VANDERBILT UNIVERSITY MEDICAL CENTER

Cryospray Therapy Versus Standard of Care for Benign Airway Stenosis (CryoStasis)

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Protocol

Version Date: 27May2025

VANDERBILT UNIVERSITY School of Medicine

Cryospray Therapy for Benign Airway Stenosis: a pilot study

(pilot-CRYOSTASIS)

Protocol

Principal investigators:

Fabien Maldonado, MD

Industry Sponsor:

US Endoscopy/STERIS

Protocol Version Date

27MAY2025

PROTOCOL SYNOPSIS

Title	Cryospray Therapy for Benign Airway Stenosis: a pilot study
Short title	(pilot-CRYOSTASIS)
Study objective	1. To assess the effectiveness of cryospray therapy used in addition to current standard of care endoscopic therapies in preventing short term recurrent airway stenosis. 2.
Study device	truFreeze Spray Cryotherapy System (US Endoscopy)
Design	, Multicenter, investigator-initiated, industry-funded, randomized 1:1, superiority study. The protocol allows for inclusion of an additional external site if recruitment is insufficient after 6 months for study.
Study Centers	Multicenter at academic and community hospitals
Number of subjects	40
Timeline	12-month recruitment, 6 months follow up
Inclusion criteria	1. Referral to interventional pulmonology or otorhinolaryngology for endoscopic management of suspected benign tracheal stenosis. 2. Significant tracheal stenosis defined by stenosis ≥ 50% of tracheal lumen assessed on chest CT or clinical symptomatology warranting evaluation. 3. Able to provide informed consent. 4. Age > 18

Exclusion criteria	1. Inability to provide informed consent. 2. Pregnancy 3. Known or suspected malignant central airway stenosis 4. Patient has already been enrolled in this study. 5. Study subject has any disease or condition that interferes with safe completion of the study including: a. Hypoxemia with need for supplemental oxygen ≥ 2L/min by nasal canula b. Pneumothorax in the previous 12 months c. Severe COPD (defined as a FEV1/FVC < 70% and FEV1 < 30% predicted) and/or severe persistent asthma. d. Hemodynamic instability with systolic blood pressure <90 mmHg or heart rate > 120 beats/min, unless deemed to be stable with these values by the attending physicians. 6. Prior complications with SCT (Spray cryotherapy) 7. Contraindication to rigid bronchoscopy 8. Significant tracheomalacia or alterations in cartilage integrity on CT (Computed Tomography) that would require stent placement or surgical referral. 9. Greater then 1 BCAS intervention, excluding cricotracheal resection, within 6 months before enrollment.
Primary endpoint	The degree of re-stenosis at 6 months, expressed as the percentage of airway lumen volume within

	the stenotic segment, at 6 months compared to personal best patency volume on CT scan performed within two weeks of the study intervention. If patients require repeat intervention before six months based on clinical course, a CT chest before this re-intervention will be analyzed for the primary outcome.
Secondary endpoint	• **Change in PEF (Secondary)** • **Change in QOL questionnaire (Secondary)** • **Incidence of complications between groups (Secondary)**
Statistical methodology	We hypothesize the patency assessed at 6 months will be 90% of baseline in the intervention group and 70% in the control group, with SD 20% in the control group. A sample size of 16 subjects in control and 16 subjects in intervention group will have 80% power to detect an increase in stenotic volume of 20% from control arm with type I error of 0.05. Eight additional patients will be recruited to account for attrition and crossover. Study will be done using the intention-to-treat principle. Continuous and categorical variables will be analyzed using the appropriate methodology accounting for non-normal distributions. Patients will be blinded to intervention including those evaluating the volumetric CT's and those performing statistical analysis.

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1. Abbreviations

NoAAC: North American airway collaborative

VICTR: Vanderbilt Institute for Clinical and Translational Research

Clinical and Translational Science Award (CTSA)

REDCap: Research Electronic Data Capture

FDA: Food and Drug Administration

BCAS: Benign central airway stenosis

COVID19: Cornavirus-19

LN2: Liquid nitrogen

SOC: Standard of care

LMA: Laryngeal mask airway

iSGS: Idiopathic subglottic stenosis

QOL: Quality of life

PEF: Peak expiratory flow

FEV1: Forced expiratory volume in one second

FVC: Forced vital capacity

6MWD: 6-minute walk distance

COPD: Chronic Obstructive Pulmonary Disease

CCQ: COPD Clinical Questionnaire

VUMC: Vanderbilt University Medical Center

ITS: Idiopathic tracheal stenosis

BMI: Body mass index
ROI: Region of interest

GSE: General safety events

SAE: Serious adverse events

eCRF: Electronic case report form

IRB: Institutional review board

HIPPAA: Health Information Portability and Accountability Act

GCP: Good clinical practice

2. General Study Information

Principal Investigators: Fabien Maldonado, MD.

Statistician: Heidi Chen, PhD

Industry Sponsor: US Endoscopy/STERIS

Title: Cryospray Therapy for Benign Airway Stenosis: a pilot study

Protocol Version Date: 27, May, 2025

2.1 Investigate team and research environment

Vanderbilt has a complex airway center including interventional pulmonology, otorhinolaryngology, and thoracic surgery.. The Vanderbilt University Institute of Imaging Science is a trans-institutional initiative within Vanderbilt University serving physicians, scientists, students, and corporate affiliates. Vanderbilt Institute for Clinical and Translational Research (VICTR) VICTR is Vanderbilt's virtual home for clinical and translational research. Supported by the Vanderbilt University Medical Center's Office of Research and the NIH sponsored Clinical and Translational Science Award (CTSA), the mission of the institute is to transform the way ideas and research discoveries make their way from origin to patient care. REDCap (Research Electronic Data Capture) is a secure web application for building and managing online surveys and databases. While REDCap can be used to collect virtually any type of data (FISMA (Federal Information Security Management Act), and HIPAA- compliant environments), it is specifically geared to support online or offline data capture for research studies and operations.

3. Statement of compliance

This human subject study will comply with all applicable federal, state, and local laws and regulations, including accepted standards of good clinical practice as adopted by current Food Drug Administration (FDA) regulations and statutes. Participating study sites shall only allow individuals who are appropriately trained and qualified to assist in the conduct of the study.

4. Purpose of the study

4.1: Overview

Benign central airway stenoses (BCAS) are an important cause of pulmonary morbidity and mortality.

[1]. Because of the Cornavirus-19 (COVID19) pandemic we anticipate a substantial increase in BCAS in the coming years primarily due to post intubation tracheal stenosis. There are two general options available for management of BCAS: surgical resection or endoscopic management. Surgery is the preferred definitive option; however, the first therapeutic attempt is typically endoscopic with surgery reserved for recurrence[2]. In addition, many patients are not candidates for surgical resection due to comorbid conditions, anatomical characteristic such as length or complexity of stenosis. Symptomatic stenosis frequently recurs resulting in numerous airway procedures[3]. For example, at our institution, we have been doing 3-4 balloon dilation procedures a week for BCAS. Procedures such as dilation, thermal ablative therapies, and stenting may result in additional airway injury due to disruption of extracellular matrix[4]. Spray cryotherapy (SCT) is a novel FDA cleared technique which allows for Liquid nitrogen (LN2) to be delivered in a metered fashion via a 7F catheter used and studied extensively in the esophagus[5]. The proposed advantage of SCT in the treatment of BCAS is the possibility to decellularize the tracheal mucosa, leaving the extracellular matrix intact, which may then serve as a scaffold for appropriate wound repair[6], shifting the abnormal wound response after conventional endoscopic management to a more normal healing process[7]. Thus, SCT has the potential for long-lasting endoscopic management of BCAS and is used as part of routine clinical care by many physicians, but there is a relative paucity of peer-reviewed data, and no randomized controlled trial has been performed. Management decisions require adequate assessment of the position, extent, and severity of the obstructing or stenotic segment. This has historically been done with endoscopic measurements which are subjective, recurrent symptoms or abnormal pulmonary function tests[8] which are universally a late finding in airway stenosis, typically occurring when the stenosis exceeds 70%[9].

4.2: Hypothesis

We hypothesize that the addition of SCT to standard endoscopic treatment of benign airway stenosis will result in decreased stenosis recurrence at 6 months as estimated by quantitative radiologic assessment of the stenotic volume.

4.3: Aims and Objectives

1. To assess the effectiveness of cryospray therapy used in addition to current standard of care endoscopic therapies in preventing short term recurrent airway stenosis with a multicentric outcome evaluation

5. Study Design

5.1 : General Study Design

Subjects will be randomly allocated in a 1:1 fashion to intervention standard of care (SOC) + SCT and control (SOC) groups. Participants will be blinded to the use of SCT to prevent knowledge of their group assignment from biasing their subjective assessments. Adjudicators in the primary outcome measurements (quantitative CT) will be blinded to group assignment as well. Patient will be excluded who have received greater then 1 BCAS intervention within 6 months of enrollment. All operators will be experienced trūFreeze users with proper passive venting training as defined by trūFreeze protocol. All operators will have a minimum experience of 5 prior procedures.

5.1.2 : Intervention group

Patients with BCAS randomized to the intervention group will undergo conventional endoscopic treatment and SCT. The airway will be managed with a Dedo laryngoscope, rigid bronchoscope, or a laryngeal mask airway (LMA) per operator preference and lesion location. Airway pictures will be documented through our propriety bronchoscopy software. The truFreeze spray cryotherapy system will be used to apply liquid nitrogen spray through a flexible catheter passed though the working channel of a therapeutic bronchoscope or rigid laryngoscope. Typical dosimetry with passive venting without suction starts with up to 2 cycles with standard endoscopy, with one cycle after. Spray applications will be adjusted as needed based on the size and region being ablated for up to a 10 second spray time. Standard of care endoscopic management may include radial mucosal cuts using a thermal modality, and mechanical dilation.

5.1.3 : Control group

Patients randomized to the control group will undergo the same airway management and standard of care interventions without use of SCT before and after intervention.

5.1.4 : Both groups

Peak expiratory flow (PEF) as well as a quality of life (QOL) questionnaires will be obtained within 2 weeks before and 2 weeks after the procedure. A repeat thin-cut chest CT or neck to quantitatively estimate of the patency volume will be obtained within 5 weeks of the first procedure to establish personal best patency volume (baseline patency volume, see below). A final CT scan will be done at 6 months after the index study procedure to evaluate the degree of restenosis. A QOL questionnaire and check-in survey will be done at the at the 3-month. Patients will have a final QOL questionnaire at the 6-month mark. Patients will be doing continuous PEF measurements weekly throughout the study with a mobile application as mentioned below.

Throughout the follow up period if a patient meets one or both of the following criteria: 1) drop in peak flow to 30% of previous value 2) drop in dyspnea score by 30% then the investigators will review if the patient needs an early CT and possibly repeat bronchoscopy. If patients undergo a repeat procedure before the 6-month CT they will be analyzed in an early reintervention subgroup. These patients will be including in the final analysis as per intention-to-treat and thereafter followed as per follow-up procedures outlined in the protocol. Patients in the SCT group may get repeat SCT as per discretion of the proceduralist. Patients in the SOC will not be able to get SCT and may only have repeat dilation with laser radial cuts.

6. Subject Information

Inclusion criteria:

1. Referral to interventional pulmonology or ENT for endoscopic management of suspected benign tracheal stenosis.
2. Significant tracheal stenosis defined by stenosis ≥ 50% of tracheal lumen assessed on chest CT or clinical symptomatology warranting evaluation.
3. Able to provide informed consent
4. Age > 18

Exclusion criteria:

1. Inability to provide informed consent.
2. Pregnancy
3. Known or suspected malignant central airway stenosis
4. Patient has already been enrolled in this study.
5. Study subject has any disease or condition that interferes with safe completion of the study including:

    e. Hypoxemia with need for supplemental oxygen ≥ 2L/min by nasal canula

    f. Recent pneumothorax in the previous 12 months

    g. Severe COPD (defined as a FEV1/FVC < 70% and FEV1 < 30% predicted) and/or severe persistent asthma.

    h. Hemodynamic instability with systolic blood pressure <90 mmHg or heart rate > 120 beats/min, unless deemed to be stable with these values by the attending physicians.

6. Prior complications with SCT
7. Contraindication to rigid bronchoscopy
8. Significant tracheomalacia or alterations in cartilage integrity that would require stent placement or surgical referral as assessed by CT imaging.
9. Greater then 1 BCAS intervention within 6 months before enrollment

7. Outcomes

Primary endpoint:

The primary endpoint will be the degree of re-stenosis at 6 months expressed as the percentage of airway lumen volume within the stenotic segment at 6 months compared to personal best patency volume on CT scan performed within five weeks of the study intervention. If patients require repeat intervention before six months based on clinical course, a CT chest before this re-intervention will be analyzed for the primary outcome. All analyses will be performed using OsiriX (Pixmeo, Geneva, Switzerland, see below).

Secondary and exploratory endpoints:

- **Change in PEF (Secondary)**
- **Change in QOL questionnaire (Secondary)**
- **Incidence of complications between groups (Secondary)**

8. Statistical Design

8.1: Expected Outcomes

- We anticipate being able to enroll 40 patients over 1-year period at VUMC with the possibility of opening the study at a second site if enrollment is deemed insufficient at 6 months (15 patients enrolled or less)
- We expect to observe a significant difference in percent change of stenotic volume between SCT-treated patients and controls suggesting decreased recurrence in SCT-treated patients.

8.2: Randomization

Patients will be randomly allocated in a 1:1 ratio in permuted blocks of 4 and 6 to intervention or control arms via randomization module in REDCap. Randomization tables will be set up by a study statistician with no role in recruitment, enrollment, or subsequent randomization and will not be available to study personnel performing these tasks.

8.3: Power Calculation and target accrual

A search of the literature in idiopathic tracheal stenosis (ITS) shows that clinical recurrence after first intervention can happen as early as 6 months. A study by Perotin et al out of 9 institutions of 23 patients with ITS found recurrence of stenosis to be 30% at 6 months, 59% at 2 years, and 87% at 5 years with a delay of 14 to 16 months [10]. Further a study by Gelbard et al evaluated 603 patients who underwent endobronchial dilation in ITS and found the time to stenosis reoccurrence averaged 1.3 years, which was considerably shorter than cricotracheal resection [11]. We hypothesize that the earliest signs of loss of airway patency will be evident at 6 months by radiologic CT-based volumetric assessment, which will detect early (as opposed to clinical) recurrence. Our results will inform the design of future larger studies.

We hypothesize the patency assessed at 6 months will be 90% of baseline in the intervention group and 70% in the control group, with SD 20% in the control group. The sample size estimation was completed using two-sample t-test. For the pilot study, a sample size of 16 subjects in control and 16 subjects in intervention group will have 80% power to detect an increase in stenotic volume of 20% from control arm with type I error of 0.05. An additional 8 patients will be enrolled (attrition), for a total target accrual of 40 patients. Likert scale will be used after treatment to determine the minimal clinically important difference for future studies using this endpoint.

8.4: Analytical Plan

Descriptive statistics including means, standard deviations, and ranges for continuous parameters, as well as percentages and frequencies for categorical parameters will be presented. Investigations for outliers and assumptions for statistical analysis, e.g., normality and homoscedasticity will be made. If necessary, data will be transformed using Box-Cox power transformation. Comparisons between groups, i.e. intervention vs control, will be made using either the t-test or Wilcoxon Rank Sum test for continuous variables (such as patency volume, visual analog scales) and Chi-square test for categorical variables (such as incidences of pneumothorax). Other than the primary end point of percentage change of patency volume at 6 month, we will apply linear regression to assess the 6-month patency volume difference between control and intervention groups with the adjustment of baseline patency volume. Patients will be analyzed according to the per protocol and intention to treat principle. For peak flows, COPD questionnaire, intermediatory time points will be analyzed using mixed model analysis for repeated continuous variables starting from 2 weeks pre-procedure, 3 months and then to 6 months post procedure.

8.5 Measurements:

PEF, quality of life using the Clinical COPD CCQ. Incidence of complications (hypoxemia, bleeding, pneumothorax rate, barotrauma). Baseline characteristics (age, sex, body mass index (BMI), smoking status, underlying disease process). CT Chest without contrast with 3D quantitative reconstruction.

Peak expiratory flow monitoring: Evidence supports the use of PEF as a simple, efficient, and accessible way of monitoring progression of tracheal stenosis. PEF has previously been captured in clinical encounters as component of the decision-making algorithm for surgical intervention in tracheal stenosis patients. Mobile Device Software 'App' can robustly capture longitudinal patient reported outcome measures..

> **CQS mobile computing group**: Established expert programmer and data scientist support dedicated to mobile application development and support. Prior work with the CQS team successfully created and deployed a mobile app (Airflo™) for tracheal stenosis patient PEF reporting. Leveraging a Research Electronic Data Capture (REDCap) foundation, mobile apps are built on open-source mobile computing "MyCap" infrastructure (https://projectmycap.org/). MyCap leverages REDCap, ResearchKit (Apple: iOS), and ResearchStack (Android) to capture patient reported outcome measures, and physiologic tasks (such as maximum phonation time, voice samples, and peak expiratory flow rate) using iOS and Android mobile devices. REDCap is used to define tasks/instruments/surveys to be completed by participants. MyCap translates REDCap task metadata into a structure compatible with ResearchKit and ResearchStack. When a project participant completes a task, MyCap converts the results into a format compatible with REDCap before synchronizing back to the REDCap project.

9. Outcome Measure Synopsis

9.1: Quantitative imaging of stenotic volume

Airway segmentation and measurement of the patency volume will be performed as follows by two experienced operators (FM and R). DICOM images of CT scans will be transferred to the LungCloud™ platform.

The LungCloud™ is provided by Neurotargeting LLC, a spin-off of Vanderbilt University. The LungCloud™ is a health IT clinically approved system for the collection of complete data sets in the clinical flow, including but not limited to clinical outcome, radiology notes and scans. The LungCloud™ is set up as a network of accounts that can be interconnected around a clinical study. The managing institution creates a Clinical Trial inside the LungCloud™ and ties it to each of the institutions. Each institution is then able to collect and share data for the clinical study. Data is collected in the clinical flow and from PACS and stays at all time in the LungCloud™ instance of the institution owning the data.

Image data is encrypted and sent to the institution's LungCloud™. Automatic uploading of images is achieved through what is referred to as the LungDrive. This is a process that runs on the end user's machine and works in a way similar to Box or Dropbox, i.e., any new image volume placed in a specific folder is automatically encrypted and uploaded to the account repository. The non-imaging data can be entered into the system and associated with the imaging data through web-based forms customized to the end users. Images are reconstructed in 3D and can be reviewed in any view (sagittal, coronal or axial). The system offers a series of web-based user interfaces that permit inspecting and visualizing both imaging and non-imaging data.

Deanonymized DICOM images will be transferred and analyzed using the OsiriX (Pixmeo, Geneva, Switzerland) threshold-based region growing algorithm function for volume segmentation. Briefly, the process is as follows: The operator identifies proximal and distal ends of the tracheal stenotic and use the region of interest (ROI) function to segment the trachea in 3 to 4 different areas spanning the length of the stenotic segment using the closed polygonal function (Figure 5). The ROI volume function will then be used to generate missing intervening ROIs and the repulsor function will be used to manually adjust the boundaries of the ROI. The volume of the final airway will then be computed and a three-dimensional image saved for documentation (Figure 6).

To classify the degree of tracheal stenosis, we will manually annotate the trachea (wall, stenosis, and patency) on all scans. Expert manual review will be performed by one of two experienced investigators (FM, AR) In an exploratory analysis to enable routine automated analysis of stenotic volumes, we will perform transfer learning using our abdomen/thoracic model using leave-one-out cross-validation. Mixture modeling with image intensity classification will be used to separate the tracheal wall, stenosis region, and patency following our work in the abdomen.

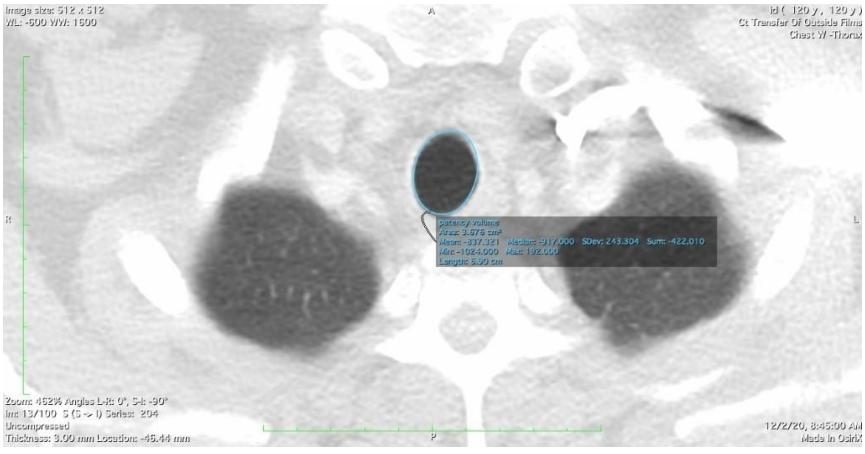

Figure 5. Closed polygonal function-derived 2D-tracheal segmentation

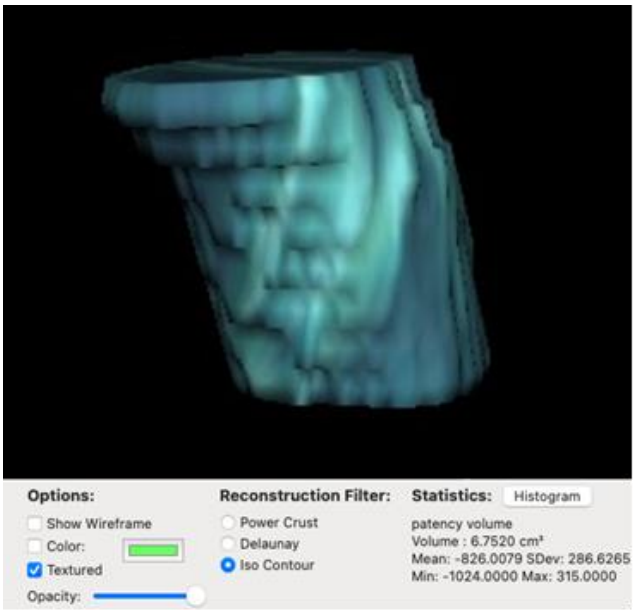

Figure 6. 3D reconstruction and computation of the patency volume

10. Clinical COPD Questionnaire

Nouraei and colleagues successfully validated the Clinical Chronic Obstructive Pulmonary Disease Questionnaire in a group of patients with laryngotracheal stenosis. Total and domain-specific scores also corrected with FEV1 and PEFR. The authors found statistically significant differences between total and domain specific CCQ scores across populations of patients with different preoperative stenosis severities ($P < 0.001$ in all cases; one-way analysis of variance). Furthermore, there were statistically significant differences between preoperative and postoperative total and domain-specific CCQ scores ($P < 0.001$ in all cases; Student's t-test)[18].

We plan to use this questionnaire at carefully selected timepoints throughout the study timeline to assess symptom improvement pre- and post-intervention.

11. Safety

General Safety Events (GSE):

General safety events will be reported within our data collection protocol which includes safety events associated with the procedure (Spray Cryotherapy or Ballon Dilation): bleeding, pneumothorax, infection, airway trauma, air embolism.

Serious adverse event (SAE)

A serious adverse event (SAE) is an undesirable sign, symptom, or medical condition.

which:

- is fatal or life-threatening;

- requires or prolongs inpatient hospitalization;
- results in persistent or significant disability/incapacity;
- constitutes a congenital anomaly or birth defect; or
- jeopardizes the participant and requires medical or surgical intervention to prevent one of the outcomes listed above.

Events not considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above cannot resulting in inpatient admission.
- respite care

General Instructions for Reporting Serious Adverse Events

The Institutional Review Board and Industry Sponsor be notified of all SAEs, within 7 business days after the treating institution becomes aware of the event. Only SAEs related to research procedures will be reported to the IRB.

12. Data Handling

12.1 Data Collection

The Vanderbilt University Office of Research will be used as a central location for data processing and management. Vanderbilt University, with collaboration from a consortium of institutional partners, has developed a software toolset and workflow methodology for electronic collection and management of research and clinical trial data. REDCap (Research Electronic Data Capture) is a secure, web-based application that is flexible enough to be used for a variety of types of research. REDCap provides an intuitive user interface that streamlines project development and improves data entry through real-time validation rules (with automated data type and range checks).

REDCap also provides easy data manipulation (with audit trails for reporting, monitoring and querying patient records) and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). In addition to traditional data capture functionality, REDCap's survey capabilities are a powerful

tool for building and managing online surveys. The research team can create and design surveys in a web browser and engage potential respondents using a variety of notification methods. All data collection projects rely on a thorough, study-specific data dictionary, defined by all members of the research team in an iterative, self-documenting process. This iterative development and testing process results in a well-planned and individualized data collection strategy.

REDCap servers are housed in a local data center at Vanderbilt, and all web-based information transmission is encrypted. REDCap was developed specifically around HIPAA-Security guidelines and is recommended to Vanderbilt researchers by both our Privacy Office and Institutional Review Board. REDCap has been disseminated for local use at more than 940 other academic/non-profit consortium partners in 75 countries. Vanderbilt leads the REDCap Consortium, which currently supports more than 99,000 projects and 128,000 users.

12.2 Data and Safety Monitoring

The Independent Data Monitoring Committee (iDMC) will be comprised of two members with experience in airway stenosis, thoracic surgery and interventional pulmonary. Dr. Samira Shojaee, and Dr. Eric Grogan both from Vanderbilt University Medical Center (VUMC). Both members will meet periodically to discuss data and any SAEs. The group maintains authority to intervene in the conduct of studies as necessary to ensure clinical-research is performed at the highest quality standards.

Both members have a clinical expertise include interventional pulmonology, minimal invasive diagnostics, airway stenosis and lung cancer. They also both have extensive experience in the minimally invasive management of malignant airway obstruction, complex airway management and difficulty airways. In addition, Dr. Shojaee specializes in the diagnosis and management of pleural diseases and symptom relief with minimally invasive procedures such as indwelling pleural catheters and pleurodesis. Dr. Eric Groan is also NIH R01 funded and co-chair of the MAS Lung Cancer Lab

12.3 Data Handling and Record Keeping

An electronic case report form (eCRF) is required and must be completed for each included participant. The completed dataset should not be made available in any form to third parties, except for authorized representatives of proper Health/Regulatory Authorities, without written permission from Vanderbilt.

To enable evaluations and/or audits from health authorities and Vanderbilt, the site investigator agrees to keep records including: The identity of all participants (sufficient information to link records; e.g., hospital records), all original signed informed consent forms, copies of all source documents, and detailed records of any study drug or device disposition. To comply with international regulations, the records should be retained by the investigator in compliance with regulations.

Queries resulting from review of the eCRFs will be generated for the site and corrections will be made by the study site personnel. This will be done on an ongoing basis.

13. Regulatory Considerations

13.1 Protocol Review and Amendments

Information regarding study conduct and progress will be reported to the Institutional Review Board (IRB) per current institutional standards.

The trial will not be initiated until there is approval by the IRB of the protocol, informed consent document and any other material used to inform the patient about the nature of the trial. The IRB should be duly constituted according to regulatory requirements. The investigator will inform the IRB of the progress of the trial at least yearly.

Any changes to the protocol will be made in the form of a written amendment and must be approved by the sponsor-investigator and the IRB prior to implementation. Protocol changes to eliminate an immediate hazard to a trial patient may be implemented by the investigator immediately. The investigator must then immediately inform any applicable local IRB and the sponsor-investigator (or designee).

The sponsor-investigator (or designee) is responsible for the coordination and development of all protocol amendments. Once approved by the sponsor-investigator, Vanderbilt will disseminate this information to the participating study sites.

13.2 Informed Consent

The investigator (or his/her designee) will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each participant will be informed that participation in the study is voluntary, that s/he may withdraw from the study at any time, and that withdrawal of consent will not affect subsequent medical treatment or relationship with the treating physician(s) or institution.

The informed consent will be given by means of a standard written or in electronic format, written in non-technical language, which will be IRB approved. The participant should read and consider the statement before signing and dating it and will be given a copy of the document. No patient will enter the study or have study-specific procedures done before his/her informed consent has been obtained.

In accordance with the Health Information Portability and Accountability Act (HIPAA), the written informed consent document (or a separate document to be given in conjunction with the consent document) will include a subject authorization to release medical information to the study sponsor and supporting agencies and/or allow these bodies, a regulatory authority, or Institutional Review Board access to subjects' medical information that includes all hospital records relevant to the study, including subjects' medical history.

13.3 Ethics and Good Clinical Practice

This study will be carried out in compliance with the protocol and Good Clinical Practice (GCP), as described within:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.

2. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees to adhere to the instructions and procedures described within the above and thereby to adhere to the principles of Good Clinical Practice with which the above conform.

13.4 Confidentiality

It is the responsibility of the investigator to ensure the confidentiality of patients participating in the trial and all of their medical information is maintained. CRFs and other documents submitted to regulatory authorities must not contain the name of a trial patient. All patients in the trial will be identified by a unique identifier which will be used on all CRFs and any other material submitted to regulatory authorities. All case report forms and any identifying information must be kept in a secure location with access limited to the study staff directly assisting with the trial.

13.5 Study Termination

The sponsor-investigator reserves the right to terminate the study at any site and at any time. Reasons for study termination may include, but are not limited to, the following:

• Investigator non-compliance with the protocol, GCP or regulatory requirements.

• Insufficient enrollment if not supplemented adequately by additional site as defined by 15 patients enrolled or less at 6 months.

• Safety concerns.

• Decision by suppliers to modify or discontinue the availability, development or manufacture of protocol-indicated treatment or device.

• A request to discontinue the study by the IRB or a recognized regulatory authority.

14. Cost

Subjects will not be paid to participate in the study.

There will be no additional costs to subjects for participating in this study. Subjects and/or their insurance companies will be responsible for all care provided as part of the standard of care stated above.

Full study budget can get found in the separate budget allocation sheet.

15. Study Coordination

15.1 Trial Compliance

This is an investigator-industry sponsored study. The Principal Investigators, Fabien Maldonado, M.D. are conducting the study with the sponsor US Endoscopy with the device truFreeze Spray Cryotherapy.

Vanderbilt is the Lead site for this study. All aspects of the study will be carefully monitored by the Lead site for compliance with applicable government regulations with respect to current GCP and standard operating procedures.

15.2 Changes to Protocol and Informed Consent Document

Any change to the protocol and informed consent document must be reviewed and approved by the Lead site before being submitted to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) at participating institutions and the industry sponsor. Amendments should not be implemented until all necessary approvals have been obtained, except when necessary to eliminate an immediate hazard to study subjects.

15.3 Protocol Deviations

The Lead site is responsible for implementing and maintaining quality assurance and quality control to ensure that studies are conducted according to the protocol, GCP, and all applicable regulatory requirements. A protocol deviation is any noncompliance with the protocol. Noncompliance can be on the part of the study participant, the investigator, or the study site staff. Deviations to the protocol are not permitted except when necessary to eliminate an immediate hazard to study subjects.

15.4 Monitoring and Quality Assurance

As the Lead site, Vanderbilt has responsibilities to health authorities to take all reasonable steps to ensure the proper conduct of the study with regard to ethics, protocol adherence, integrity, validity of the data recorded on the CRFs, and adherence to regulations regarding GCP and the protection of human subjects.

15.5 Data Verification

Data will be collected via eCRFs and entered into the database per Lead site guidelines. The Lead site will check data accuracy by performing source data verification. Source data verification is a direct comparison of the entries made on the CRFs against the appropriate source documentation. This will be conducted remotely, with the possibility of on-site verification periodically. Discrepancies in the data will be brought to the attention of the investigator and/or the investigator's staff. Any necessary corrections will be made directly to the eCRFs or via queries by the investigator and/or the investigator's staff.

15.6 Study Documentation

Each participating site is responsible for submitting copies of all relevant regulatory documentation to the Lead site. The required documents include but are not limited to the following: IRB approvals (i.e., protocol, consent form, amendments, patient brochures, recruitment material, etc.), each participant's informed consent, enrollment form, eligibility checklist, summary of unanticipated problems or protocol deviations, and documentation of expertise of the investigators. The Lead site will provide each participating site with a comprehensive list of the necessary documents. Specified members at each participating site will submit all pertinent regulatory documents to the Lead site, for storage in a secure location. It is the responsibility of the participating sites to maintain copies of all documentation submitted to the Lead site.

15.7 Closure of the Study

The Lead site reserves the right to discontinue a site at any time during the study for medical or administrative reasons such as:

· Unsatisfactory enrollment;

· GCP noncompliance;

· Inaccurate or incomplete data collection;

· Falsification of records;

· Failure to adhere to the study protocol.

15.8 Records Retention

FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study, including CRFs, consent forms, laboratory test results and physical exam, imaging and medical procedure records, must be retained by each site's Principal Investigator for 2 years after marketing application approval. If no application is filed, these records must be kept 2 years after the study is discontinued and the applicable national and local health authorities are notified.

Following closure of the study, each participating site will maintain a copy of all site study records in a safe and secure location. The Lead site will inform the investigator at each site at such time that the records may be destroyed.

16. Publications

Any manuscript or releases resulting from the collaborative research must be approved by the investigator and will be circulated to applicable participating sites/investigators prior to submission for publication or presentation. A publication plan consistent with the international Committee of Medical Journal Editors (ICMJE) will be created prior to analysis ad publication of any data. All data will be made available to authors as required. The publication of sub-studies, post-hoc analyses, or single-center experiences will not precede the primary multicenter publication. Publication of results will be determined by the investigators, without limitations from the funder. Input (non-binding) will be obtained from the funder who will be given access to the data after closure of the study. All authors are expected to disclose financial or affiliations that could be considered conflicts of interest per journal or medical society requirements.

17. Citations

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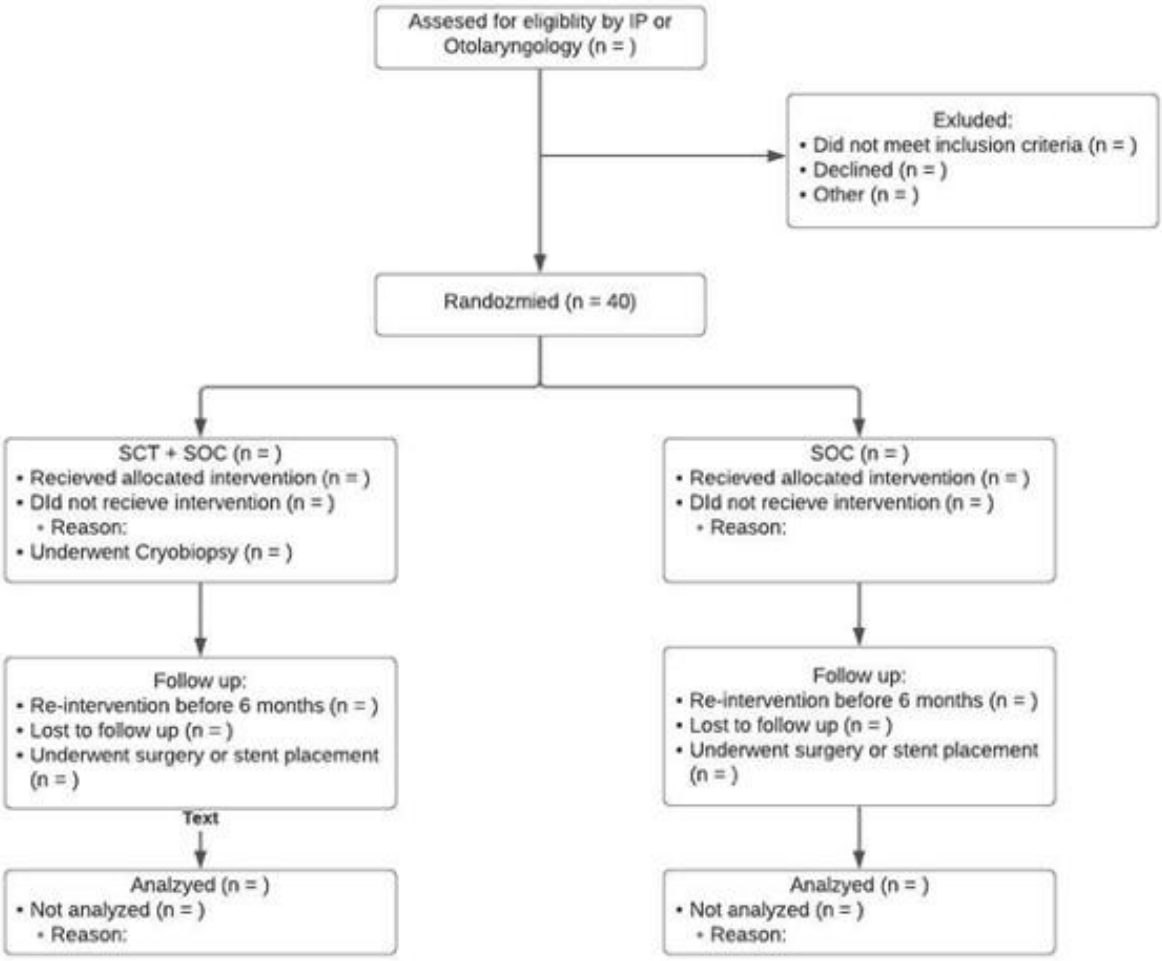
18. Consort Diagram



20. Study Assessment Table				
Study Procedures (Subject level)	Pre-Procedure	Post Procedure	3 months	6 months
Pathology review/Cryobiopsy		Non-SOC (1)		
CT scan (Chest)		Non-SOC (1)		SOC (1)
Physician's Time/Exam	SOC (1)			SOC (1)
Bronchoscopy	SOC (1)			
Questionnaire Delivery	Non-SOC (1)	Non-SOC (1)	Non-SOC (1)	Non-SOC (1)
PEF	Non-SOC (1)	Non-SOC (1)	Non-SOC (1)	Non-SOC (1)

Study Procedures (Site level)	
Site start up	Non-SOC (1)
IRB Cost	INV
Radiology Review setup	Non-SOC (1)

Serious adverse events	INV
Archiving/Document storage	Non-SOC (1)
Statistician	Non-SOC (1)
Segmentation and Volumetric Analysis	Non-SOC (1)
RedCAP Development	Non-SOC (1)

21. COPD Questionnaire (CCQ)

CLINICAL COPD QUESTIONNAIRE

Please **circle** the number of the response that best describes how you have been feeling during the **past 7 days**. (Only **one** response for each question).

On average, **during the past 7 days**, how often did you feel:	never	hardly ever	a few times	several times	many times	a great many times	almost all the time
1. Short of breath while **at rest**?	0	1	2	3	4	5	6
2. Short of breath while **doing physical activities**?	0	1	2	3	4	5	6
3. **Concerned** about getting a cold or your breathing getting worse?	0	1	2	3	4	5	6
4. **Depressed (down)** because of your breathing problems?	0	1	2	3	4	5	6
In general, **during the past 7 days**, how much of the time:							
5. Did you **cough**?	0	1	2	3	4	5	6
6. Did you **produce sputum or phlegm (chest mucus)**?	0	1	2	3	4	5	6
On average, **during the past 7 days**, how limited were you in these activities **because of your breathing problems**:	not limited at all	very slightly limited	slightly limited	moderately limited	very limited	extremely limited	totally limited /or unable to do
7. **Strenuous physical activities** (such as climbing stairs, hurrying, participating in sports)?	0	1	2	3	4	5	6
8. **Moderate physical activities** (such as walking, housework, carrying things)?	0	1	2	3	4	5	6
9. **Daily activities at home** (such as dressing, washing yourself)?	0	1	2	3	4	5	6
10. **Social activities** (such as talking, being with children, visiting friends/relatives)?	0	1	2	3	4	5	6