Spiration

CPR-05184, Revision AB,

ANET Electrosurgery Applicator

Pilot Evaluation Study

Health Canada Application #268397

Gyrus ACMI, Inc., d/b/a Olympus Surgical Technologies America (OSTA)
(formerly Spiration, Inc.)

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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 Spiration, Inc. products 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 or metastatic lung tumor with a pulmonary nodule/tumor suitable for ablation by the ANET device via EBUS bronchoscopy, prior to planned surgical resection
STUDY SIZE	Up to 10 subjects will be treated, with interim evaluation after 5 subjects have been treated.
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 immunohistochemical and microenvironment assessment for immune response.
INCLUSION CRITERIA	1. Subject with Stage I or Stage II primary lung cancer or metastatic lung tumor 2. Pathological proof of target nodule/tumor type and malignancy 3. Target nodule/tumor which can be accessed via EBUS bronchoscopy 4. Resection/surgical candidate 5. Participants must be at least 18 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. 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 a pre-defined treatment algorithm 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 immune-histochemical methods. Routine staining will be performed with hematoxylin and eosin (H&E). Analysis of cellular vitality may be performed withimmunostaining, 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 immune response indicators. The microenvironment of the resected lung 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-surgical visit.

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 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, 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 (distal electrode.) The distal electrode coils into 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 coil and the needle to thermally coagulate the tissue. Once the target energy has been reached, ablation is stopped and the coil and needle are retracted from the target. Additional ablations can be performed to achieve a larger coagulation zone.

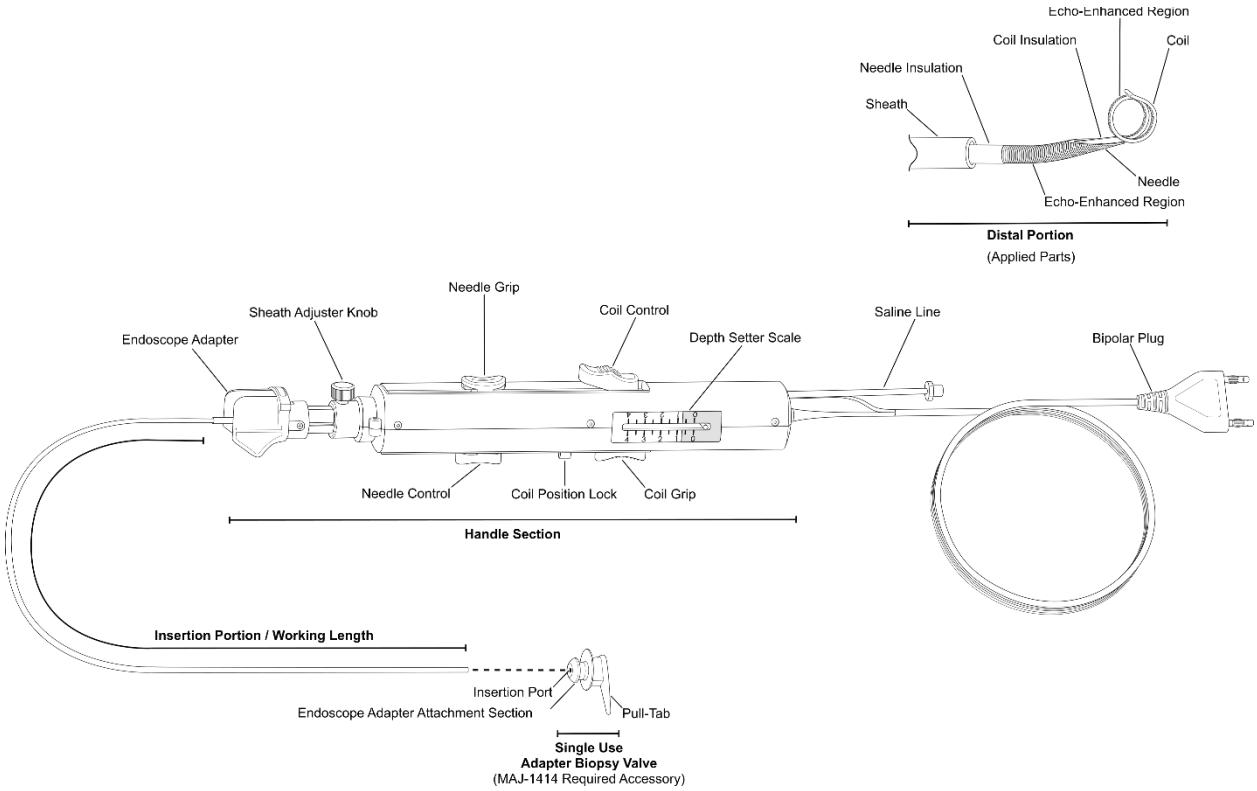


Figure 1: ANET Applicator with Identification of All Components

2.2 Handle

The handle consists of the electrical and fluid connectors, the coil control, the proximal electrode control, the sheath control, and the endoscope connector (**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 connecting-slider, which locks the device onto the biopsy valve adapter. The needle control controls the needle's extension out from the sheath. The proximal electrode can be advanced up to 35mm from the sheath. The position of the needle relative to the sheath is indicated by the depth setter scale 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**).

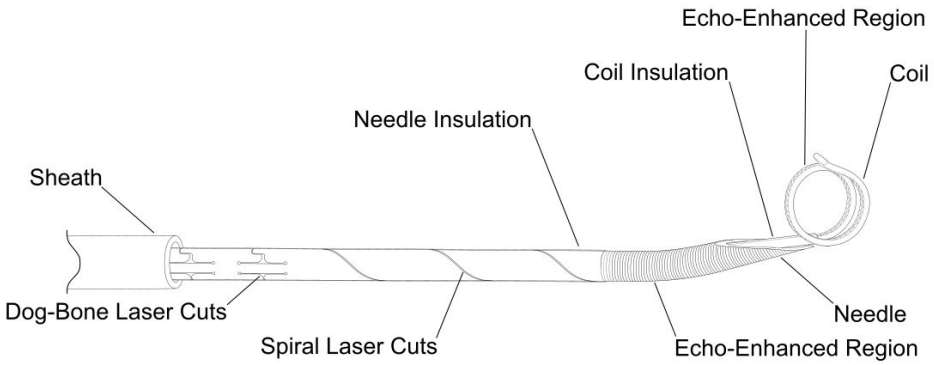


Figure 2: Sheath/Needle Tip

2.3 Needle (Proximal Electrode)

The needle is the proximal electrode. It is a19 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 needlerelative 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/electrode, 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 (Distal Electrode)

The coil 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.

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 portion 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 Electrosurgical Generator. Additional Olympus generators will be added via the 510(k) process as compatibility is established.
- ***Ultrasound endoscope*** – The ANET Applicator is compatible with an ultrasound endoscope with an inner working channel of 2.2mm.
- ***Olympus biopsy valve*** – Model # MAJ-1414

2.6 Packaging and Sterilization

In its packaged configuration, the device is held down to a card-stock backer card with ties and tabs. The device and backer-card is captured within a sealed pouch (made of LDPE and HDPE [Tyvek]), which is packaged inside a dust cover box. Each dust cover box contains one device inside a sealed pouch and an Instruction for Use (IFU).

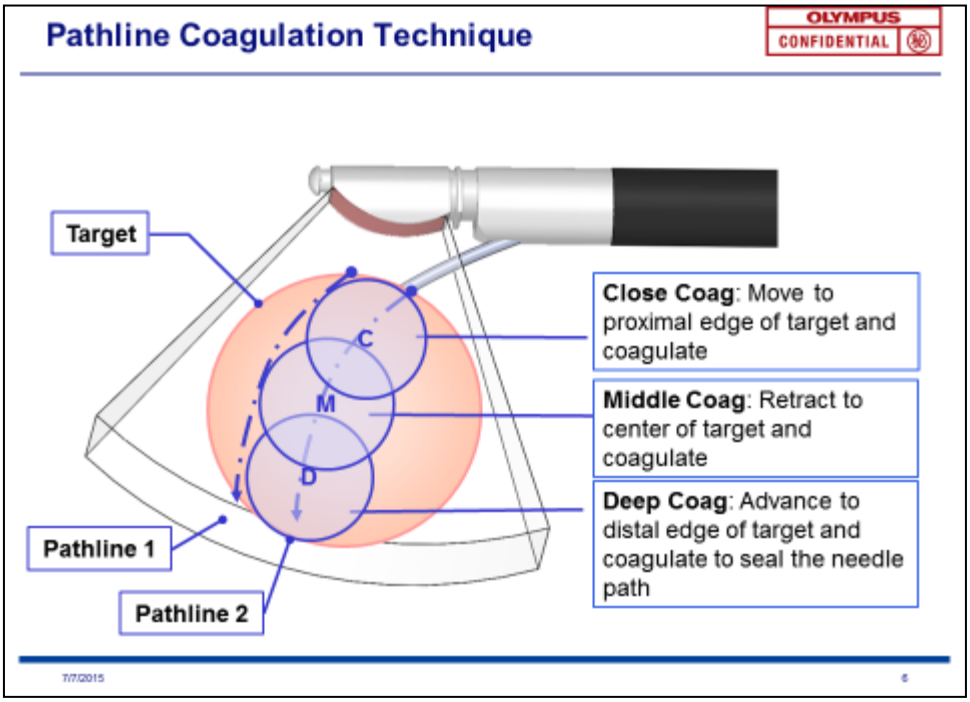
The device is sterilized by ethylene oxide (EO) sterilization.

3 PRIOR ANIMAL STUDIES

3.1 Rabbit Cancer Study

Six rabbits (Oryctolagus cuniculus or similar) were inoculated with a VX2 cancer cells in the lower right lung approximately 10 days before the study. The inoculation was performed according to previously validated methods1. The test of ANET was performed under the TGH Preclinical Animal Study Protocol AUP4152.2.

The power level for the ANET ablation was 10W and the saline flow rate was 30 or 60 ml/hr. The target was evaluated using the EBUS bronchoscope (e.g. by ultrasound) to ensure visibility and accessibility for needle penetration and coil deployment. The number of pathlines was determined as shown in Figure 1. Once the ANET-EBUS needle was in the desired location, the electrode coil was advanced out of the needle into the target. Energy was continuously applied until 1 kJ or the ESG stopped. After the desired energy was applied, the coil electrode was retracted into the needle. The needle was repositioned within the target to deliver additional energy applications as planned. After testing with the ANET device, the lungs were sectioned, fixed and stained at TGH. The sections were cryogenically fixed and stained using standard methods at TGH to evaluate the extent of coagulation necrosis[12,13].



[12]Neumann, R. A., Knobler, R. M., Pieczkowski, F. & Gebhart, W. Enzyme histochemical analysis of cell viability after argon laser-induced coagulation necrosis of the skin. Journal of the American Academy of Dermatology 25, 991–8 (1991).

[13]Marcovich, R. et al. Optimal lesion assessment following acute radio frequency ablation of porcine kidney: cellular viability or histopathology? The Journal of urology 170, 1370–4 (2003).

Figure 3: Description of the Pathline technique used in this test

Five of the rabbits had tumors that were cohesive and clearly visible under CT, and one animal had a very diffuse tumor (#214). Four of the 5 non-diffuse tumors were completely coagulated according to the histopathology data. In one animal, the target was well centered but did not coagulate enough tissue (#218).

Target No	Energy /Number of Activations	Ablation time (minutes)	Saline volume (ml)	Ablated area measurement (long axis x short axis)	Ablated Area* (cm2)	Ablated area of tumor
#212	3.3kJ / 4 activations	13.6	7.8	1.8x0.8	2.26	100%
#216	4.3kJ /6 activations	14.8	9.7	2.0x1.4	2.20	100%
#217	4.6kJ / 6 activations	18.3	13.6	2.1x1.4	2.30	100%
#218	5.9kJ / 8 activations	15.3	15	2.6x0.8	1.63	45%
#219	2.2kJ / 4 activations	6.6	7.2	1.8x0.8	1.13	100%
#214	2.0 kJ / 2 activations	8.0	4.1	1.3x0.7	0.741	92%

Table 1: Rabbit model ablation data

The rabbit model data also showed that the saline flow did not result in any undesirable in or off-target effects and that the coagulation effect was confined as predicted. The rabbit data showed that the ANET device design and methods are capable of a significant and predictable thermal effect that inactivates tumor tissue.

3.2 GLP Swine Study

The primary goal of this GLP study was to evaluate the safety and efficacy 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.

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 protocol was executed without deviations and all amendments to the protocol after the release date were approved in advance by the study director and the sponsor's representative and handled according to the released SOPs of CARE Research.

The test articles were ANET RF Applicator's produced by Spiration Inc. 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.

In conclusion, peri and post procedural clinical observations, gross pathology and histopathological analysis indicate that the RF ablation provided by the ANET applicator is safe and effective in this model of pseudotumors, 7 days after its use.

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 histologicalevaluation 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

1) 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 of their target nodule/tumor will be treated with the ANET device. Following ANET ablation, the subject will undergo their scheduled lung resection. The ablated target nodule/tumor will undergo gross histopathologic assessment and may undergo immunohistochemical and microenvironment assessment for immune response.

5.2 General Description of Study

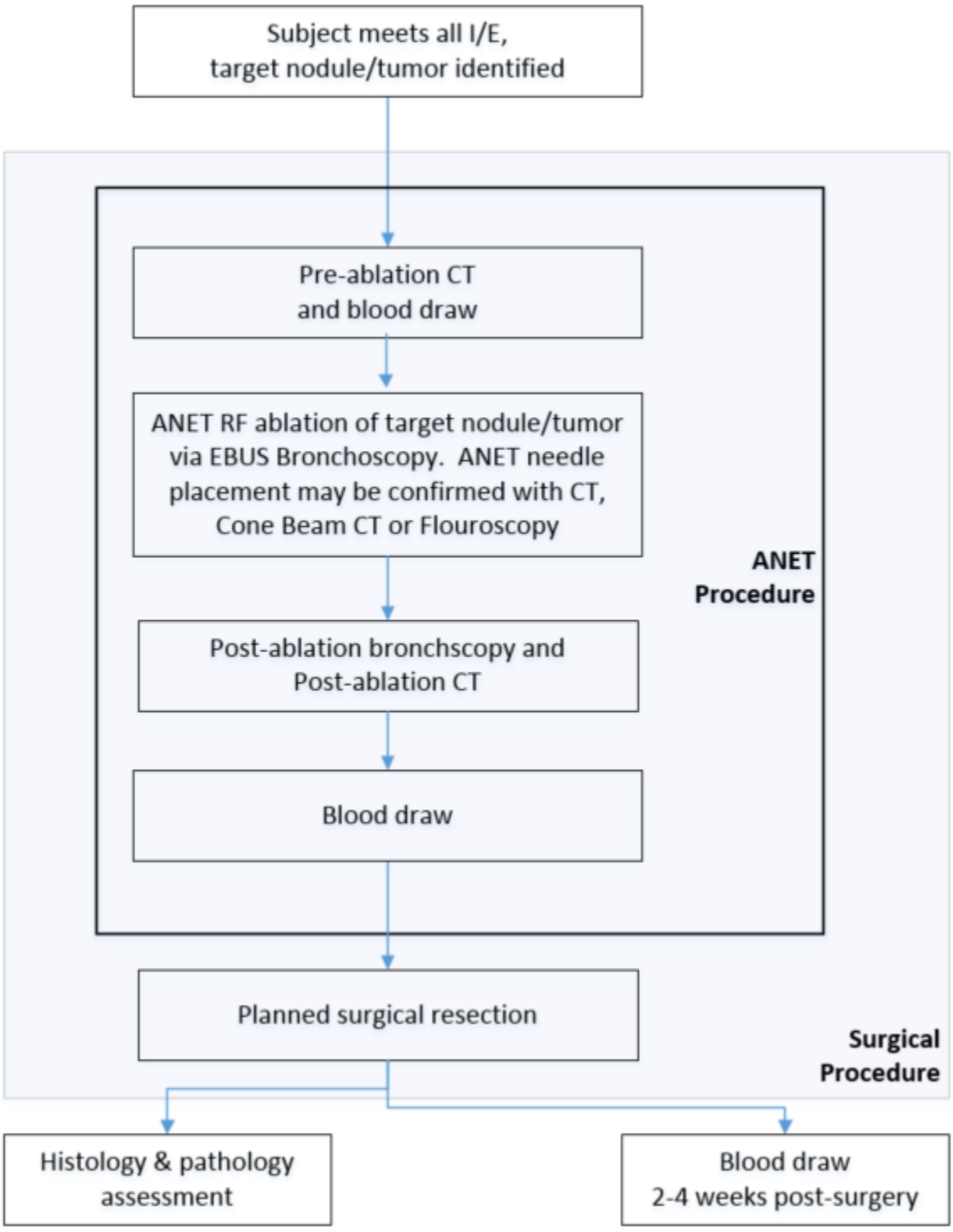
After consent and evaluation of all inclusion and exclusion criteria, subjects will be enrolled in the study. The subject will undergo standard pre-operative testing, per the study institutional requirements. At that time, subject vital signs will be recorded. The subject will be prepped for bronchoscopy.

A pre-ablation chest CT will be taken. The target nodule/tumor will be accessed via EBUS bronchoscopy and ablated using the ANET Electrosurgical Applicator. Following ablation, there will be a bronchoscopic exam of the treated region and a 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 may be prepared for immunohistochemistry 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 to evaluate for potential immune response indicators.

A study diagram is shown in Figure 4 below.



6 SUBJECT POPULATION

Adult subjects with confirmed Stage I or Stage II primary lung cancer or metastatic lung tumor 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 or metastatic lung tumor
2. Pathological proof of target nodule/tumor type and malignancy
3. Target nodule/tumor which can be accessed via EBUS bronchoscopy
4. Resection/surgical candidate
5. Participants must be at least 18 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. 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 The CT may be taken while the subject is under general anesthesia. A pre-ablation blood draw will be also 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). The nodule/tumor will be accessed via EBUS bronchoscopy. If the nodule/tumor cannot be accessed via EBUS, or the 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 needle 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. If an acceptable ablation zone cannot be determined, the subject will be considered a treatment failure and will not count against enrollment. 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 flouroscopy. 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, the target nodule/tumor will be ablated per the energy application guidelines in the IFU

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.

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 pathologic 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 immunohistochemistry.

The specimens will be examined by gross pathology, histopathology, and may be examined by immunohistochemical methods. Routine staining will be performed with hematoxylin and eosin (H&E). Analysis of cellular vitality be performed of immunostaining, including mouse antihuman mitochondria monoclonal antibody (MAB 1273; Millipore UK, Hertfordshire, UK)[14,15], Terminal deoxynucleotidyl transferase-mediated nick end-labeling (TUNEL; Boehringer Ingelheim, Germany),[16] antibody MiB1(Ki67; Dianova, Hamburg Germany)[17,18]and PHH3[19], and Masson's trichrome[20,21]. The

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

[15] 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.

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

[17] Ibid.

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

[19] Ozturk Sari et al. Endocr Pathol. 2016 Ju=n;27(2):162-70

[20] 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.

[21] T.D. Koreckij et al., Low Dose, Alternating Electric Current Inhibits Growth of Prostatic

immunohistochemical 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.

The micro environment of the resected tissue may have pathological assessment for immune cell population to explore a potential mechanistic effect of immune response.[22].

The pathologic and immunohistochemical 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.

Cancer, The Prostate, Accepted 5 October 2009, Published online in Wiley InterScience (www.interscience.wiley.com).

[22] 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 ADVERSE EVENTS

Peri- procedural adverse events, defined as from the start of the EBUS bronchoscopy through the planned surgical resection, up to 30 days post procedure will be collected,. Adverse events shall be reported in accordance with study institution requirements, local vigilance systems, sections 59 to 62 of the Canadian Medical Device Reporting Regulations, and ISO 14155:2011, sections 8.2.5 and 9.8.

8.1 DEFINITIONS

8.1.1 Adverse event: 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.

8.1.1 Serious Adverse Event (SAE)

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.

8.2 Protocol Defined Potential Adverse Events

The following list of adverse events are anticipated given the nature of the EBUS procedure and the investigational device.

Table 2: Potential Adverse Events

MedDRA term	Term type	Term ID
Ablation site reactions including…	HLT	10021544
Ablation site reaction	PT	10021542
Abscess formation	LLT	10058042
Anesthetic complications	HLT	10002111
Arrhythmia	PT	10003119
Atelectasis	PT	10003598
Bronchopulmonary hemorrhage	PT	10065746
Bronchospasm	HLT	10006482
Bronchial obstruction	PT	10006440
Burn or charring outside of ablation zone	PT	10006706
Cardiac arrest	PT	10007515
Death	PT	10011906
Device failure	PT	10018065
Dyspnea	PT	10013968
Electric shock	LLT	10014357
Hematoma	PT	10055370
Hemoptysis	PT	10018964
Hemorrhage	PT	10019524
Hemothorax	PT	10019614
Hypoxemia	LLT	10021142
Infection (acute)	PT	10076200
Laryngeal spasm	LLT	10023854
Pleural effusion	PT	10035598
Pleural hemorrhage	PT	10055319
Pleuritic pain	PT	10035623
Post-procedural hemorrhage	LLT	10055322
Post-procedural thoracic complication	PT	10056745
Pneumonia	PT	10035664
Pneumonitis	PT	10035742
Pneumo-mediastinum	LLT	10050184
Pneumothorax	PT	10035759
Pulmonary edema	PT	10037375
Pulmonary embolism	PT	10050071
Pulmonary fistula	PT	10065873
Respiratory failure	HLT	10038695
Tissue necrosis	PT	10065769
Traumatic lung injury	PT	10022117
Tumor seeding	LLT	10049737
Tumor recurrence	LLT	10029097

8.3 **Adverse Event Reporting**

The investigator is required to report a serious adverse event, regardless of whether the event is considered anticipated or unanticipated based on the defined potential adverse event list in Table 2, to Health Canada and to the manufacturer and importer within 72 hours of discovery. This includes cases in which the incident:

- is related to a failure of the device or a deterioration in its effectiveness, or any inadequacy in its labelling or in its directions for use, and
- has led to the death or a serious deterioration in the state of health of a patient, user or other person, or could do so were it to recur.

For an incident that occurs in Canada, the manufacturers and importers are required to provide a preliminary and a final report in respect of the incident.

The preliminary report shall be submitted:

- Within 10 days after the manufacturer or importer of a medical device becomes aware of an incident, if the incident has led to the death or a serious deterioration in the state of health of a patient, user or other person, or
- Within 30 days after the manufacturer or importer of a medical device becomes aware of an incident, if the incident has not led to the death or a serious deterioration in the state of health of a patient, user or other person, but could do so were it to recur.

<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 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.

10 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 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.

10.1 Investigator Reports

All study investigators shall prepare and submit the following complete, accurate, and timely reports.

1) *Serious adverse events:* An investigator shall submit to the sponsor, Health Canada and to the reviewing REB a report of any serious adverse event occurring during an investigation as soon as possible, but in no event later than 72 hours after the investigator first learns of the effect. See section 8.3 for reporting parameters.
2) *Deviations from the investigational plan.*

10.2 Sponsor Reports

The study sponsor shall prepare and submit the following complete, accurate, and timely reports and revised authorizations:

1) *Adverse events:* Please see section 8.3 for reporting parameters.
2. Manufacturers or importers may submit a request for a revised authorization to address changes made to the device, study investigation plan, or institutional information, such as those listed below:

a. A change to the device
   - A change involving modifications to device design (where the device generally maintains its original functionality and intended use), sterilization, software, materials, and/or labelling
   - A change in device name or device identifier
   - A change to the number of devices requested
   - A change to the manufacturer name

b. A change to the study documents (protocol and ICF)
   - A change to the study protocol that will not bias the data previously collected (see list of examples below)
   - ICF revisions (clarifications to language/wording)

- Additional study subjects
- Change to duration of study

c. A change to the institutional information
   - Addition or removal of institutions where the testing is being conducted
   - Change to the list of qualified investigator(s)
   - Updated REB approval information

Examples of protocol changes that would require a revised authorization are listed below. When in doubt whether an application is required, sponsors should contact the Investigational Testing Division at: hc.it-ee.sc@canada.ca.

1. Protocol revisions to permit the enrolment of additional study subjects and longer term patient follow-up.
2. Any protocol amendment that requires REB approval will also require a Health Canada authorization.
3. Inclusion and exclusion criteria modifications. These include changes to eligibility criteria, tests or procedures for selecting the study population, as well as tests, procedures, or criteria for dismissing clinical trial subjects prematurely or at the end of the trial.
4. Changes to the patient selection criteria, tests or procedures required for the ongoing assessment of clinical trial subjects, including assessment of safety, or evaluation of safety and effectiveness. This includes protocol changes as a result of serious unexpected adverse reactions.
5. Inclusion of sub-studies.
6. Changes to sample size estimation or addition of interim analyses that will affect the analysis and interpretation of the study results.
7. Changes to the post-treatment follow-up period that may affect the safety evaluation of the device.
8. Use of ancillary medical devices for the treatment or monitoring of the study subjects that may have an impact on the analysis of effectiveness or increase the risk to clinical trial subjects.
9. Changes to the requirements or procedure for reporting of serious, unexpected adverse reactions.

10.3 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.

10.3.1 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
- REB/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.

10.3.2 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 REB/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.

10.3.3 Study Closeout Visits

The study closeout visit will occur once all subjects have completed the study through the peri-procedure follow-up phase and all data is collected (i.e.; pathology). 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 REB 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.

10.4 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.

Study data will be collected in accordance with Good Clinical Practice. Written Case Report Forms (CRFs) will be used to collect subject data. The bronchoscopy procedure may be video recorded.

A core pathology lab may be used for gross pathology, histopathology, immune-histopathology, and micro CT analysis of the ablation zone. A core lab will be used for analyzing blood for possible immune response. The core labs will collect data in accordance with Good Clinical Practice.

11 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.