randomization assignment. The study team will be instructed to maintain blinding for participants until after their EGD unless a participant has a clinical need to know their randomization assignment. The decision to unblind will be at the discretion of the study PIs and only if unblinding would change clinical care. Unblinding for safety reasons will be recorded in study records and it will be reported to the IRB and the funding sponsor at the time of continuing review. Though we have found it hard to ensure participants stay blinded while in the hospital, we have instructed the extended care team to limit disclosure to the patient until after the EGD at the earliest. The EGD images will be de-identified and batch reviewed to ensure reviewers are not aware of the treatment arm, and all other endpoints are objective/quantitative and thus would not change based on blinding.

8.7 Data and Safety Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study. The Principal Investigator and designated members of the study team will be responsible for monitoring subject safety and applicable reporting to the IRB and study sponsor. This safety monitoring will include careful assessment of eligibility and detailed assessment and appropriate reporting of adverse events as noted above. Grading of EGDs will be done by a single physician contracted to perform the reads. Data collected on the study will be reviewed after 10 people are enrolled in the study. Another data review will occur at 20 and 40 patients enrolled.

9 Study Administration, Data Handling and Record Keeping

9.1 Confidentiality

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

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information

- The rights of a research subject to revoke their authorization for use of their PHI.

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

9.2 Data Collection and Management

Data will be collected by trained research staff using source documents and CRFs. Source and CRFs will be entered into a RedCap data management system (DMS). Participants will be assigned a Participant ID "PID" for use on CRF data collection in for entry into the DMS to protect and ensure confidentiality.

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

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

The study data will be stored indefinitely. A de-identified data set may be shared with the funding sponsor.

9.3 Records Retention

Study records, including administrative and participant related source and CRFs, will be retained for 7 years after the completion of the research (often marked by a final progress report).

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator and research team will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. clinical EP laboratories), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities.

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

11 Ethical Considerations

This study is to be conducted in accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the funding sponsor before commencement of this study if required. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the appropriately delegated research staff obtaining the consent.

11.1 Risks

Risks of the ablation procedure will be explained using the clinical procedure consent.

Additional risks of the study procedures are described below:

EnsoETM device risks: Placement of the EnsoETM device, similar to any device in the esophagus, can result in or exacerbate esophageal tissue damage, particularly in patients with known esophageal deformity or evidence of esophageal trauma. We believe these risks are similar to those whom receive the standard of care temperature monitor probes inserted into the esophagus during routine clinical procedures.

EGD procedure risks: Diagnostic upper endoscopy procedures are considered very safe and rarely can result in complications including bleeding, infection, and/or perforation. Large studies have reported the occurrence of any adverse event anywhere from 1 in 200 to 1 in 10,000 patients with major complications or death extraordinarily rare[8].

Loss of Confidentiality Risks: there is the potential for loss of confidentiality during data collection.

11.2 Benefits

There are no known direct benefits to the participants in this study. There may be a benefit to cooling the esophagus to reduce or prevent damage during the ablation procedure. The knowledge gained by participation in this study may benefit society as a whole in the future and potentially lead to additional studies.

11.3 Risk Benefit Assessment

The risks of participating in the study are outweighed by the potential benefits of participating in the study.

11.4 Informed Consent Process / HIPAA Authorization

Participants will be provided an IRB approved consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. A verbal review of the consent form will take place with the participant and delegated personnel. This consent form will include HIPAA authorization language which will also be reviewed with the participant. The formal consent of a subject, using the IRB-approved consent form, will be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent. The participant will be consented in a private clinical space and will be given ample time to ask questions and have questions answered. As the majority of the procedures that will occur are standard of care, we will clearly discuss what the research part of the day and days after will include. The voluntary nature of the study will be reviewed and participants will be told that should they choose not to consent, their clinical care will not be effected.

12 Study Finances

12.1 Funding Source

This study is financed through a contract with Attune Medical. Attune Medical is considered the funding sponsor who will provide financial support to conduct the study and the EnsoETM device free of charge.

12.2 Conflict of Interest

All University of Pennsylvania Investigators will follow the University of Pennsylvania Policy on Conflicts of Interest Related to Research.

12.3 Participant Stipends or Payments

Participants will receive payment for their additional time, effort & inconvenience of being in the trial in the amount of $100. Reimbursements for participation in the study will be administered using a Greenphire ClinCard, a reloadable prepaid card provided by the University of Pennsylvania. Payment will be loaded on the day of discharge after completing the EGD procedure.

13 Publication Plan

Investigators will follow all University of Pennsylvania applicable policies and guidelines relating to publishing study results. The research team will share materials planned for publication to the Funding Sponsor according to the terms of the Clinical Trial Agreement.

14 References

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