Spiration

CPR-05394, Revision AD,
ANET Electrosurgery Applicator
Pilot Evaluation Study

Gyrus ACMI, Inc., d/b/a Olympus Surgical Technologies America (OSTA)
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CERTIFICATION AND CONFIDENTIALITY AGREEMENT:

I have read and agree to conduct this study in compliance with the approved investigational plan, Good Clinical Practices, and applicable regulatory requirements, as indicated by the investigator and the ethics committee. This investigational plan contains confidential proprietary information with respect to Gyrus ACMIproducts and this clinical study.

_____
Print Name

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Signature of Investigator *Date*

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Institution Name

ANET ELECTROSURGERY APPLICATOR PILOT EVALUATION STUDY

STUDY OBJECTIVES	Evaluate the preliminary safety and performance of the ANET Electrosurgery Applicator (ANET) during and after bronchoscopic ablation of a target pulmonary nodule/tumor.
PRIMARY ENDPOINT	Safety provided by the evaluation of peri-procedural, device-related adverse events produced by the ANET device used to ablate a target pulmonary nodule/tumor.
SECONDARY ENDPOINTS	1. Pathological and histological evaluation of the acute thermal effect, produced by the ANET device, on targeted pulmonary nodule/tumor and surrounding tissue to determine if the observed zone of ablation is localized and consistent with the predicted zone of ablation. 2. Characterize any effects on surrounding tissue outside the zone of predicted ablation.
OBSERVATIONAL ENDPOINT	Characterization of possible immune response changes.
STUDY POPULATION	Adult subjects with confirmed Stage I or Stage II primary lung cancer with a pulmonary nodule/tumor suitable for ablation by the ANET device via EBUS bronchoscopy, prior to planned surgical resection (lobectomy or greater).
STUDY SIZE	Up to 10 subjects will be treated, at up to 5 sites.
STUDY DESIGN	This is a first in human use pilot study. Study subjects who are already scheduled for a surgical resection of their target nodule/tumor will be treated with the ANET RF Applicator. Blood will be drawn pre and post ablation, and at 2-4 weeks post surgery. Following ANET ablation, the subject will undergo lung resection as scheduled. The ablated target nodule/tumor will undergo gross histopathologic assessment. and may undergo immuno-histochemical assessment and microenvironment assessment for immune response.
INCLUSION CRITERIA	1. Subject with Stage I or Stage II primary lung cancer 2. Pathological proof of target nodule/tumor type and malignancy with specimen considered adequate for mutational analysis per institutional laboratory standards 3. Target nodule/tumor which can be accessed via EBUS bronchoscopy 4. Resection/surgical candidate (lobectomy or greater) as standard of care 5. Participants must be at least 22 years old and able to provide consent

EXCLUSION CRITERIA	1. Subjects in whom flexible bronchoscopy is contraindicated 2. Target nodule < 1.0 cm 3. Prior radiation or neo adjuvant chemotherapy of the target nodule/tumor 4. Any comorbidity that the investigator feels would interfere with the safety of the subject or the evaluation of study objectives 5. Pacemaker, implantable cardioverter, or other electronic implantable device 6. Patients with coagulopathy 7. Patients in other therapeutic lung cancer studies 8. Subject is pregnant or breastfeeding 9. Known or suspected sensitivity or allergy to nickel
TREATMENT ALGORITHM	The target nodule/tumor depth will be measured under EBUS visualization. The applied energy will be determined based on the nodule/tumor size using guidance from the Instructions for Use (IFU) to create a measurable and predictable ablation zones. A single ablation zone will be created in the first 3 subjects. The applied energy and number of ablation zones for the remaining subjects will be determined based on the nodule/tumor size and physician defined target ablation zone(s).
PATHOLOGY ASSESMENT	The ablated target nodule/tumor and surrounding tissue will be resected. The specimens will be examined by gross, histopathologic, and may be assessed by immuno-histochemical methods. Routine staining will be performed with hematoxylin and eosin (H&E). Analysis of cellular vitality may be performed with different methods of immunostaining which may include one or more of the following: including mouse antihuman mitochondria monoclonal antibody (MAB 1273), TUNEL, Ki167 PHH3, and Masson's trichrome. The pathology assessment will be performed by an experienced pathologist.
IMMUNE RESPONSE	Blood will be drawn pre and post ablation to assess for immune response indicators The micro environment of the resected tissue may have pathological assessment for immune cell population to explore a potential mechanistic effect of immune response.
FOLLOW-UP	Subjects will be followed peri-operatively for safety. There is no long-term follow-up since the ablated tissue will be resected during the scheduled post-ablation surgical procedure, however adverse events will be captured 30 days post-procedure.

	There will be a blood draw 2-4 weeks following surgery, during the standard post-surgery visit,
STUDY DURATION	Approximately 2 years

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1 INTRODUCTION

The American Cancer Society's estimates for lung cancer in the United States for 2013 are:

- About 228,190 new cases of lung cancer will be diagnosed (118,080 in men and 110,110 in women).
- There will be an estimated 159,480 deaths from lung cancer (87,260 in men and 72,220 among women), accounting for about 27% of all cancer deaths.

Lung cancer is by far the leading cause of cancer death among both men and women. Each year, more people die of lung cancer than of colon, breast, and prostate cancers combined.

Early detection, diagnosis and treatment of lung cancer is the largest unmet need in pulmonary medicine. Surgical resection is the treatment of choice for patients with Stage I and Stage II lung cancer[1]. However, over 20%[2] are not suitable for resection due to comorbidities[3].

Localized energy destruction of tumors (ablation or coagulation) in other organs is a well-established technique, and initial feasibility pre-clinical and clinical evaluations have been performed in lungs.[4,5] Percutaneous RF ablation is currently a therapeutic option for patients not appropriate for surgery, particularly individuals with poor pulmonary reserve[6], however, treating central and hilar nodules and tumors with percutaneous RF is a contraindication for this therapy.

Bronchoscopic ablation of lung cancer may provide benefits for patients not appropriate for or who decline surgery with Stage I or Stage II hilar/central tumors and nodules.

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[1] Dennis A. Casciato, ed., *Manual of Clinical Oncology*, Seventh edition (Philadelphia: LWW, 2012).

[2] Amgad El-Sherif et al., "Outcomes of Sublobar Resection versus Lobectomy for Stage I Non-Small Cell Lung Cancer: A 13-Year Analysis," *The Annals of Thoracic Surgery* 82, no. 2 (August 2006): 408-415; discussion 415-416, doi:10.1016/j.athoracsur.2006.02.029.

[3] Jean Deslauriers MD FRCPS, F. G. Pearson MD, and Farid M. Shamji MD, *Lung Cancer, Part I: Screening, Diagnosis, and Staging, An Issue of Thoracic Surgery Clinics, 1e*, 1 edition (London: Elsevier, 2013).

[4] Hidemi Suzuki et al., "Innovative Technique of Transbronchial Radiofrequency Ablation for Intrapulmonary Tumors: A Preliminary Study in a Rabbit Model," *Journal of Bronchology & Interventional Pulmonology* 18, no. 3 (July 2011): 211–17, doi:10.1097/LBR.0b013e318229671b.

[5] Ralf Eberhardt, Nicolas Kahn, and Felix J. F. Herth, "'Heat and Destroy': Bronchoscopic-Guided Therapy of Peripheral Lung Lesions," *Respiration* 79, no. 4 (2010): 265–73, doi:10.1159/000284015.

[6] Thierry de Baere, Geoffroy Farouil, and Frederic Deschamps, "Lung Cancer Ablation: What Is the Evidence?," *Seminars in Interventional Radiology* 30, no. 2 (June 2013): 151–56, doi:10.1055/s-0033-1342956.

Lethal RF ablation zones extending beyond a tumor cannot adequately be characterized by imaging[7]. Therefore, "Treat and Resect" studies have been used to characterize tissue response to RF ablation of lung tissue. Jaskolka, et al[8] studied patients in which metastases were resected 2-4 weeks after percutaneous RF treatment and demonstrated support for RF ablation as an effective treatment for select pulmonary metastases. Clasen, et al[9] characterized the pathomorphology of tissue response for pulmonary malignancies treated with RFA, followed by resection 3 days later confirmed in their study that 10 (90.9%) cases of the tumor tissue was completely ablated, however in 2 cases a safety margin was absent. Schneider, et al[10],[11] completed studies in which RF neoplasms were treated with RF intraoperatively, then immediately resected. In both studies they were able to histopathically characterize the ablation zone. Local control of the neoplasms was determined effective in 38% and 89% of the tumors treated, demonstrating that it should not replace surgery, but RFA was safe and may be an option for patients who are not candidates for surgery.

[7] Sophie Chheang et al., "Imaging Features Following Thermal Ablation of Lung Malignancies," *Seminars in Interventional Radiology* 30, no. 2 (June 2013): 157–68, doi:10.1055/s-0033-1342957.

[8] Jeffrey D. Jaskolka et al., "Pathologic Assessment of Radiofrequency Ablation of Pulmonary Metastases," *Journal of Vascular and Interventional Radiology: JVIR* 21, no. 11 (November 2010): 1689–96, doi:10.1016/j.jvir.2010.06.023.

[9] Stephan Clasen et al., "Pathomorphologic Evaluation of Pulmonary Radiofrequency Ablation," *Cancer* 113, no. 11 (December 1, 2008): 3121–29, doi:10.1002/cncr.23882.

[10] Thomas Schneider et al., "Intraoperative Radiofrequency Ablation of Lung Metastases and Histologic Evaluation," *The Annals of Thoracic Surgery* 87, no. 2 (February 2009): 379–84, doi:10.1016/j.athoracsur.2008.10.088.

[11] Jaskolka et al., "Pathologic Assessment of Radiofrequency Ablation of Pulmonary Metastases."

2 DEVICE DESCRIPTION

2.1 Device Overview

The Active Needle Endoscopic Treatment (ANET) Applicator is a disposable bipolar electrosurgical applicator used for the coagulation and necrosis of soft tissue. Each Applicator consists of a 19 gauge needle (proximal electrode) with a coil (distal electrode). The needle and coil serve as the bipolar electrodes, so there is no need for an external ground pad.

The ANET Applicator works with ancillary equipment which include an ultrasound endoscope and a compatible electrosurgical generator. The ANET Applicator has been shown to be compatible with the previously cleared/approved Olympus ESG-100 or Olympus ESG – 300 Electrosurgical Generator. Other radiofrequency generators will be added as compatibility is established.

A standard infusion pump can be used to infuse small quantities of saline during active ablation. Saline is administered to cool the electrodes during ablation, and to prevent the tissue surrounding the target tissue from dehydration.

To perform ablation, the flexible portion of ANET device is first inserted into an ultrasound endoscope with a working channel of 2.2mm or greater, then locked onto the adapter biopsy valve on the endoscope using the endoscope adapter on the handle. The handle facilitates advancement of the sheath and needle/proximal electrode during puncture of the target tissue. Once the needle is positioned in place, the handle is used to facilitate advancement and of the coil. The coil in the target tissue and helps to hold the device in place during treatment.

To perform ablation, the bipolar plug on the ANET Applicator is connected to the compatible electrosurgical generator. The generator is set to the appropriate power level included in the Instructions for Use (IFU). During ablation, radiofrequency (RF) current is passed between the distal electrode and the proximal electrode to thermally coagulate the tissue. Once the target energy has been reached, ablation is stopped, and the electrodes are retracted from the target. Additional ablations can be performed to achieve a larger coagulation zone.

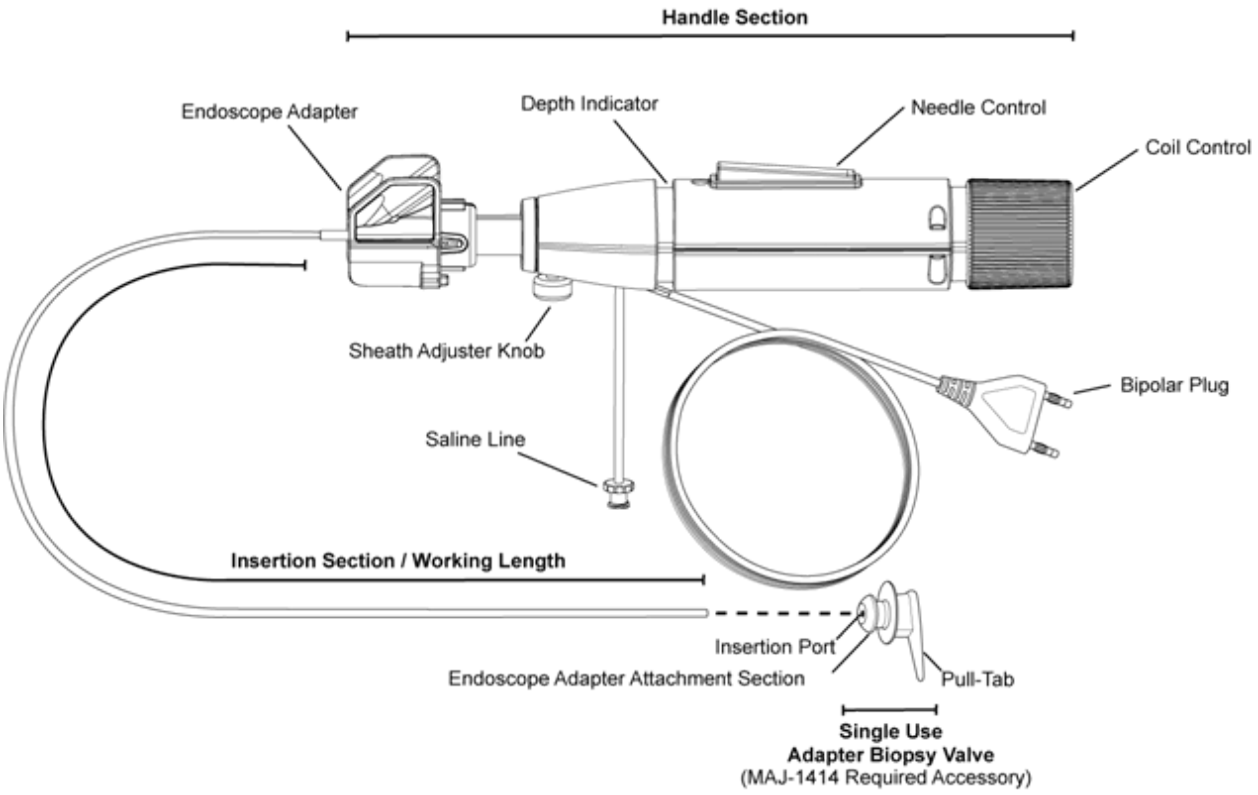


Figure 1: ANET Applicator with Identification of All Components

2.2 Handle

The handle consists of the electrical and fluid connectors, the coil control, the needle control, the sheath adjuster knob, and the endoscope adaptor (**Figure 1**). The handle includes error-proofing features assist the user in performing the advancement and retraction steps in sequence.

An insulated bipolar plug connects the ANET to a compatible electrosurgical generator which delivers RF energy to the applicator. The saline line consists of a flexible silicon tube with standard luer fitting which delivers small quantities of saline through the needle tip during active ablation to cool the electrodes and to prevent tissue dehydration adjacent to the electrodes. The handle contains a saline manifold which routes saline from the saline line to the inside of the needle for delivery.

The handle affixes to the endoscope via the endoscope adaptor, which locks the device onto the biopsy valve adapter. The needle control controls the needle's extension out from the sheath. The needle can be advanced up to 35mm from the sheath. The position of the needle relative to the sheath is indicated by the depth indicator on the handle.

The coil control advances and retracts the coil relative to the needle. Sheath

The sheath, which houses the needle, consists of a single lumen, extruded PTFE tube. The sheath protects both the needle tip and endoscope during device

insertion into and retraction from the endoscope. The sheath also electrically insulates the electrodes from the endoscope (**Figure 2**).

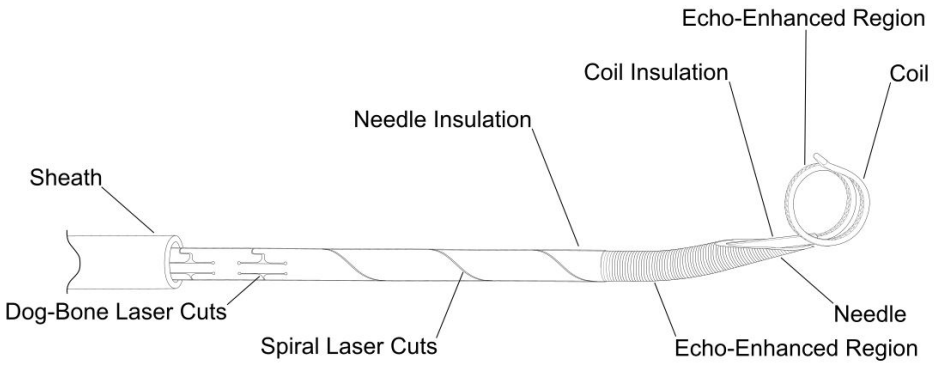


Figure 2: Sheath/Needle Tip

2.3 Needle Electrode

The needle electrode is a 19 gauge hypotube needle assembly located within the sheath. To penetrate the target tissue, the hypotube assembly is advanced beyond the sheath. The needle's distal end is machined to a Huber point bevel form to easily penetrate through the tissue with minimal coring.

The Huber tip is bent on the distal end to create a curved path. The curve is designed to orient the coil as it is advanced into the tissue. The 19G hypotube has several different laser cut patterns for added flexibility. A spiral cut on the distal portion of the 19G hypotube provides strength and flexibility during penetration into the target tissue. A laser cut "dog bone" pattern provides single plane bending and aligns the needle relative to the endoscope during penetration. The laser cut region is fully covered by a layer of heat-shrink tubing to prevent saline leakage through the laser cuts and ensure that saline is delivered to the very distal end of the needle, where ablation is occurring. The most distal 9mm of the 19G hypotube has a shallow spiral groove on the outer surface, which reflects the endoscope's ultrasound waves. The echo-enhanced region is intended to increase visualization at the tip, e.g., where the needle is located within the target tissue, and thereby help prevent penetration beyond the targeted tissue.

After the needle tip has been inserted into the target tissue, the coil is advanced. The position of the needle tip determines where the coil will penetrate tissue.

2.4 Coil Electrode

The coil electrode is composed of an insulated Nitinol flat wire and extends the entire length of the device, through the inner lumen of the handle and needle electrode.

The distal end is formed into a coil shape to anchor the electrodes into the target tissue during coagulation. The tip of the coil is cut at a 45 degree angle to aid in tissue penetration. The coil has many shallow divot features, which reflects the

endoscope's ultrasound waves. The echo-enhanced region is intended to increase the visualization of the coils, e.g., where the coil is located within the target tissue, and thereby help prevent penetration beyond the targeted tissue structure.

The coil is gold plated from the proximal end point of the divots to the proximal end of the device; the purpose of the gold plating is to improve the conductivity of the coil. Due to the bipolar nature of the ANET device and the co-axial construction of the electrodes, the coil is electrically insulated from the needle with a polyethylene terephthalate (PET) heat shrink tubing.

2.5 Ancillary Supplies

- ***Electrosurgical generator*** - The ANET Applicator is compatible with the Olympus ESG-100 and Olympus ESG-300 Electrosurgical Generator.
- ***Ultrasound endoscope*** – The ANET Applicator is compatible with an ultrasound endoscope with an inner working channel of 2.2mm or greater.
- ***Olympus biopsy valve*** – Model # MAJ-1414

2.6 Packaging and Sterilization

In its packaged configuration, the device is held in a plastic tray with a lid (made of LDPE and HDPE [Tyvek]).The sealed tray is packaged inside a dust cover box. Each dust cover box contains one device inside and an Instruction for Use (IFU).

The device is sterilized by ethylene oxide (EO) sterilization.

3 PRIOR ANIMAL STUDIES

These GLP studies were conducted in a swine model with lung pseudotumors for early evaluation of the ANET-RFA Applicator, produced by Spiration Inc. (test device) via bronchoscopy access. The primary goal of these studies was to evaluate the ability of the test device (ANET RF applicator) to deliver energy into pseudo-tumors in swine lungs and produce a thermal effect without substantial concurrent damage to the surrounding healthy tissue. Two studies were performed. A short term study, in which the animals were followed for 7 days post ANET ablation procedure, and a long term study, in which the animals were followed for 3 months post procedure. After the follow-up period, the animals were euthanized and the treated tissue was resected and underwent pathologic assessment.

Study Limitations

There are no large animal lung cancer models available for testing, therefore a pseudotumor model was used. The pseudotumors used in the animal model for these studies are mostly a dense proliferation of macrophages, which do provide a dense tissue target but are not cancer and therefore, while representative of the thermal cytocidal response pattern, may not represent the actual ablation of cancer cells.

These studies were done in a small number of healthy animals (6). This number is too small for any meaningful statistical analysis.

Short Term Study

This GLP study was conducted under an IACUC approved protocol, SP-1602 GLP: RF Energy Ablation of Lung Pseudotumors Study in Yorkshire-Cross Farm Swine via Bronchoscopy (One Week Evaluation) and in accordance with Spiration's protocol NCS-05097.AA. The study was conducted at the Care Research and Colorado Histo-Prep facilities in Fort Collins, CO.

The test subjects were 6 domestic swine with induced pseudo-tumors in the lungs. A total of 6 pseudo-tumors were ablated, 1 in each swine with 1 pseudo-tumor left un-ablated in the opposite lung as a control. One extra animal was kept in reserve for the duration of the study as a backup. The pseudo-tumors were located and measured by EBUS bronchoscope and one per animal selected for RF ablation using the ANET RF applicator. The amount of energy delivered was based on the depth of the lesion as measured by EBUS, and determined by a predefined algorithm. During the thermal ablation the applicator electrodes are cooled with a flow of sterile physiological saline solution which conducts some of the heat away from the center of the bipolar ablation zone while also helping to maintain conductivity of the ablated tissue. With the rapid cooling of the saline as it expands away from the electrodes, no addition thermal tissue damage can be detected and the saline is absorbed by the tissue vasculature. The ANET applicators all functioned properly, and delivered the pre-determined amount of energy to the target location. Placement, visualization and subsequent removal of the electrodes in the pseudotumor targets was accomplished without complication.

After ablations, the swine were recovered and monitored for peri- or post-procedural complications and general health for 7 days, which according to the study pathologist was the optimal time to observe any cytocidal effects in the tissue. Each animal was then euthanized and a gross necropsy performed by the Study Board Certified Veterinary Pathologist. Lung tissue was removed, fixed, trimmed and processed into stained slides. The Study Pathologist then performed histopathological and histomorphometric evaluations of the H&E and trichrome stained slides and evaluated a panel of health screen organs for any findings.

The animals were healthy for the duration of the study; all gained weight, and had hematology and clinical chemistry values which were consistent with animals in good health.

The bronchoscopic follow up at 7 days revealed normal airways with only minimal hyperemia noted at the ablation penetration sites. At necropsy, direct observation of the thoracic cavity revealed no significant findings in 5 of the 6 swine, with only mild irregular consolidations in the lungs which were determined by the pathologist to be related to anesthesia and not the ablation procedure. One swine (1506) had chronic resolved pleuritis with adhesions and chronic resolved pericarditis with adhesions, both of which resulted from a prior infection that had resolved with no adverse effect on this study. This animal also had marked bilateral peri-acute alveolar hemorrhage with dorsal gravitational congestion due to the animal being on its back terminally. These findings were compatible with a terminal overdose of IV heparin with an extended period between the injection and euthanasia. No other gross findings were detected in either the thorax or the abdomen of the animals.

Histopathology of the ablated pseudotumors and surrounding tissues revealed a localized thermal ablation effect with no evidence of adverse effects. There was some variation in the pattern of the thermal effect between different swine, however the surrounding vascularized pulmonary tissue rapidly cools the infused saline so that the ablation effects are limited to the target area and immediate vicinity. There was no evidence of secondary bacterial infection in any of the bronchus penetration sites or in the ablated areas of the induced pulmonary pseudotumor. Where bronchoscopic observations implied thermal damage to the mucosa at the ANET penetration site, there was healing of the bronchus wall with active mucosal re-epithelization. This study demonstrates that even with site to site variation there is overall consistency in the ablation effects. These changes were focal and limited to the pseudotumor itself and the immediately surrounding tissues.

Long Term Study

This study was a long-term version of CARE Study SP-1602 that evaluated minimally invasive interventions over a short term (one week). This GLP 3 month study employed 6 (plus 1 extra) female Yorkshire-Cross farm swine from a previous Non-GLP study conducted at CARE (SP-1601). In that study, bronchoscopic techniques and instrumentation were used along with fluoroscopy to inject paste mixtures of barium sulfate and talcum powder into accessible lung

parenchyma locations of female Yorkshire-Cross farm swine to produce lung pseudotumors. Six of these animals that had been identified as having a minimum of one 1 – 3 cm in diameter pseudotumor accessible in both the right and left lung were assigned to this study. Details regarding the creation of the lung pseudotumors are in the CARE Non-GLP protocol and Summary Document for that project (CARE Study SP-1601).

RFA with the test device was performed by the sponsor in 2 staggers with Study Day 1 as the day that each animal was subjected to the ablation procedure as follows: Each animal was anesthetized. Fluoroscopy was used to identify the location of the lung pseudotumors and bronchoscopic examination of the airways was done using a diagnostic scope. Lung pseudotumors in the lung parenchyma were located and their depth determined by ultrasound techniques using the EBUS bronchoscope. Any targets that could be accessed and seen by the ultrasound function of the EBUS bronchoscope were selected for possible bronchoscopic ANET- RFA Applicator treatment. Energy was delivered to one pseudotumor per animal per a predefined algorithm based on the size of the lesion as determined by EBUS, using the applicator (ANET-RFA) developed by the sponsor (test device) to the test pseudotumor. A similar pseudotumor was selected in the opposite lung and left untreated (non-ablated) to serve as control. Once completed, the energy device was retracted and removed. No treatment was provided to the selected pseudotumor in the opposite lung. No acute adverse events were observed or recorded. Each animal was recovered from anesthesia and kept alive for 3 months before being euthanized 85 days post-ablation for animal 1501 and 86 days for animals 1502-1506. Prior to euthanasia, each animal was anesthetized and subjected to bronchoscopy and fluoroscopy to observe and document the condition of the ablated and non-ablated control pseudotumor locations. Clinical observations were conducted daily. Body weights were recorded on Study Day 1, 3, and weekly thereafter post-RFA, excluding Week 12.

Following euthanasia, the pathologist conducted the necropsy and tissue collection, and grossly evaluated the tissue samples, including those collected from the pseudotumor treated with the ANET-RFA Applicator and the non-ablated control sites. The extent of thermal effects to the ablated pseudotumor and pulmonary parenchyma tissue surrounding the primary treatment site were characterized for comparison with the non-ablated control sites by gross examination, pre-fixation fluoroscopic imaging and post histology examination. This included any possible effects due to the perfusion of heated saline coolant within the lung parenchyma. Other evaluations included blood hematology and chemistry, and standard tissue pathological evaluations.

Based on the results of this study, the minimally invasive bronchoscopic radiofrequency ablation (RFA) of induced lung pseudo-tumors in female Yorkshire-Cross farm swine using the ANET-RFA Applicator (test device) was well tolerated when the animals were evaluated for 3 months following the ablation procedure. This study demonstrates that even with site to site variation there was overall consistency in the ablation effects, and the rapid cooling of the heated saline by the surrounding pulmonary tissue so any complete or selective ablation effects were limited to the BNTM target area and immediate vicinity. All animals remained

essentially normal throughout the study and did not exhibit abnormal clinical signs, body weight effects, hematology or chemistry changes from Day 1 to the end of the study, or toxicologically relevant gross necropsy observations. In addition, pathology evaluation of health screen tissues that were collected (included kidneys, liver, gallbladder, spleen, urinary bladder, adrenals, stomach, small intestine, pancreas, large intestine, heart, lung, parabronchus lymph nodes, and mesenteric lymph nodes) did not reveal adverse treatment related effects.

The gross pathology and histopathological analysis of the lung tissues in this study indicates that the ANET RFA Applicator provided by Spiration was safe and effective in this BNTM model of pseudotumors when evaluated 3 months after its use. The ANET ablation of the BNTM histiocytic granulomas had similar predictable identifiable patterns of localized thermal ablation effects. During the thermal ablation the coil area of the device was flushed with sterile physiological saline as a coolant to prevent overheating of the device tip. This very small amount of heated saline flowed along a path of least resistance, and as it flowed it is cooled rapidly by the viable vascularized pulmonary tissue and the movement of air through the respiratory tree. Within a short distance from the ANET electrodes, the heated saline cooled uniformly and destroyed tissue by thermal coagulation, and then as the saline cooled to the tumoricidal temperature, the thermal damage became selective, and created a zone where only the most sensitive cells such as carcinoma, skeletal, and smooth muscle cells were selectively destroyed. With the rapid cooling of the saline no additional morphological thermal tissue damage could be detected, and the saline was absorbed by the tissue vasculature. The flow of this cooled saline appeared to contain proteinaceous debris from the "up-stream" ablated tissue, which stimulated the alveolar macrophages and fibroblast in the lung parenchyma to slightly thicken without any thermal effects, and this morphological change allowed the tracking of the cooled saline flow pattern in the pulmonary tissue; however, this subtle change was almost completely resolved in this long term chronic study as compared to the short term study conducted earlier SP-1602. There was no evidence of secondary bacterial infection in any of the bronchus penetration sites or in the ablated areas of the induced BNTM pulmonary pseudo-tumor. Where sections were collected through the bronchus wall at the ANET RF Applicator penetration site, there was occasionally bronchus mucosal thermal damage due to back flow of the heated saline along the needle electrode, but this was limited to the immediate site of penetration, and there was early healing of the bronchus wall with active mucosal re-epithelization and submucosal fibrosis.

4 STUDY OBJECTIVES

4.1 Objectives

Evaluate the preliminary safety and performance of the ANET Electrosurgery Applicator (ANET) during and after bronchoscopic ablation of a target pulmonary nodule/tumor.

4.1.1 Primary Endpoints

Safety provided by the evaluation of peri-procedural device related adverse events produced by the ANET device used to ablate a target pulmonary nodule/tumor.

4.1.2 Secondary Endpoints

1) Pathological and immuno-histochemical evaluation of the acute thermal effect, produced by the ANET device, on targeted pulmonary nodule/tumor and surrounding tissue to determine if the observed zone of ablation is localized and consistent with the predicted zone of ablation.

2) Characterize any effects on surrounding tissue outside the zone of predicted ablation.

4.1.3 Observational Endpoint

Characterization of possible immune response changes.

5 STUDY PLAN

5.1 Study Design

This is a first in human use pilot study. Study subjects who are already scheduled for a surgical resection (lobectomy or greater) of their target nodule/tumor, as standard of care, will be treated with the ANET device. The target ablation zone will be entirely within the planned resection. Following ANET ablation, the subject will undergo their scheduled lung resection. The ablated target nodule/tumor will undergo gross, immuno-histochemical and histopathologic assessment.

5.2 General Description of Study

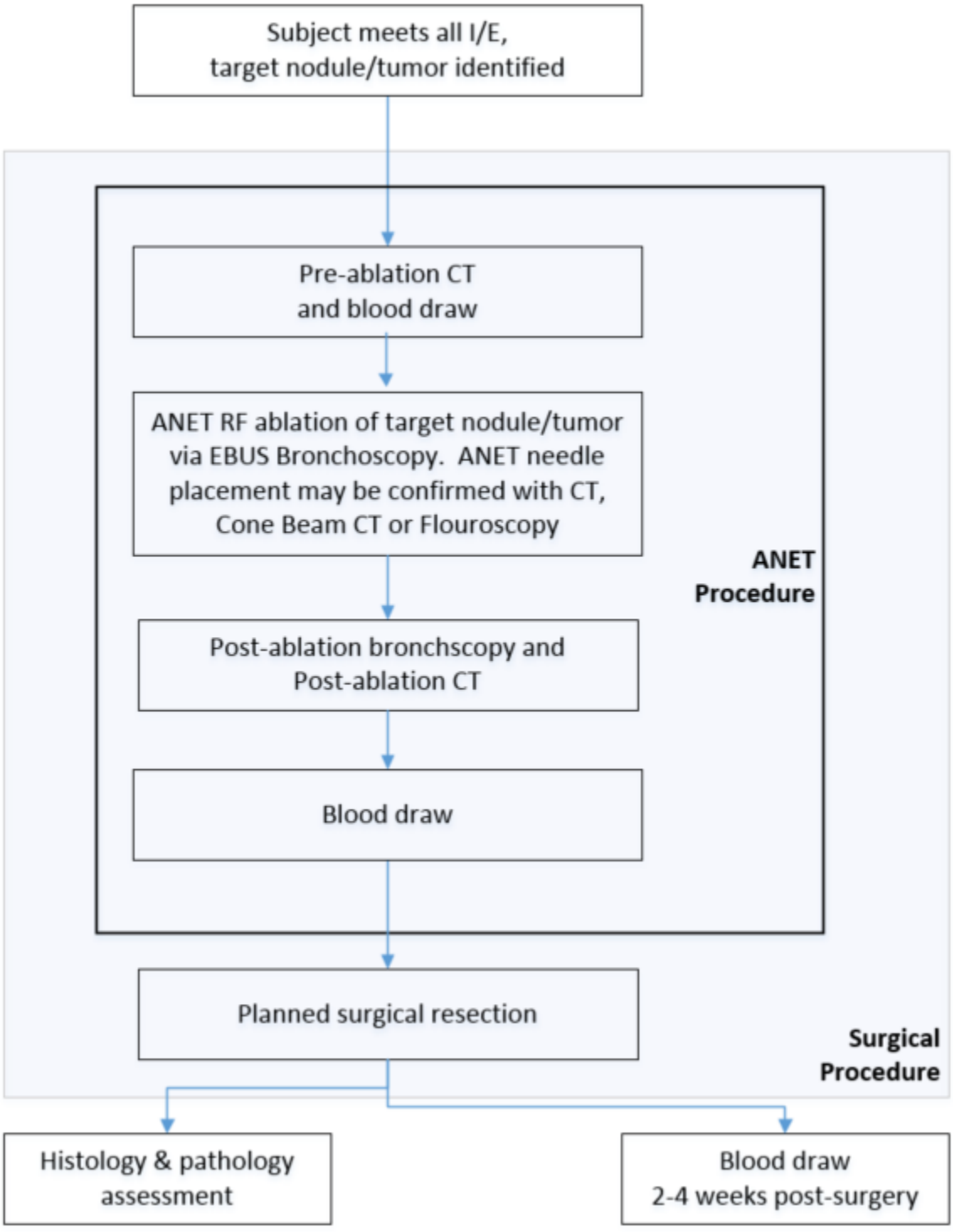
After consent and evaluation of all inclusion and exclusion criteria, subjects will be enrolled in the study. The subject will undergo standard pre-operative testing, per the study institutional requirements. At that time, subject vital signs will be recorded. The subject will be prepped for bronchoscopy.

A pre-ablation chest CT will be taken. The target nodule/tumor will be accessed via EBUS bronchoscopy, which may be guided and confirmed with Cone Beam CT, Intra-operative CT, or fluoroscopy, and ablated using the ANET Electrosurgical Applicator. Following ablation, there will be a bronchoscopic exam of the treated region and repeat chest CT will be taken.

Immediately following bronchoscopy and ablation of the target tissue, the subject will undergo the planned surgical resection. The ablated tumor will be prepared for histopathological and immuno-histochemistry evaluation to assess the area of ablated tissue. Subjects will be followed peri-operatively. There is no long-term follow-up since the ablated tissue will be resected during the surgical procedure, however adverse events will be captured through 30 days post procedure.

Blood will be drawn pre and post-ablation and 2-4 weeks post-surgery, per the ANET Blood Draw Lab Manual, DN0042775, to evaluate for potential immune response indicators. Blood specimens will be analyzed by a core laboratory to characterize cells important in immune responses and the disease process.

A study diagram is shown in Figure 4 below.



5.3 Study Duration

The study duration is anticipated to be approximately 2 years.

6 SUBJECT POPULATION

Adult subjects with confirmed Stage I or Stage II primary lung cancer with a pulmonary nodule/tumor suitable for ablation, by the ANET device via EBUS bronchoscopy, prior to planned surgical resection.

6.1 Eligibility Criteria

The criteria listed below shall be used to determine if an individual is eligible for enrollment in this clinical study.

6.1.1 Inclusion Criteria

1. Subject with Stage I or Stage II primary lung cancer
2. Pathological proof of target nodule/tumor type and malignancy with specimen considered adequate for mutational analysis per institutional laboratory standards
3. Target nodule/tumor which can be accessed via EBUS bronchoscopy
4. Resection/surgical candidate (lobectomy or greater) as standard of care
5. Participants must be at least 22 years old and able to provide consent

6.1.2 Exclusion Criteria

1. Subjects in whom flexible bronchoscopy is contraindicated
2. Target nodule < 1.0 cm
3. Prior radiation or neo adjunctive chemotherapy of the target nodule/tumor
4. Any comorbidity that the investigator feels would interfere with the safety of the subject or the evaluation of study objectives
5. Pacemaker, implantable cardioverter, or other electronic implantable device
6. Patients with coagulopathy
7. Patients in other therapeutic lung cancer studies
8. Subject is pregnant or breastfeeding
9. Known or suspected sensitivity or allergy to nickel

6.2 Assignment of Subject Identification

Each subject who has signed an informed consent will be assigned a subject ID number unique to the subject and study site.

7 STUDY PROCEDURES AND PATHOLOGY METHODS

7.1 Informed Consent

Subjects must sign the informed consent prior to enrollment. The original consent form will become an integral part of each case report form file. One copy will be retained with the subject's medical records and one copy will be provided to the subject.

7.2 Procedure

All study eligible subjects will be prepared for the bronchoscopic/surgical procedure according to the policies and procedures established by the institution.

Before starting the procedure, a "time out" review will take place. During the "time out" session, the nodule/tumor to be treated will be noted by all in attendance.

7.2.1 Pre-ablation CT and blood draw

A pre-ablation chest CT will be taken prior to ANET ablation, may be performed while the subject is under general anesthesia.

A pre-ablation blood draw will be taken to assess for immune response indicators

7.2.2 ANET Procedure

The ANET Applicator and RF generator will be prepared for use per the ANET Instructions for Use (IFU). ASA guidelines for the Prevention and Management of Operating Room fires will be followed, including:

- Discuss the use of RF prior to the procedure with the anesthesiologist and collaborate with procedure team throughout the procedure
- Use cuffed tracheal tube
- Before RF ablation:
    - Announce the intent to start RF ablation
    - Reduce the oxygen concentration to the minimum required to avoid hypoxia, guided by patient monitoring
    - Stop the use of nitrous oxide
- Wait a few minutes before authorizing the use of RF ablation
- Refer to institutional requirements

The nodule/tumor will be accessed via EBUS bronchoscopy. If the nodule/tumor cannot be accessed via EBUS, or nodule/tumor is considered inappropriate for ablation by the treating physician, the subject will proceed to surgery and will not count against enrollment.

Under visualization with the EBUS bronchoscope, the ablation zone path length of the nodule/tumor will be measured using the EBUS measurement tool. If the path length is less than 1 cm, then a new ablation zone in the target nodule/tumor will be identified. The center of the ablation zone, should be at least 2 cm away from any critical structures, as determined by the investigator.

If an acceptable ablation zone cannot be determined, the subject will be considered a treatment failure. Tissue ablation parameters will be determined by the IFU.

The ANET applicator will be advanced to the target nodule/tumor under EBUS visualization. After the needle has been advanced into the target location, position may be confirmed using cone beam CT, intra-operative CT or fluoroscopy. The needle should be deep enough in the tissue and not extend past the distal margin of the target nodule/tumor. If it is not possible to place the needle appropriately, the subject will be considered a treatment failure.

Once the needle is placed correctly, a fixed electrode spacing of 1mm should be maintained for ablation. The target nodule/tumor can then be ablated per the energy application guidelines in the IFU. Saline flow post-RF delivery must be maintained to bring the tissue next to the electrode to baseline temperatures (~ ≥ 120 s) and to arrest thermal damage to stated margins.

If the tumor is greater than 3.1 cm, than the energy parameters for no greater than a 3.1 cm ablation zone will be used.

7.2.3 Post-ablation bronchoscopic evaluation and CT

A post-ablation bronchoscopic evaluation and chest CT will be taken following ANET ablation, while the subject is under general anesthesia.

7.2.4 Post-ablation blood draw

A post-ablation blood draw will be taken to assess for immune response indicators. The blood draw should occur prior to the surgical resection.

7.2.5 Surgical Resection

Surgical resection will be performed as scheduled, per institutional guidelines.

Following resection, the ANET ablated tumor will be grossly evaluated and prepared for histopathologic and immuno-histochemistry evaluation of the treated tumor.

7.2.6 Pathology

The tumors will be bisected through the largest diameter in a right angle referring to the RFA electrode direction and marked to determine orientation of coagulation electrode immediately after the resection procedure. The specimens will be fixed in 10% neutral buffered formalin-solution and prepared for routine diagnostics and immuno-histochemistry.

The specimens will be examined by gross pathology, histopathology, and may be examined by immuno-histochemical methods. Routine staining will be performed with hematoxylin and eosin (H&E). Analysis of cellular vitality may be performed with different methods of staining, which may include one or more of the following: mouse antihuman mitochondria monoclonal antibody (MAB 1273; Millipore UK, Hertfordshire, UK)[12,13], Terminal deoxynucleotidyl transferase-mediated nick end-labeling (TUNEL; Boehringer Ingelheim, Germany),[14] antibody MiB1(Ki67; Dianova, Hamburg Germany),[15,16] PHH3[17], and Masson's trichrome[18][19] The immuno-histochemical criterion for cell death in is the lack of expression or proliferation/mitotic index markers in the treated and surrounding tissue. The intent of the pathology assessments is to determine if the observed zone of ablation is consistent with the predicted zone of ablation and to characterize any effects on surrounding tissue outside the zone of predicted ablation.

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[12] Schneider et al., "Intraoperative Radiofrequency Ablation of Lung Metastases and Histologic Evaluation."

[13] Thomas Schneider et al., "The Efficacy of Bipolar and Multipolar Radiofrequency Ablation of Lung Neoplasms - Results of an Ablate and Resect Study," *European Journal of Cardio-Thoracic Surgery: Official Journal of the European Association for Cardio-Thoracic Surgery* 39, no. 6 (June 2011): 968–73, doi:10.1016/j.ejcts.2010.08.055.

[14] Clasen et al., "Pathomorphologic Evaluation of Pulmonary Radiofrequency Ablation."

[15] Ibid.

[16] Pelosi et al. J Thorac Oncol. 2014;9: 273–284

[17] Ozturk Sari et al. Endocr Pathol. 2016 Jun;27(2):162-70

[18] Jie Ouyang et al. Utility of desmin and a Masson's trichrome method to detect early acute myocardial infarction in autopsy tissues, Int J Clin Exp Pathol 2010;3(1):98-105.

[19] T.D. Koreckij et al., Low Dose, Alternating Electric Current Inhibits Growth of Prostatic Cancer, The Prostate, Accepted 5 October 2009, Published online in Wiley InterScience (www.interscience.wiley.com).

The micro environment of the resected tissue may have pathological assessment for immune cell population to explore a potential mechanistic effect of immune response.[20].

The pathologic and immuno-histochemical effect of RFA on nodules/tumors and surrounding tissue will be evaluated by an experienced pathologist.

7.2.7 Post-surgical blood draw

A post-surgical blood draw will be taken 2-4 weeks post-surgery to assess for immune response indicators.

[20] M.A.J. Gorris, et al. Eight-Color Multiplex Immunohistochemistry for Simultaneous Detection of Multiple Immune Checkpoint Molecules within the Tumor Microenvironment J Immunol January 1, 2018, 200 (1) 347-354

8 RISK/BENEFIT ANALYSIS

Spiration has conducted an analysis of the potential benefits and risks of the ANET procedure and device operation on the patient population as defined in section 6 of this protocol.

8.1 Justification for Investigation

The safety of subjects participating in first in man studies is the paramount consideration in proceeding to clinical trials in man. Such subjects would not normally be expected to derive any therapeutic benefit.

The lack of observed safety effects from the pre-clinical studies have provided the benchmark for safety in this first in human study. The study has been designed to optimize knowledge gained regarding ablation therapy in human cancerous tissue without involving excessive numbers of subjects and while ensuring the safety of patients.

The study design is a treat/resect design wherein the ablated tissue is fully within the part of the lung being surgically removed and represents the standard of care for these patients with primary lung cancer.

Lastly, the study has limited the number of trial sites being used to incorporate centers of excellence in the treatment of lung cancer. All of these sites will be further trained on the use of the ANET device to minimize safety events.

8.2 Benefits

There are no direct benefits to patients in this study. However, information from this study may help patients in the future with Stage I, II lung cancer and with pulmonary metastasis who are not suitable for surgical intervention.

8.3 Risks

- Risks of ANET ablation procedure and device are listed in Table 2, section 9.2 of this protocol.
- Patients will receive up to 3 additional CT's as a result of this study. These include a CT prior to and after ANET ablation. An additional CT may be taken to confirm needle location during the ANET ablation procedure.

8.3.1 Risk Management

Risks associated with the use of the ANET device are minimized through device design, Investigator selection and training, pre-specified subject eligibility requirements and study monitoring to ensure adherence to this investigational plan. Specific strategies include:

- Clearly defining the subject inclusion/exclusion criteria

- Prior to treatment, adequate cancer specimen sufficient for mutational analysis will be collected
- AEs will be reported per section 9 of this investigational plan and will be reported and monitored throughout the course of the study
- The sites will be notified if unexpected adverse device effects and other safety concerns that could negatively impact the safety of subjects if such issues arise
- Ensuring that treatment and follow-up of subjects is consistent with standard and current medical practice.

9 ADVERSE EVENTS

9.1 DEFINITIONS (from ISO/FDIS 14155)

9.1.1 Adverse Event (AE)

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 which includes:

a. events related to the investigational medical device or the comparator; and

b. events related to the procedures involved

For users or other persons, this definition is restricted to events related to investigational medical devices.

9.1.2 Adverse Device Effect (ADE)

Adverse event related to the use of an investigational medical device.

NOTE 1- This includes any adverse event resulting from insufficiencies or inadequacies in the instructions for use, the deployment, the implantation, the installation, the operation, or any malfunction of the investigational medical device.

NOTE 2- This includes any event that is a result of a use error or intentional misuse.

9.1.3 Device deficiency

Inadequacy of a medical device related to its identity, quality, durability, reliability, safety or performance, such as malfunction, misuse or use error and inadequate labeling.

9.1.4 Investigational medical device

Medical device being assessed for safety or performance in a clinical investigation

NOTE: This includes medical devices already on the market that are being evaluated for new intended uses, new populations, new materials or design changes.

9.1.5 Serious Adverse Device Effect (SADE)

Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

9.1.6 Serious Adverse Event (SAE) (see Table 3.0 of Protocol Defined Serious

Adverse Events, below)

Adverse event that:

a) led to a death,

b) led to a serious deterioration in health that either:

1) resulted in a life-threatening illness or injury, or

2) resulted in a permanent impairment of a body structure or a body function, or

3) required in-patient hospitalization or prolongation of existing hospitalization, or

4) resulted in medical or surgical intervention to prevent life threatening illness or injury or permanent impairment to a body structure or a body function.

c) led to fetal distress, fetal death or a congenital abnormality or birth defect.

NOTE 1: This includes device deficiencies that might have led to a serious adverse event if a) suitable action had not been taken or b) intervention had not been made or c) if circumstances had been less fortunate. These are handled under the SAE reporting system.

NOTE 2: A planned hospitalization for pre-existing condition, or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered to be a serious adverse event.

9.1.7 Unanticipated Serious Adverse Device Effect (USADE)

Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report.

NOTE: Anticipated: an effect which by its nature, incidence, severity or outcome has been previously identified in the risk analysis report. Adverse events listed in Table 2 below are considered anticipated for this study.

9.2 Protocol Defined Potential Adverse Events

The following list of adverse events are anticipated given the nature of the EBUS procedure and the investigational device. Potential adverse event terms in Table 2 are referenced from MedDRA release Version 23.0. All terms are Preferred Terms (PT).

Table 2: Potential Adverse Events

MedDRA term
Ablation site reactions

MedDRA term
(MedDRA term: Administration site reactions)
Abscess formation (MedDRA term: Wound Abscess)
Anaesthetic complication
Arrhythmia
Atelectasis
Bronchial obstruction
Bronchopulmonary hemorrhage
Bronchospasm
Burning or charring – unintended (MedDRA term: Burn local)
Cardiac arrest
Death
Device failure
Dyspnea
Electric shock
Esophageal hemorrhage
Hematoma (MedDRA term: Post-procedural hematoma)
Haemolysis
Haemoptysis
Hemorrhage
Hemothorax
Hypoxemia
Infection (acute)
Laryngeal spasm
Localized allergic reaction or irritant reaction (MedDRA term: Administration site hypersensitivity)
Microbubble/air embolism
Pleural effusion
Pleuritic pain
Pneumo-mediastinum
Pneumonia
Pneumonitis
Pneumothorax
Post-procedural hemorrhage
Postoperative thoracic procedure complication

MedDRA term
Pulmonary edema
Pulmonary embolism
Pulmonary fistula
Respiratory failure
Systemic toxicity
Soft tissue necrosis
Tumor recurrence (MedDRA term: Neoplasm recurrence)
Tumor seeding (MedDRA term: Treatment related secondary malignancy)

Table 3: Adverse Event Categories

<table>
<tr><th>ADVERSE EVENTS</th><th>Non-device-related</th><th colspan="2">Device- or procedure-related</th></tr>
<tr><td>Non-serious</td><td>Adverse Event (AE)[a]
(3.2)</td><td colspan="2">Adverse Device Effect (ADE)
(3.1)</td></tr>
<tr><td rowspan="3">Serious</td><td rowspan="3">Serious Adverse Event (SAE)[b]
(3.37)</td><td colspan="2">Serious Adverse Device Effect (SADE)
(3.36)</td></tr>
<tr><td>Anticipated</td><td>Unanticipated</td></tr>
<tr><td>Anticipated Serious Adverse Device Effect (ASADE)
(3.42, Note)</td><td>Unanticipated Serious Adverse Device Effect (USADE)
(3.42)</td></tr>
<tr><td colspan="4">a Includes all categories.
b Includes all categories that are serious.</td></tr>
</table>

9.1 Adverse Event (AE) Reporting by Investigator

Peri-procedural adverse events, defined as from the start of the EBUS bronchoscopy through the planned surgical resection, will be collected up to 30 days post procedure using the AE paper case report form. AEs shall be reported in accordance with study institution requirements, local vigilance systems, 21CFR 803 21CFR 812.46, 21CFR 812.140, and 21CFR 812.150, and ISO 14155:2011, sections 8.2.5 and 9.8.

Since this is a limited pilot study, there is no Data Safety Monitoring Board. In the event of an incidence of massive hemoptysis, acute respiratory distress, or a death that is device or ANET procedure related, as defined by the Principal Investigator, the study will be paused and the IRB and FDA will be notified.

Onset of Multiple Adverse Events

If AEs occur concurrently, each event should be recorded on separate AE case report forms. However, if known at the time of reporting, a diagnosis should be recorded on the case report form rather than individual signs and symptoms (e.g., record congestive heart failure rather than dyspnea, rales and cyanosis). If a diagnosis is established from subsequent follow-up information post initial SAE reporting, the event term should be updated on the corresponding case report form to reflect the final diagnosis term.

If a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual adverse event should be recorded as a separate AE. Signs and symptoms that are considered unrelated to an encountered syndrome or disease should be recorded as individual AEs (e.g., if congestive heart failure and severe headache are observed at the same time, each event should be recorded as an individual AE on separate AE case report forms).

AEs occurring secondary to other events (e.g., sequelae) should be identified by the primary cause; a "primary" event, if clearly identifiable, and generally represents the most accurate clinical term to record as the AE event term.

Event Causality

All AEs will be assessed for relationship to the investigational device and/or the procedure on 2-point scale as follows:

Related:	The AE has a timely relationship to the device or procedure.
Not related:	There is no clear evidence that the AE has a relationship to the investigational device or procedure.

9.2 Serious Adverse Event (SAE) Reporting by Investigator

Investigators are required to report a serious adverse event (see criteria in Section 9.1.6), regardless of whether the event is considered anticipated or unanticipated to the Spiration Clinical Field Specialist (i.e. site monitor) assigned to investigational site within 2 working days from the time of initial awareness.

9.3 Unanticipated Serious Adverse Device Effect (USADE) Reporting by Sponsor

All SAEs that satisfy USADE criteria (see Section 9.1.7) will be reviewed by the study Medical Monitor, an independent physician consultant with subject matter expertise. The sponsor must report the results of an evaluation of an USADE to FDA and all reviewing IRBs and investigators within 10 working days after the sponsor first receives notice of the adverse device effect.

10 PROTOCOL DEVIATIONS AND VIOLATIONS

During the course of the study, any deviations or violations from this protocol will be properly logged and documented by the site.

11 Study Management

The clinical study will be conducted in accordance with the applicable Sponsor SOPs. The sponsor will establish a clinical study plan that is appropriate to this study and that ensures compliance with Food and Drug Administration (FDA and Health Canada) regulations, Good Clinical Practice (GCP), and any other applicable regulations.

The clinical study plan will establish and document practical working methods for the management and execution of the pivotal study. The plan will address study-specific: definitions, roles, responsibilities, documents, clinical site and investigator selection, site qualification, training, inventory management, CRO management, data management, clinical data and CRFs, safety reporting/AEs/SAEs, monitoring, randomization, record retention, clinical complaints and product returns, and applicable sponsor SOPs and work instructions.

11.1 Investigator Reports

All study investigators shall prepare and submit the following complete, accurate, and timely reports per 21 CFR 812.150(a):

1) *Serious Adverse Events.* Please see Section 9.2 for reporting parameters.
2) *Withdrawal of IRB approval.* An investigator shall report to the sponsor, within 5 working days, a withdrawal of approval by the reviewing IRB of the investigator's part of an investigation,
3) *Progress.* An investigator shall submit progress reports on the investigation to the sponsor, and the reviewing IRB at regular intervals, but in no event less often than yearly,
4) *Deviations from the investigational plan.* An investigator shall notify the sponsor and the reviewing IRB (see 56.108(a) (3) and (4)) of any deviation from the investigational plan to protect the life or physical well-being of a subject in an emergency. Such notice shall be given as soon as possible, but in no event later

than 5 working days after the emergency occurred. Except in such an emergency, prior approval by the sponsor is required for changes in or deviations from a plan, and if these changes or deviations may affect the scientific soundness of the plan or the rights, safety, or welfare of human subjects, FDA, and IRB approval is also required,

5) *Informed consent.* If an investigator uses a device without obtaining informed consent, the investigator shall report such use to the sponsor and the reviewing IRB within 5 working days after the use occurs,

6) *Final report.* An investigator shall, within 3 months after termination or completion of the investigation or the investigator's part of the investigation, submit a final report to the sponsor and the reviewing IRB, and

7) *Other.* An investigator shall, upon request by a reviewing IRB or FDA, provide accurate, complete, and current information about any aspect of the investigation.

11.2 Sponsor Reports

The study sponsor shall prepare and submit the following complete, accurate, and timely reports per 21 CFR 812.812.150(b):

1) *Unanticipated adverse device effects.* Please see section 9.3 for reporting parameters.

2) *Withdrawal of IRB approval.* A sponsor shall notify FDA and all reviewing IRBs and participating investigators of any withdrawal of approval of an investigation or a part of an investigation by a reviewing IRB within 5 working days after receipt of the withdrawal of approval,

3) *Withdrawal of FDA approval.* A sponsor shall notify all reviewing IRBs and participating investigators of any withdrawal of FDA approval of the investigation, and shall do so within 5 working days after receipt of notice of the withdrawal of approval,

4) *Current investigator list.* A sponsor shall submit to FDA, at 6-month intervals, a current list of the names and addresses of all investigators participating in the investigation. The sponsor shall submit the first such list 6 months after FDA approval,

5) *Progress reports.* At regular intervals, and at least yearly, a sponsor shall submit progress reports to all reviewing IRBs. In the case of a significant risk device, a sponsor shall also submit progress reports to FDA,

6) *Recall and device disposition.* A sponsor shall notify FDA and all reviewing IRBs of any request that an investigator return or otherwise dispose of any units of a device. Such notice shall occur within 30 working days after the request is made and shall state why the request was made,

7) *Final report.* In the case of a significant risk device, the sponsor shall notify FDA within 30 working days of the completion or termination of the investigation and shall submit a final report to FDA and all reviewing the IRBs and participating investigators within 6 months after completion or termination,

8) *Informed consent.* A sponsor shall submit to FDA a copy of any report by an investigator of use of a device without obtaining informed consent, within 5 working days of receipt of notice of such use, and

9) *Other.* A sponsor shall, upon request by a reviewing IRB or FDA, provide accurate, complete, and current information about any aspect of the investigation.

11.3 Clinical Study Monitoring Plan

Regular monitoring of study data at each site will be performed as defined by the study specific clinical plan. Individual sites will be monitored to verify that enrollment rate, data recording, and protocol adherence are satisfactory. The frequency of monitoring individual sites will fluctuate depending on enrollment rate and site performance. The details of frequency of visits, activities to be performed at each visit, etc., are part of the conduct of the study, and will be implemented on an ongoing basis during the course of the study, in accordance with the monitoring procedures and the study specific clinical plan.

Monitoring visits will be performed for the purposes of verifying adherence to the protocol and the completeness and accuracy of data entered on the CRF. The study monitor will verify CRF entries by comparing them with the primary source documents (hospital/clinic/office records), which will be made available for this purpose. The monitor will review the maintenance of regulatory documentation, device accountability, and any protocol deviations and/or violations. The monitor will also review the progress of the study with the investigator and other site personnel on a regular basis. At the end of the study, a close-out monitoring visit will be performed. Monitoring visits will be arranged in advance with site personnel at a mutually acceptable time. Sufficient time must be allowed by the site personnel for the monitor to review CRFs and relevant source documents. The coordinator and/or investigator should be available to answer questions or resolve data clarifications.

Monitoring visits will include study initiation visits, ongoing monitoring visits and study close-out visits, as described in the following sections.

11.3.1 Study Initiation Visits

The study monitor or designee will communicate with study site and identify a date, time and location to meet with the clinical investigator(s), coordinator, and other key personnel involved in the study. A memo will be distributed to all key personnel confirming the date, time, location and purpose of the visit along with a detailed agenda.

Prior to the study initiation site visit, the clinical site should submit the following documents to the study monitor, or have them available for collection during the visit:

- Signed Investigator Agreement,
- Curriculum vitae of investigator,
- Medical license of investigator,
- Signed Financial Disclosure Form, and
- IRB/Ethics committee approval documentation of protocol and informed consent form.

The study monitor will review the study protocol requirements with special emphasis on the inclusion and exclusion criteria, study objectives, safety and efficacy endpoints, AE reporting, participant withdrawal criteria, the visit schedule and required assessments, special clinical procedures, study stopping rules, and other key site requirements for study support.

The study monitor will review the site's procedures for obtaining and documenting the participant's informed consent, including who is responsible for this process and the location of the completed forms. A thorough review of the regulatory binder with site staff and investigator will be performed for completeness. The study monitor will review the protocol case report forms and the site's plans for maintaining adequate source documentation.

11.3.2 Study Interim Visits

The first monitoring visit after site activation normally will be conducted when the first few participants have been enrolled at the site. Since study monitoring is based on the rate of patient enrollment, the number of patients enrolled at each site, and a number of other variables, monitoring will be adjusted to reflect the needs of the study and the participating sites.

Prior to the site visit, the study monitor will review the protocol for critical aspects of the study for monitoring compliance. The study monitor will also review outstanding queries and missing forms, unresolved issues from the previous site monitoring visit (if

applicable), study signature log, regulatory checklist, and device accountability log, as well as any other project specific documents.

The study monitor will review demographic and eligibility criteria source documentation to ensure that all participants who have been enrolled in the trial were eligible. All data or events that: 1) are critical to the reliability of the study findings, specifically those data that support primary and secondary endpoints, 2) are critical to the safety and ethical treatment of study subjects, or 3) affect the integrity of critical data will be 100% source verified.
If possible, identified discrepancies will be corrected by the site staff during the visit, but may be corrected afterwards. Documentation will also be reviewed for AEs. Any protocol deviations or unreported AEs will be brought to the attention of the coordinator and/or investigator for immediate action. The study documentation to be kept in the study regulatory binder will also be reviewed routinely.

Informed consent forms will be reviewed to verify that the appropriate IRB/IEC approved consent has been signed by both patient and the person administering the consent, dated, and properly witnessed if this is required by the form used at the site.

Test articles will be reviewed at each visit and compared to the test article accountability log. The study monitor will be responsible to review the delivery and the inventory records to ensure that the quantity of the study product was shipped, received or returned as specified. The test article records will be compared with the participant records to ensure that proper device was received. The monitor will also verify that the test article is kept in a secure locked place.

The study monitor will conduct a summary meeting with the study investigator and/or the study coordinator to discuss the findings of the monitoring team, discrepancies found, corrected, and remaining, and any recommendations to improve data quality for future site visits. An attempt should be made to correct any discrepancies found on the case report forms or in the source documentation. These discrepancies will still be included in the monitoring report, as well as whether resolved or in need of resolution after the monitoring visit. In addition, the overall status of enrollment, amendments, and other protocol adherence issues are to be addressed.

After the interim site monitoring visit, the site will receive a summary of any data discrepancies as well as any other issues encountered. The summary will also describe any outstanding issues that will require the site’s attention.

11.3.3 Study Closeout Visits

The study closeout visit will occur once all subjects have completed the study through the long-term follow-up phase. A study closeout visit will include all of the elements identified as part of the interim monitoring visit information. It will also include a review of data not previously audited.

The study monitor will conduct a summary meeting with the study coordinator and the study investigator to discuss any discrepancies or other findings by the monitoring team.

At this meeting, an attempt should be made to resolve discrepancies in the source documentation requiring the investigator's revision. The study monitor will also review all regulatory requirements the site may have for closing this study with their IRBs and for the maintenance and retention period of study documentation.

A closeout site visit report will be prepared, with all outstanding issues listed. The site should resolve all issues within a reasonable time after the visit. Once the study monitor verifies that all open issues are resolved, the site will be considered closed.

11.4 Data Management Plan

The sponsor will establish a data management plan that is appropriate to this study and that ensures the protection of human subjects, protocol compliance, data accuracy, and data integrity.

12 STATISTICAL METHODS AND ANALYSIS

This is a pilot study with primary outcomes aimed at collecting adverse event information. Summary results will be reported, as there is no proposed hypothesis. The sample size of 10 has been arbitrarily chosen to provide procedural and device related adverse event information. Subjects who are considered "Treatment Failures" will not be counted against the sample size of 10.