

Safety of a Sheath Cryoprobe Bronchoscopic Transbronchial Biopsy Technique

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ACRONYM : FROSTBITE

Principal Investigator: Yarmus, Lonny

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STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the ICH GCP E6, the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and the STTR Terms of Award. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator: _____
Print/Type Name

Signed: _____ Date: _____
Signature

STUDY CONTACT INFORMATION

Coordinating Center	Core Lab
<p>Johns Hopkins University</p> <p>Principle Investigator (Lead PI):</p> <p>Lonny Yarmus, DO Associate Professor of Medicine Johns Hopkins University Phone: 410-502-2533 Email: lyarmus@jhmi.edu</p> <p>Research Coordinator:</p> <p>Jenna Los Research Program Manager Phone: 410-955-5288 Email: jlos1@jhmi.edu</p>	<p>Pathology:</p> <p>Peter Illei, MD Associate Professor of Pathology Johns Hopkins University pillei1@jhmi.edu</p> <p>Radiology:</p> <p>Cheng Tin (Tony) Lin Associate Professor of Radiology Johns Hopkins University clin97@jhmi.edu</p>
Additional Site Contact	
<p>Fabien Maldonado, MD Associate Professor of Medicine Vanderbilt University Phone: 615-322-3412 Email: fabien.maldonado@vanderbilt.edu</p>	

PROTOCOL SYNOPSIS

Title:	Safety of a Sheath Cryoprobe Bronchoscopic Transbronchial Biopsy Technique (The FROSTBITE Trial)
Study Objective:	To determine the safety profile of a 1.1mm flexible single-use cryoprobe with oversheath used for transbronchial lung biopsy via a bronchoscopic approach
Study Device:	ERBE 1.1mm flexible single-use cryoprobe with oversheath
Design:	A multi-center, non-randomized, single-arm, prospective trial designed to determine the safety profile of a novel cryoprobe and generate preliminary efficacy data.
Phase:	Post-Market
Number of Sites enrolling participants:	2
Number of Subjects:	50
Study Participant Duration:	30 days
Study Duration:	3 months
Population:	<p>Inclusion Criteria:</p> <ul style="list-style-type: none"> • Male or Female, ≥ 18-years-old • Scheduled to undergo bronchoscopy with transbronchial biopsy as the standard medical care determined by their treating pulmonologist <p>Exclusion Criteria:</p> <ul style="list-style-type: none"> • Pregnant or nursing females, or females of child bearing potential who refuse to take a pregnancy test prior to enrollment • Individuals with current or recent systematic conditions, such as uncontrolled hypertension (systolic > 200 mmHg or diastolic > 110 mmHg), type 1 diabetes, severe pulmonary hypertension, acute kidney injury, stroke (within the last 6 months) or myocardial infarction (within the last 3 months) • Presence of bleeding disorder • Platelet count $< 50,000$ • Current use of systemic anticoagulation or antiplatelet therapy without the ability to hold therapy for the recommended amount of time prior to an invasive procedure (aspirin monotherapy is acceptable)

	<ul style="list-style-type: none"> • International Normalized Ratio (INR) <1.5 • Robotic Bronchoscopy • Do Not Resuscitate (DNR) status • Do Not Intubate (DNI) status
Endpoints:	<p>The primary endpoint will be the number of serious adverse events in those enrolled in the study. Serious adverse events are defined as:</p> <ul style="list-style-type: none"> • Grade 3 or 4 bleeding • Pneumothorax requiring chest tube placement (Grade ≥2) • 30-day respiratory failure and death. <p>Secondary endpoints include minor adverse events (bleeding Grade 1 or 2), pneumothorax not requiring chest tube placement (Grade 1), histologic accessibility, diagnostic yield</p>
Statistical Methodology	<p>This study is designed to ensure the serious adverse event rate using the 1.1mm flexible single-use cryoprobe with oversheath will not exceed the expected serious adverse event rate of transbronchial biopsy using standard biopsy procedures (30%)</p>
Safety Monitoring	<p>After each 10 consecutive patients are enrolled, the DSMB will review safety. Additionally, after each serious adverse event, the study will be halted until adjudicated by the DSMB.</p>

1. BACKGROUND

The current standard method for obtaining bronchoscopic lung biopsy specimens is transbronchial forceps biopsy (FBx). However, the utility and diagnostic capability of FBx is limited by poor specimen quality due to small biopsy size, crush artifact and cellular artifact.¹ In part due to these restraints, the diagnostic yield of FBx has remained limited to 34-58% in diffuse lung disease¹⁻³ and 54-58% for pulmonary nodules^{4,5}. With the promise of obtaining higher quality tissue samples, cryoprobe biopsy (CBx) offers a similarly minimal invasive alternative to standard FBx.

Cryoprobes have been used in the bronchoscopic management of malignant endobronchial disease and for transbronchial biopsy. The CBx operates using the Joule-Thomson effect, in which compressed gas undergoes adiabatic expansion and rapidly cools the probes' tip. This allows for adequate anchoring of the probe to lung parenchyma and biopsy specimen retrieval. There have been numerous recent studies evaluating the performance of CBx. Higher diagnostic yields and larger biopsy sizes with better quality have been observed using CBx over FBx^{2,3,6-8}. However, these benefits might come at a cost of higher complication rates, specifically, higher bleeding risks.

1.1. STUDY RATIONALE

One of the drawbacks of the current available CBx system (Erbe Elektromedizin GmbH) is the need for *en bloc* removal of the cryoprobe, specimen, and bronchoscope out of the patient's airway to retrieve the sample. This timeframe when the scope is removed may increase bleeding complications usually mitigated by keeping the scope wedged after each biopsy. This necessitates a secure airway to permit rapid re-entry of the bronchoscope, and placement of a prophylactic bronchial blocker which limited this procedure to advanced bronchoscopists. To overcome this obstacle, a novel, smaller 1.1mm flexible single-use cryoprobe (SCBx) with oversheath has been developed that can retract the tissue sample through the oversheath via the working channel of the bronchoscope while the scope remains wedged in the airway. This permits a technically less cumbersome biopsy while the scope remains in place to manage potential complications. It is reasonable to hypothesize that due to these advancements in procedural technique, the safety profile of this new SCBx would be superior to traditional CBx. That said, no prospective trials have been performed evaluating the safety of the SCBx. Here, we propose the first human studies to evaluate the safety of the SCBx for bronchoscopic transbronchial lung biopsy.

1.2. DEVICE DESCRIPTION

The ERBE 1.1mm flexible single-use cryoprobe with oversheath has been approved by the US Food and Drug Agency (FDA) under a 510(k) application (ERBECRYO® 2 Cryosurgical Unit and Accessories - K190651). It is to be used with the ERBECRYO 2 unit. The ERBECRYO 2 is a device which was designed and optimized to address many of the clinical variables known to affect previous generations of the technology. This device contains an advanced flow-control system which is intended to provide optimized and reproducible CO2 gas flow for each individual probe specification, including the miniaturized 1.1mm cryoprobe. This optimized gas flow is an important variable to support standardization of the technical and clinical performance.

The use of an oversheath with the 1.1mm single-use flexible cryoprobe enables the working channel of the bronchoscope to be protected during retrieval of cryobiopsy specimens. This enables retrieval of the biopsy specimen through the working channel of the bronchoscope in a similar fashion to transbronchial forceps, brush and needle biopsy, permitting the bronchoscope to remain in place while biopsy samples are retrieved. In this study, the 1.1mm single-use flexible cryoprobe with oversheath will be used according to the associated Instructions for Use (IFU).

1.3. PRIOR CLINICAL DATA

Preliminary studies were performed in a porcine model to better evaluate the performance of SCBx.⁶ In a prospective, randomized, single blinded study, a 1.1mm SCBx probe, 1.9mm CBx probe, and standard 2.0mm FBx forceps were compared, assessing histologic adequacy and accessibility, sample quantity and quality, and procedure time. Samples adequate for standard pathologic processing were retrieved with 82.1% of the SCBx specimens, 82.9% of the CBx specimens, and 30% of the FBx specimens. The histologic accessibility and biopsy area of both SCBx and CBx was superior to FBx (Figure 1⁶). Procedure time for FBx was faster than for both SCBx and CBx, but SCBx was significantly faster than CBx ($P < .0001$). Fluoroscopy time was lower for both SCBx and CBx compared with FBx. This preliminary study demonstrated SCBx is a feasible technique to retrieve specimens through the working channel of a bronchoscope resulting in higher quality biopsy specimens compared with FBx.

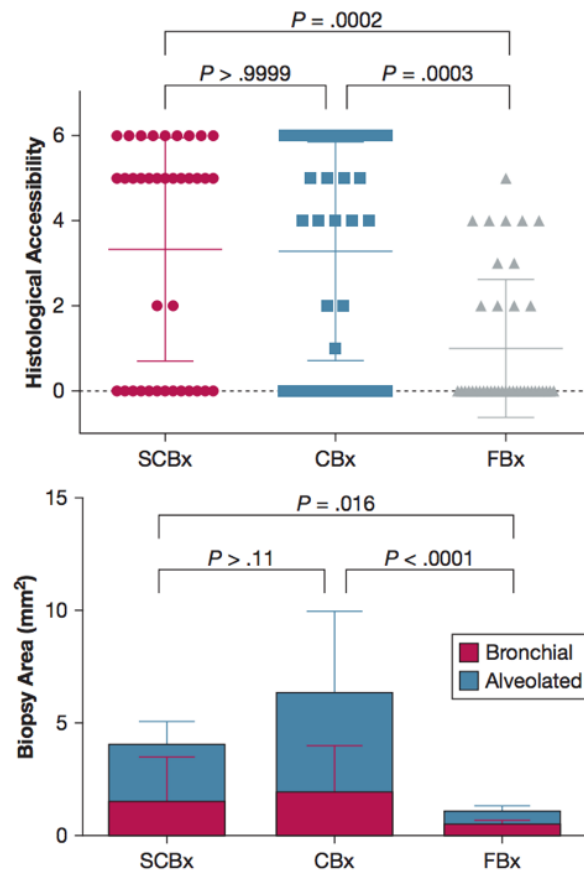


Figure 1: Comparison of histologic accessibility and biopsy area between SCBx, CBx and FBx

1.4. POTENTIAL RISKS AND BENEFITS

This is the first-in-human dedicated post-market safety study using the new 1.1mm single-use flexible cryoprobe with oversheath. Prior larger reusable cryoprobes (1.9mm, 2.4mm) of the prior generation have been shown to be safe and efficacious. The risks of using the smaller 1.1mm single-use flexible cryoprobe with oversheath are similar to use of the larger probes. The risks associated with bronchoscopy and cryoprobe transbronchial biopsy include:

- Alteration of vocal cords (hoarse)
- Hypertension
- Bronchospasm
- Injury to mouth or pharynx
- Injury to teeth or related soft tissues
- Chills/fever
- Laryngospasm
- Coughing
- Lung parenchymal infection
- COPD exacerbation
- Myocardial ischemia
- Dyspnea
- Pharyngitis
- Dysrhythmias
- Pneumonia
- Hemoptysis
- Pulmonary edema
- Empyema
- Pulmonary infiltrate
- Excessive bleeding
- Puncturing of the airway
- Thromboembolism
- Spasm of the airway

Only trained and experienced bronchoscopists will perform the study procedure.

Possible Risks of Participation in this Clinical Study

There are standard risks of participating in a research study such as accidental disclosure of subject's confidential information. Every effort will be made to protect the privacy of research subjects. Subject names and protected health information (PHI) will be kept confidential to the extent possible and as required by applicable laws and regulations. All records and data related to the study will be maintained in a secure protected space, with access restricted to study personnel only.

1.5. POTENTIAL BENEFITS

It is possible that subjects will not receive any benefits from transbronchial cryobiopsy. Subjects may benefit from optimized medical management of their disease. Potential benefits of the procedure may be increased size of the biopsy specimen, providing additional tissue for pathologic analysis. This may lead to increases in the diagnostic yield of the bronchoscopy.

2. RESEARCH DESIGN AND METHODS

This is a multi-center, non-randomized, single-arm, prospective trial designed to evaluate the safety of a novel 1.1mm single-use flexible cryoprobe with oversheath. Consecutive patients who have been referred for transbronchial biopsy by their treating pulmonologists will be enrolled. Transbronchial biopsy will be performed using the ERBE 1.1mm single-use flexible cryoprobe with oversheath together with the ERBECRYO 2 unit.

2.1. OBJECTIVE AND PURPOSE

The objective of this study is to evaluate the safety profile of the new 1.1mm single-use flexible cryoprobe with oversheath for the sampling of lung tissue. The safety profile will be assessed across the

various indications for transbronchial lung biopsy (i.e. lung transplant rejection surveillance, lung nodule biopsy, diffuse parenchymal lung disease, pneumonitis). Safety will be assessed by collecting AE and SAE information up to 30 days.

2.2. PRIMARY SAFETY ENDPOINT

The primary safety outcome will be the proportion of participants enrolled that underwent a device related Serious Adverse Events. A complication will be considered 'device related' if it occurs following the index procedure of an attempt at cryoprobe insertion into the working channel of the bronchoscope.

A device related Serious Adverse Event is defined by the presence of any of the following:

- bleeding grade ≥ 3 (see Adverse Events for bleeding grading system)
- pneumothorax requiring chest tube placement (Grade ≥ 2 , see Adverse Events for pneumothorax grading system)
- post-procedure respiratory failure defined as the need for non-invasive or mechanical ventilation requiring ICU admission within 30 days after procedure
- Death within 30 days after procedure

2.3. SECONDARY ENDPOINTS

Secondary Outcomes will include (for definitions, see Data Collection):

- 1) Mild or Moderate Adverse Events: Rate of bleeding grade ≤ 2 , pneumothorax not requiring chest tube placement (Grade 1)
- 2) Histologic Accessibility Grade
- 3) Total Histologic area (mm^2)
- 4) Alveolated Area (mm^2)
- 5) Open alveoli percent
- 6) Percent crush artifact
- 7) Artifact free lung parenchyma percent
- 8) Activation time
- 9) Diagnostic Yield: defined as proportion of patients for which the cryobiopsy sample led to a specific diagnosis on histologic examination
- 10) Procedure time

2.4. STUDY SITE PARTICIPATION

This study will require a minimum of two participating sites. Each site will have a sufficient patient population to enroll 20-30 subjects over the enrollment period (3 months). Each site must have sufficient transbronchial biopsy volume (defined as at least 100 cases per year). To ensure adequate access to patients across the various indications for transbronchial biopsy, each site must have institutional centers for Lung Cancer, Lung Transplantation and Interstitial Lung Disease. In addition, the principal investigator and each participating co-investigator at each site must have cryobiopsy experience, defined as completion of at least 10 cryobiopsies.

2.5. SUBJECT ENROLLMENT

Patients presenting to their pulmonologist in need of a standard of care assessment of a transbronchial lung abnormality via bronchoscopic biopsy and who meet inclusion/exclusion criteria will be invited to participate. The PI will confirm subject eligibility.

Patients who sign the informed consent form will be considered enrolled. Patients who undergo bronchoscopy with at least one cryobiopsy attempt will be included within the analysis population.

2.6. PARTICIPANT INCLUSION CRITERIA

- Male or Female, ≥ 18 -years-old
- Scheduled to undergo bronchoscopy with transbronchial biopsy as the standard medical care determined by their treating pulmonologist

2.7. PARTICIPANT EXCLUSION CRITERIA

- Pregnant or nursing females, or females of child bearing potential who refuse to take a pregnancy test prior to enrollment
- Individuals with current or recent systematic conditions, such as uncontrolled hypertension (systolic > 200 mmHg or diastolic > 110 mmHg), type 1 diabetes, severe pulmonary hypertension, acute kidney injury, stroke (within the last 6 months) or myocardial infarction (within the last 3 months)
- Presence of bleeding disorder
- Platelet count $< 50,000$
- Current use of systemic anticoagulation or antiplatelet therapy without the ability to hold therapy for the recommended amount of time prior to an invasive procedure (aspirin monotherapy is acceptable)
- International Normalized Ratio (INR) < 1.5
- Robotic Bronchoscopy
- Do Not Resuscitate (DNR) status
- Do Not Intubate (DNI) status

2.8. WITHDRAWAL OF SUBJECTS

Subjects who have signed an informed consent form may be withdrawn from this study if they withdraw their consent, or if the investigator determines the subject should no longer continue in the study. Subjects may be excluded early from the study at the discretion of the investigator if continued participation in the trial would in some way jeopardize the subject's health or welfare, or as a result of voluntary withdrawal, being lost to follow-up (LTFU), death, or other reasons not related to SAEs.

Regardless of the reason for withdrawal, data available for the subject at the time of withdrawal, including the reason for withdrawal, will be entered on the Case Report Forms (CRFs). Data collected up to the time of withdrawal may be used by the sponsor and supporting medical device manufacturer for regulatory submissions, publications and other study related data analyses. In cases of withdrawal or LTFU, the study Investigator should document the contact attempts and reasons for subject withdrawal or lost to follow up with other supporting information as requested on the appropriate CRFs.

3. STUDY PROCEDURES

3.1. SCREENING AND INFORMED CONSENT

Patient screening will follow each participating institution's standard of care. A member of the Research Team at the site will review the subject's previously documented standard of care medical information (including CT from the referring doctor) for eligibility and inclusion into the study. Subjects will be approached to obtain informed consent prior to any study-specific procedure. Informed consent will take place in a private environment (e.g. patient exam room), free from distractions. The PI or study

team member will approach the subject at their standard of care clinic appointment or prior to their scheduled bronchoscopy and will explain the study to qualified subjects prior to obtaining consent. All individuals who obtain consent (PI, Sub-investigators, and study coordinators) must have completion certificates for the CITI Human Subjects Research Education Course. Interviews to obtain consent will not follow any stressful situation (e.g. patient being informed he/she may have cancer) and will not be conducted if the patient has received any mind-altering medications or anesthesia. Patients will be assessed for their capacity to consent by the ability to show comprehension of the procedure, ask appropriate questions, and appear properly oriented. A signed copy of all consents and the HIPAA authorization document will also be given to consenting subjects.

3.2. SCREEN FAILURES

Subjects who provide informed consent, but are determined to be ineligible prior to the index procedure (insertion of the cryoprobe into the working channel of the bronchoscope) will be considered screen failures. The reason for screen failure will be documented.

3.3. SEDATION/AIRWAY

Patients will undergo the procedure under deep sedation with an artificial airway (endotracheal tube, laryngeal mask airway or tracheostomy) in place. The use of a rigid bronchoscope will be left to the discretion of the physician performing the procedure. Deep sedation medications and route of administration are at the discretion of the sedating physician.

3.4. BRONCHOSCOPIC BIOPSY

Prior to induction of anesthesia, the 1.1mm flexible single-use cryoprobe will be connected to the ERBECRYO 2 unit via the plug (see IFU for full details). The cryoprobe should be tested as described in the instruction for use (IFU) by immersing the cryotip to a depth of at least 5 cm in sterile warm water at a temperature of approx. 20°C (68°F) and by activating the freeze function of the ERBECRYO 2 for approximately 5 seconds. A clearly visible, homogenous ball of ice must form at the cryotip when freezing capacity is sufficient. Gas bubbles must not escape under any circumstances. Once tested, induction of anesthesia may be performed.

Once appropriate sedation has been achieved and a secure airway has been placed, an airway inspection is to be performed. Following airway inspection and after the appropriate airway has been selected, the cryoprobe multiadapter will be secured to the working channel connector of the bronchoscope. The oversheath can then be inserted and secured to the multiadapter. The cryoprobe can then be inserted through the oversheath via the bronchoscope into the targeted airway using tactile control until in the targeted location is reached. It is optional to place a deflated prophylactic bronchial blocker prior to biopsy. The freeze function is then activated. The initial freeze time will be 4 seconds as measured on the device timer. At the discretion of the bronchoscopist, should inadequate tissue be collected due to incomplete freeze, the freeze time can be extended by 1 second on each consecutive attempt, up to a maximum of 8 seconds. The cryoprobe along with the biopsy specimen is removed with a jerk motion analogous to the same motion used for standard forceps. The sample is then extracted through the oversheath with the bronchoscope remaining in the airway, thereby maintaining freezing activation until the specimen is completely out of the bronchoscope. It is important, that the specimen is pulled quickly into the oversheath with the first movement as far as possible in order to squeeze soft tissue in the oversheath. The freeze function is then deactivated and the specimen should immediately

be placed in saline. The removal tool accompanying each cryoprobe can be used to carefully remove the specimen from the tip of the cryoprobe only after the specimen was defrosted. Specimens will then be transferred into a fixative (i.e. formalin) per institutional policy and sent to the local institutional pathology lab for processing and interpretation. Each biopsy sample will then be sent for central pathology interpretation. If tissue cultures are clinically indicated, an additional specimen may be placed in sterile saline

For non-lung transplant rejection related biopsies, a minimum of 3 biopsies must be taken using the 1.1mm sheath cryoprobe. For lung transplant rejection surveillance biopsies, a minimum of 5 cryobiopsies must be taken (more if needed), as per IHSLT guidelines, at least 5 pieces of well-expanded alveolated lung parenchyma are required for an assessment of acute rejection.⁹ As part of this study, only cryobiopsy with the 1.1mm single-use flexible cryoprobe with oversheath is permitted for transbronchial lung biopsy. This excludes the use of needle, forceps, and brush biopsy. In case of cryoprobe failure a new 1.1mm sheath cryoprobe should be used. A bronchoalveolar lavage or wash is allowed if clinically indicated but must be performed after the index procedure is performed.

Following the procedure, the patient will be returned to the post-anesthesia care unit and a post-procedural chest x-ray will be obtained and results will be documented.

3.5. COMBINED LOCALIZATION PROCEDURES

It is expected (in fact encouraged) that additional localization modalities in addition to standard optical bronchoscopy will be used in combination with the cryobiopsy as would be done with traditional biopsy techniques under standard of care. These include, but are not limited to, fluoroscopy, radial endobronchial ultrasound, and electromagnetic navigation. Robotic Bronchoscopy is excluded due to the fact that a standard flexible bronchoscope is not used. That said, as part of this trial, for tissue acquisition, the only biopsy tool permitted to be used is the cryoprobe. If convex EBUS with rapid onsite pathologic examination (ROSE) is being performed as part of the planned bronchoscopy, this should be performed prior to transbronchial cryobiopsy.

3.6. DATA COLLECTION

Collected data include: radiographic characteristics, procedural characteristics, diagnostic yield, and adverse events, as follows:

- *Radiographic Characteristics:*

If a target location is present (i.e. biopsy for lung nodule), the location of the target lesion (side, lobe and segment, i.e. right upper lobe anterior segment, etc.) will be determined and recorded. The radiographic information collected will include size of the lesion (longest axis), distance from a visible bronchus, and distance from the pleural surface perpendicular to the lesion on axial cuts. If the patient has undergone a PET scan, the metabolic activity (measured SUV) will be recorded.

If target lesions exist, to assess centrality, we will utilize proprietary software to map zones of the lung to determine if a lesion falls into the outer, middle, or central 1/3 of the lung parenchyma. "CT Pulmo 3D" workflow using Siemens Syngo via software automatically analyzes the lung parenchyma and defines the contours of both lungs (Figure 1). This will be performed by the core radiology laboratory at Johns Hopkins. External sites to Johns Hopkins (Vanderbilt University) will submit the de-identified CT scan via a secure program (Ambra) that is in clinical use for secure image sharing.

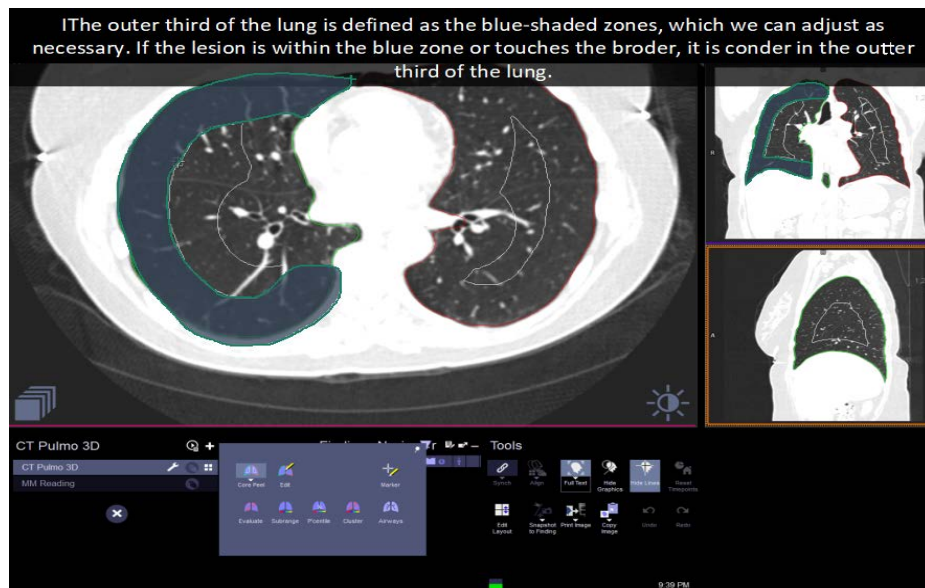


Figure 2: CT Pulmo 3D software output defining central, middle and peripheral lung zones¹⁰

For diffuse lung disease, the CT characteristic pattern will be recorded (i.e. UIP, NSIP, cystic, reticular nodular). Additionally, localization and distribution of infiltrates will be collected. If no specific lung abnormalities are noted on CT (i.e. lung transplant surveillance), this will be recorded.

- *Procedural Characteristics:*

Collected procedural information will include the number of biopsy attempts, freeze time for each biopsy, procedural time (scope in to scope out) and use of prophylactic bronchial blocker. The type (general or moderate) of sedation administered as well as artificial airway selection (ETT, LMA, rigid bronchoscopy) will also be recorded. Room temperature and gas pressure, number of probes used.

- *Pathology Core Lab Assessment:*

An independent pathology core lab will review all study cases. The core lab will be graded on a previously validated histologic accessibility on a 7-point Likert Scale^{6,11} as follows:

- Grade 0: The specimen does not contain alveolar structures and can therefore not be assessed as transbronchial biopsy specimen
- Grade 1: Due to very poor specimen quality, it is not possible to assess the relevant morphologic and histologic features
- Grade 2: Due to poor specimen quality, assessment of relevant morphologic and histologic structures and features is severely compromised and not possible
- Grade 3: Despite high limitations in specimen quality, assessment of the relevant morphologic and histologic structures and features is severely compromised but possible
- Grade 4: Despite moderate limitations in specimen quality, assessment of the relevant morphologic and histologic structures and features is compromised but possible
- Grade 5: Despite low limitations in specimen quality, assessment of the relevant morphologic and histologic structures and features are compromised but possible

- Grade 6: The specimen allows for complete and unrestricted assessment of all relevant morphologic and histologic structures and features

Additionally, the pathologic core lab will determine, the total histologic area (mm²), alveolated area (mm²), open alveoli percent, percent crush artifact, and artifact free lung parenchyma percent. The underlying diagnosis will be confirmed or rebutted.

▪ *Diagnostic Yield:*

Diagnostic yield is defined as proportion of patients for which cryobiopsy led to a diagnosis. Diagnostic yield will be determined from the results of cryobiopsy specimen only. A biopsy that results in a specific diagnosis, either malignant or benign (granuloma, inflammation, fibrosis, infection) will be assumed to be a true positive. Atypia, minimal inflammation or lung parenchyma without pathologic findings on final pathology reads are considered non-diagnostic.

▪ *Room Temperature and Gas Pressure:*

The room temperature of the OR (by a thermometer fixed to the ERBECRYO 2 unit) and the gas pressure which is displayed on the cryo unit will be collected prior to the index procedure. This ensures standardized results throughout the study. The gas pressure and the room temperature must be at least 56bar and 68°F (20°C), respectively.

3.7. Adverse Events and Serious Adverse Events

3.7.1. Adverse Events

An adverse event (AE) is any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device. Note: Pre-existing conditions are not considered to be adverse events unless there is a change in the nature or severity of the condition.

3.7.2. Serious Adverse Event

A serious adverse event (SAE) is an event that has:

- 1) Led to death,
- 2) Led to serious deterioration in the health of the subject, that either resulted in
 - a) a life-threatening illness or injury, or
 - b) a permanent impairment of a body structure, or
 - c) in-patient or prolonged hospitalization, or
 - d) medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,
- 3) led to fetal distress, fetal death or a congenital abnormality or birth defect

Note: Planned hospitalization for a pre-existing condition, or a procedure required by the study, without serious health deterioration is not considered a SAE.

3.7.3. Adverse Event Relationship

The PI will assess the relationship of an adverse event to the device and/or procedure as follows:

- *Not Related:* No relationship appears to exist between the AE and the device and/or procedure
- *Related:* The AE follows a plausible temporal sequence following administration of the study device or execution of the procedure.

- *Undetermined:* It is not possible to determine the relationship of the AE with the study treatment.

3.7.4. Adverse Event Outcome

The outcome of AEs will be recorded as follows:

- *Resolved:* The event is considered fully resolved.
- *Continuing:* At study exit the event is considered ongoing. Note: Any unresolved AE ongoing past study exit will be monitored by the physician per institutional standard of care.

3.7.5. Adverse Event Recording

AEs will be documented in the applicable source documentation (i.e. medical record). As possible, study data collection forms should capture the event diagnosis as opposed to symptoms (e.g. Infection vs. fever). The adverse events of pneumothorax and bronchopulmonary hemorrhage (including hemoptysis) will be documented as follows:

Pneumothorax: Pneumothorax will be documented by both post-biopsy CXR and chest ultrasonography. Pneumothorax severity and required intervention, such as chest tube placement, will be recorded. Severity will be classified according to Common Terminology Criteria for Adverse Events (CTCAE) grade as follows:

- *Grade 1:* Asymptomatic; clinical or diagnostic observation only; intervention not indicated
- *Grade 2:* Symptomatic; intervention indicated (e.g. tube placement without sclerosis)
- *Grade 3:* Sclerosis and/or operative intervention indicated; hospitalization indicated.
- *Grade 4:* Life-threatening consequences; urgent intervention indicated
- *Grade 5:* Death

Bleeding: Bleeding will be categorized on an adapted grading system.¹² The grades are as follows:

- Grade 0: No bleeding or only traces of blood not requiring suctioning
- Grade 1: Bleeding requiring suction to clear
- Grade 2: Bleeding requiring wedging of the biopsied segment with the flexible bronchoscope and/or iced saline
- Grade 3: Bleeding requiring inflation of a bronchial blocker
- Grade 4: Bleeding causing cardiopulmonary instability

3.7.6. SERIOUS ADVERSE EVENT REPORTING

A Serious Adverse Event is defined as the occurrence of bleeding grade ≥ 3 (see Adverse Events for bleeding grading system), pneumothorax requiring chest tube placement (Grade ≥ 2 , see Adverse Events for pneumothorax grading system), post-procedure respiratory failure defined as the need for non-invasive or mechanical ventilation requiring ICU admission, or death, which have occurred within 30 days after procedure, respectively.

All device and/or procedure related adverse events will be reported. Device related events will be collected from initiation of the index procedure (insertion of the cryoprobe into the working channel of the bronchoscope), intra-procedure, and during the standard of care follow-up period of 30 days. 30 day follow up will be assessed by chart review, if data is not able to be extracted by chart review a phone call will be made to assess any adverse events that may have occurred. Study sites are responsible to report adverse events and complications to their IRBs according to institutional requirements.

The study clinician at the site will complete a SAE Form within the following timelines:

- All deaths and immediately life-threatening events, related to the device or the procedure will be recorded on the SAE Form and submitted to the DSMB/study sponsor, lead coordinating center, and device manufacturer within 24 hours of site awareness.
- Other SAEs will be submitted to the DSMB/study sponsor and lead coordinating center within 72 hours of site awareness.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the adherence to be stable. Other supporting documentation of the event may be requested by the DSMB/study sponsor and should be provided as soon as possible. The study sponsor will be responsible for notifying FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

3.8. SAFETY OVERSIGHT

Safety oversight will be under the direction of a DSMB composed of individuals with the appropriate expertise, including interventional pulmonology. Out of an abundance of caution, the DSMB will meet to review cases and assess safety after each 10 consecutive patients have been enrolled (cases will be consecutively batched in groups of 10). The DSMB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined.

Proposed DSMB members:

Gerard Silvestri, MD: DSMB Chair, Pulmonary and Critical Care, Medical University of South Carolina
Momen Wahidi, MD: Interventional Pulmonary, Duke University
Nadia Hansel, MD, Division Chief, Pulmonary and Critical Care, Johns Hopkins University
Roy Semaan, MD, Interventional Pulmonary, University of Pittsburgh
Anil Vachani, MD, Pulmonary and Critical Care, University of Pennsylvania

3.9. STUDY HALTING RULES

Any SAE will serve as a stopping rule. The DSMB will convene to adjudicate the event. Enrollment will resume if the SAE is adjudicated by the DSMB. Additionally, if the SAE rate rises above 30% for two consecutive batches (10 cases per batch), the study will be halted.

4. CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects 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 GCP, and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by Johns Hopkins University
- Centralized, initial assessments and monthly assessments, and comprehensive review of all primary and secondary endpoint data.

- Details of clinical site monitoring are documented in a CMP. The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.
- Independent audits will be conducted by Johns Hopkins University to ensure monitoring practices are performed consistently across all participating sites and that monitors are following the CMP.

5. STATISTICAL CONSIDERATIONS

5.1. STATISTICAL AND ANALYTICAL PLANS

The study is not powered to make a statistical conclusion due to the small sample size and the safety phase in which the treatment is. As the true safety profile of the 1.1mm flexible single-use cryoprobe with oversheath is not known, we will base our statistical plan based on current standard method for transbronchial biopsy, forceps biopsy.

5.2. STATISTICAL HYPOTHESES

1. To determine the serious adverse event rate of a 1.1mm flexible single-use cryoprobe with oversheath for transbronchial bronchoscopic biopsy.

We hypothesize that the serious adverse event proportion using the novel smaller, 1.1mm flexible single-use cryoprobe with oversheath will not exceed 30%, the adverse event proportion for the current standard method of transbronchial biopsy using forceps.

2. To determine baseline performance characteristics for the use of the 1.1mm flexible single-use cryoprobe with oversheath.

We hypothesize that data generated during the performance of this safety trial will generate meaningful descriptive statistics for pathologic evaluation (histologic adequacy), procedural characteristics (activation time, number of biopsies taken, type of airway used), and diagnostic yield across various disease states necessitating transbronchial biopsy. This descriptive data will be crucial in the planning of future clinical trials.

5.3. ANALYSIS DATASETS

All analyses will be done using intention-to-treat analyses to properly identify safety and baseline performance characteristics. As participants will not be randomized, the index action for inclusion will be insertion of the cryoprobe into the working channel of the bronchoscope.

5.4. DESCRIPTION OF STATISTICAL METHODS

5.4.1. GENERAL APPROACH

This is a multi-centered, prospective, single arm trial aimed at establishing the safety profile of a novel 1.1mm flexible single-use cryoprobe with oversheath. Secondary outcomes will be descriptive procedural characteristics that will be used to power future trials.

All continuous data will be presented as means with standard deviations. Categorical data will be represented by percentages. Statistical comparisons will be made using linear and logistic regression

analysis where applicable. Sensitivity, specificity, positive and negative predictive value and accuracy of all procedures will be calculated.

5.4.2.ANALYSIS OF THE PRIMARY SAFETY ENDPOINT

The primary endpoint is the incidence of serious adverse events defined as occurrence of bleeding grade ≥ 3 (see Adverse Events for bleeding grading system), pneumothorax requiring chest tube placement (Grade ≥ 2 , see Adverse Events for pneumothorax grading system), post-procedure respiratory failure defined as the need for non-invasive or mechanical ventilation requiring ICU admission, or death, which have occurred within 30 days after procedure, respectively. The incidence will be calculated as the proportion of patients in whom a serious adverse event occurred divided by those who underwent the index procedure (insertion of the cryoprobe into the working channel of the bronchoscope).

As the true safety profile of the 1.1mm flexible cryoprobe with oversheath is not currently known, and no standard minimally significant difference in a combined bleeding and pneumothorax outcome (such as in this study) is known, we will use currently available literature on a comparable biopsy method, (forceps biopsy and the larger 1.9mm and 2.4mm cryoprobes).

Based on review of the published literature (**Table 1**), we conservatively estimate the current combined bleeding and pneumothorax proportion to be 30% for transbronchial biopsy in standard practice. The procedure will be considered safe if the final proportion of serious adverse events is 30% or less. This will infer a standard of safety for the 1.1mm flexible single-use cryoprobe with oversheath comparable to current transbronchial biopsy standard of care.

Table 1: Literature review of bleeding and pneumothorax rates

Author	Definition of bleeding as per study	Number of patients in CBx, n (%)	Number of patients in FBx, n (%)
Aktas et al. (2010) ¹³ , (n = 41); All performed CB and FB; CB = 41, FB = 41	No bleeding	26 (63.4)	27 (65.9)
	Simultaneous bleeding	5 (12.2)	5 (12.2)
	Mild: cold water + adrenaline	8 (19.5)	9 (21.9)
	Moderate: APC application	2 (4.9)	0 (0)
	Severe : hemodynamic compromise	0 (0)	0 (0)
	Pneumothorax	not reported	not reported
Hetzel et al. (2012) ¹⁴ , (n = 593); CB = 296, FB = 297	No bleeding	59 (19.9)	91 (30.6)
	Mild: suctioning	183 (61.8)	153 (51.5)
	Severe: ice cold saline, diluted vasoconstrictive drug, balloon tamponade, APC, MV, conversion to rigid bronchoscope.	54 (18.2)	53 (17.8)
	Pneumothorax	not reported	not reported
Pajares et al. (2014) ³ , (n = 77) ; CB = 39, FB = 38	Grade 0: no bleeding	5 (12.8)	8 (21.1)
	Grade 1: suction	12 (30.8)	17 (44.7)
	Grade 2: occlusion, use of endoscopic procedures and/or ice cold saline	22 (56.4)	13 (34.2)
	Grade 3: hemodynamic compromise, admission to ICU	0 (0)	0 (0)
	Pneumothorax	3 (7.7)	2 (5.2)
Fruchter et al. (2014) ¹⁵ , (n=75) CB single arm	No bleeding	70 (93)	NA
	Significant bleeding	3 (4)	
	Pneumothorax	2 (2.6)	
Hagmeyer et al. (2016) ¹⁶ , (n=32) CBx only	No or mild bleeding		NA
	Moderate: wedge of bronchoscope	8 (25)	
	severe: use of bronchial blocker or blood transfusion	17 (53)	
	Pneumothorax	6 (19)	NA

5.4.3.ANALYSIS OF THE SECONDARY ENDPOINTS(S)

1. Description of histologic performance. The mean histologic adequacy score, total histologic area (mm²), alveolated area (mm²), open alveoli percent, percent crush artifact, and artifact free lung parenchyma percent will be reported as mean (+/- SD) or Median (Range). This data will be used for statistical planning for future trials.
2. Procedural characteristics such as activation time, procedure time, number of biopsies taken and type of airway (proportion used LMA, ETT, rigid bronchoscope) used will be reported as mean (+/- SD) or Median (Range). Logistic regression will be used to determine if procedural characteristic significantly affect the presence of a significant adverse event (primary outcome). Additionally, linear regression will be used to determine if any of the procedural characteristics effect histologic performance characteristics.
3. Diagnostic yield of cryobiopsy will be calculated as the proportion of patients for which cryobiopsy led to a specific diagnosis. A biopsy will be considered diagnostic, if it results in a specific diagnosis, either malignant or benign (granuloma, inflammation, fibrosis, infection) will be assumed to be a true positive.

5.5. PLANNED INTERIM ANALYSES

Out of an abundance of caution for safety, following each 10 consecutively completed cases, the serious adverse event rate will be calculated. This data will only be available to the DSMB for review.

5.6. SAFETY REVIEW

After any serious adverse event, a safety review will take place and recruitment at all sites will be halted. The DSMB will meet to review the case and overall safety of the trial, once adjudicated by the DSMB, the trial can be resumed.

5.7. SAMPLE SIZE

This is a safety study. The goal of this study is to identify any high frequency adverse events, as well as collect preliminary data on the potential efficacy of the novel 1.1mm flexible single-use cryoprobe with oversheath. As no current data exists to accurately estimate the variance in SAE rates, there is no reliable data to power this study. We conservatively estimate that a stable estimation will of the SAE rate will occur after 50 cases. Thus in this initial study, we will include 50 participants.

6. MEASURES TO MINIMIZE BIAS: ENROLLMENT/RANDOMIZATION/MASKING PROCEDURES

Participants will be enrolled at each institution participating in the study on a referral basis to interventional pulmonology. Due to the nature of the study, patients and bronchoscopists will not be blinded. The treating bronchoscopist will be the outcome assessor.

7. SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Both PI's (Fabien Maldonado and Lonny Yarmus), as well as supporting manufacturer (Erbe Elektromedizin GmbH, Germany) will have access to de-identified study data. The site-specific investigators will have access to the source data available for their site only. Patients will be informed in the informed consent that their de-identified data will be accessible to third parties (monitors, industrial funder).

8. QUALITY ASSURANCE AND QUALITY CONTROL

QC procedures will be implemented at both sites beginning with the data entry system and data QC checks that will be run on the database by the lead coordinating center will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written SOPs, the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the 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.

9. ETHICS/PROTECTION OF HUMAN SUBJECTS

9.1. ETHICAL STANDARD

The principal investigator(s) will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH GCP E6.

9.2. INSTITUTIONAL REVIEW BOARD

The protocol, informed consent form(s), recruitment materials, and all participant materials – which will be consistent across both sites, except where the use of specific institutional templates is required-will be submitted to the site's local IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled at the clinical site. Any amendment to the protocol will be communicated to all sites by the lead coordinating center and will require review and approval by the local IRB before the changes are implemented to the study. All changes to the consent form will be approved by each site's local IRB; a determination will be made regarding whether previously consented participants need to be re-consented. Direct and frequent communication via email and phone with Vanderbilt's PI and research team will be maintained to ensure that both sites are using the most current version of the protocol and consent.

10. INFORMED CONSENT PROCESS

10.1. CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Before undergoing the study procedure, the subject or his legal representative must give written consent to participate in the study using the ICF approved by the clinical site's IRB, and all applicable signatures and dates must be obtained.

The background of the therapy and study, the reason for the study, possible benefits, and any inherent risks shall be carefully explained to the subject. The subject will be given adequate time to review, discuss with physician, discuss with others and to have questions answered prior to signing the IRB approved consent form.

A copy of the signed consent form shall be given to the subject, an original shall be filed in the subject's medical record, and a copy maintained with the site's research documentation.

10.2. 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. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be 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. All participants will receive a verbal explanation in terms suited to their 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 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. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. 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.

10.3. PARTICIPANT AND DATA CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents as well as the supporting manufacturer. This confidentiality is extended to cover testing of biological samples 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.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB or supporting manufacturer 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 local IRB and Institutional regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Johns Hopkins Hospital. 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 and by the Johns Hopkins Hospital research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the Johns Hopkins Hospital.

10.4. RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS OR DATA

There will be no stored human samples or specimens in this study except the taken biopsy specimens. Data collected for this study will be analyzed and stored at the Johns Hopkins Hospital. After the study is completed, the de-identified, archived data will be transmitted to and stored at the Johns Hopkins Hospital, under the supervision of Lonny Yarmus, for use by other researchers including those outside of the study. Permission to transmit data to the Johns Hopkins Hospital will be included in the informed consent.

11. DATA HANDLING AND RECORD KEEPING

11.1. DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at each site under the supervision of the site PI. The investigator at each site 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. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record. All sites will communicate any discrepancies to the lead coordinating center, sponsor, and DSMB which will also have access to the de-identified electronic study records on REDCap.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into REDCap, a 21 CFR Part 11-compliant data capture system provided by the Johns Hopkins Hospital. 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 by research staff at each site.

11.2. PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or MOP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. These practices are consistent with ICH E6:

- Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of each site to use continuous vigilance to identify and report deviations to the local IRB, the lead coordinating center, the sponsor, and the DSMB within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. Protocol deviations must be sent to the local IRB per their guidelines. The site PI/study staff is

responsible for knowing and adhering to their IRB requirements.

11.3. PUBLICATION AND DATA SHARING POLICY

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE policy, and the Section 801 of the Food and Drug Administration Amendments Act of 2007, requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. For interventional clinical trials performed under NIH IC grants and cooperative agreements, it is the grantee's responsibility to register the trial in an acceptable registry, so the research results may be considered for publication in ICMJE member journals. The ICMJE does not review specific studies to determine whether registration is necessary; instead, the committee recommends that researchers who have questions about the need to register on the side of registration or consult the editorial office of the journal in which they wish to publish.

FDAAA mandates that a "responsible party" (i.e., the sponsor or designated principal investigator) register and report results of certain "applicable clinical trials":

- Trials of Drugs and Biologics: Controlled, clinical investigations, other than Phase I investigations, of a product subject to FDA regulation;
- Trials of Devices: Controlled trials with health outcomes of a product subject to FDA regulation (other than small feasibility studies) and pediatric post-market surveillance studies.

12. CONFLICT OF INTERESTS POLICY

The independence of this study from any actual or perceived influence, such as by medical device 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 trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

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