Evaluation of Effect of CryoBalloon Focal Ablation System on Human Esophageal Epithelium (ColdPlay2)

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Evaluation of CryoBalloon Focal Ablation System on Human Esophageal Epithelium

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I, the undersigned, certify that I have reviewed this Clinical Investigational Plan (CIP) and agree to abide by the terms of the study described herein.

Print Name: Marcia Irene Canto, MD MHS

Signature: _____ Date: _____

TABLE OF CONTENTS

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ATTACHMENTS

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1.0 INTRODUCTION AND BACKGROUND

Barrett's Esophagus (BE) is a premalignant lesion which can lead to esophageal adenocarcinoma. This particular cancer is one of the most rapidly increasing and deadliest cancers in the United States.[1] Patients with BE are up to 40 times more at risk of adenocarcinoma than individuals without BE.[2]

Once diagnosed with BE, a patient enters a life-long surveillance program in which upper endoscopy (Esophagogastroduodenoscopy or EGD) with biopsy are performed to survey the progression of the Barrett's tissue to cancer. For more than 20 years, many technologies have been evaluated for ablation of BE. Elimination of BE and restoration of squamous esophageal lining has been demonstrated through ablation; however, no ablation technology currently provides the necessary attributes for wide-spread adoption into a community setting. Current BE technologies such as Radio Frequency Ablation (RFA) suffer from 'usability' drawbacks such as the need for precise sizing, multiple deployment steps, intensive training for the physician and large controller units. Even with these limitations, the market for these technologies is growing at a rate of 60% annually and has become the standard of care for certain BE patients.

Similarly, patients with dysplasia developing in the squamous-lined esophagus are at risk for developing esophageal squamous cell carcinoma (SCC). In the West, these patients often have a history of smoking, alcohol, or rare associated risk factors. In the East, SCC is more common than adenocarcinoma arising in BE. Hence, the potential worldwide clinical impact of a safe and effective esophageal ablation system for precancerous neoplasia is an important consideration for further testing and development of this novel therapy.

Cryoablation has been shown to cause cell death through two distinct mechanisms. The first is ice formation in the extracellular fluid matrix. This causes cells in that matrix to eject pure water which raises the salinity content of the cell which provides protection against intracellular ice formation by decreasing the freezing point of the cell. This action will induce apoptosis in some percentage of the cells is due to dehydration of the cell. Generally, apoptosis (i.e., programmed) occurs at freezing temperatures warmer than −15˚C under controlled cooling rates. The second mechanism is ice formation within a cell. This will typically cause necrosis of the cell due to mechanical destruction of the cell wall caused by ice crystals.

Apoptosis is the typical mechanism for cell death. Apoptotic cell death does not induce a systemic healing response as the contents of the cell are coated with a membrane and are absorbed locally. Necrosis of the cell occurs when the cell wall is ruptured. The body interprets this as injury and acts appropriately to repair the area.[i] Because of the body's response the cell necrosis and the desire for replacement of normal esophageal tissue, C2 believes this is the appropriate mechanism to ablate Barrett's Esophagus.

The CryoBalloon Focal Ablation System (CbFAS) is designed to address many of the limitations of ablation technologies that have been listed. The System has three main components – the delivery catheter with balloon probe, a handle, and a small cylinder containing the cryogenic fluid. There is no capital equipment involved – the system is completely single-patient use. The system utilizes one balloon probe for all sizes of esophagi. Access for treatment is simplified as the system utilizes the working channel of the endoscope.

The simplicity of the System allows for many potential benefits to the patient, the physician, and hospital. Some of the benefits may include a shorter and safer procedure, an easier deployment minimizing the need for anesthesiology, and smaller inventory requirements and no capital equipment improving capital resource utilization

NOTE: Please refer to the Investigator's Brochure for more extensive background information and literature search results.

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[1] Johnston MH (2005) Technology Insight: ablative techniques for Barrett's esophagus—current and emerging trends Nat Clin Pract Gastroenterol Hepatol 2: 323–330 doi:10.1038/ncpgasthep0214

[2] Jemal A, et al, Cancer statistics, 2009. CA Cancer J Clin 2009;59:225-49.

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PRELIMINARY EXPERIENCE AND DATA

The System has undergone acute and chronic animal testing. The testing was conducted to study the safety, deliverability and performance characteristics of the System. The studies were conducted for the evaluation of the device in a normal pig esophagus at dimensions very similar to a human esophagus. General follow-up time frames were either 4 days or 28 days. A summary of prior pre-clinical testing performed on the System is provided in the Investigator's Brochure. A summary of the animal data is also included in the Appendix below.

A clinical trial has been conducted to study the safety and performance characteristics of a full-circumferential version of the CryoBalloon Ablation System. The Investigator's Brochure highlights the differences between the two devices with the major difference being that the Focal System ablates a far smaller area. Data has been presented at the DDW 2012. The abstract for the study is included in the Investigator's Brochure with final results not yet published. Preliminary results show the full-circumferential system is safe and has good efficacy on the ablation of esophageal mucosa.

The CbFAS device has been cleared by the U.S. Food and Drug Administration under K101825. The device will be used in accordance with the manufacturer's labeling under this protocol.

The international single arm CbFAS trial conducted under the original protocol for this study has been completed and the manuscript submitted to Endoscopy journal for publication. See more detail on the preliminary results in separate attachment submitted. In this multicenter, prospective non-randomized trial 39 patients were treated, each subject receiving 1-2 ablations of 6, 8 or 10 seconds. Symptoms were assessed directly and 2 days post-cryoablation. Follow-up endoscopy was performed after 6-8 weeks to assess the ablation response. Outcome parameters were incidence of adverse events, pain, esophageal stricture formation, and ablation response by cryogen dose.*Results:* 56 ablations (10 with 6-sec, 28 with 8-sec, 18 with 10-sec) were performed. Six ablations failed due to device malfunction (n=3) or other reasons (n=3). Median (IQR) procedure time was 7 min (4-10) min. No major adverse events occurred; six patients had a minor mucosal laceration requiring no intervention. Mild pain was reported in 27% of patients immediately after cryoablation and in 14% after 2 days. After 6-8 weeks no strictures had developed. Full squamous regeneration was seen in 6 [60%] of 6s-areas; 23 [82%] of 8s-areas, 100% of 10s-areas). These study results suggest that focal cryoablation of BE with the newly developed CbFAS is feasible, safe, and results in squamous regeneration in the majority of patients. These results were recently published (Scholvinck et al, Endoscopy July 2015 – paper uploaded into eIRB separately)

Furthermore, Dr. Canto has treated 41 BE patients and 10 patients with esophageal squamous low grade and high grade dysplasia (neoplasia) within this study and more treated within the clinical cryotherapy program at Johns Hopkins Hospital) with successful ablation in 1-3 outpatient procedures using multifocal cryoballoon ablations, in keeping with standard of care treatment approach to ablation. Research and clinical patients have tolerated cryoablation well, with visible treatment response.
Based upon the results of 2 clinical trials completed under this IRB-approved study,

Dr. Marcia Canto plans to continue this single center (industry sponsored) study to continue the evaluation of safety, effectiveness and 5-year durability of cryoballoon ablation for esophageal neoplasia.

These two publications from Johns Hopkins Hospital by Canto et al document the safety and efficacy of the cryoballoon ablation for esophageal neoplasia. These treatments have become standard-of-care at Johns Hopkins as well as elsewhere in the United States and Europe (more references can be provided). More clinical trials are underway with expanded indications, including resistant Barrett's esophagus (separate IRB submitted)..Copies of the published papers have been uploaded.

1. Canto MI, Abrams JA, Kunzli HT, et al. Nitrous oxide cryotherapy for treatment of esophageal squamous cell neoplasia: initial multicenter international experience with a novel portable cryoballoon ablation system (with video). Gastrointest Endosc 2018;87:574-581.
2. Canto MI, Shaheen NJ, Almario JA, et al. Multifocal nitrous oxide cryoballoon ablation with or without EMR for treatment of neoplastic Barrett's esophagus (with video). Gastrointest Endosc 2018

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2.0 SUMMMARY DEVICE DESCRIPTION AND USE

The System has three main components – the delivery catheter with balloon probe, a handle, and a small cylinder containing the cryogenic fluid. Deployed through the working channel of an endoscope, the operation of the System is very similar to the deployment of dilatation balloons. The balloon is made of a conformable material. Once deployed, the balloon is simultaneously inflated and cooled with cryogenic fluid delivered from the handle. Due to the conformability of the balloon material, no sizing procedure is required. Additionally, one balloon can ablate a full range of esophagi (approximately 20mm to 32mm in diameter). Esophageal cells are ablated as the balloon comes into contact with the esophagus for less than 15 seconds. After ablation, the System is repositioned for additional ablation or withdrawn.

Cryoballoon ablation system includes

- CryoBalloon Focal Ablation Catheter (FG-1009) - Sterile single-patient use with a balloon at end of Catheter
- CryoBalloon Focal Ablation Handle (FG-1012) - Single-patient use and battery-powered
- CryoBalloon Ablation Cartridge (FG-1010) - Nitrous oxide, single-patient use
- Sidecar External Working Channel (FG-1011)- Single-patient use

Proposed System Benefits include the following:

- Completely Disposable System
    - No investment in equipment
    - No service, maintenance or set-up of equipment
    - No special storage requirements

- One-sized fits all balloon catheter
    - Minimize disposable devices necessary for procedure
    - Lower inventory costs

- Minimal steps of operation
    - Shorter procedure times
    - Improve resource utilization

- Working channel access of system
    - Less discomfort to patients

Potential move from than general or heavy sedation to conscious sedation

Latest device improvements:

Principle of pre-inflation and self-sizing

The balloon pre-inflates and contacts the esophagus wall prior to the actual ablation. Pre-inflating the balloon against the esophagus creates an intimate contact with the esophagus and enables the user to visualize and identify targeted ablation areas through the inflated balloon.

The self-sizing feature of the balloon allows inflation to a range of esophageal diameters without applying pressures beyond ~6 psi. Concurrently, the inflation does not exceed beyond the esophagus inner diameter. The self-sizing balloon concept is also similar to the Barrx™ Halo 360 Sizing Balloon, where it uses a 33.7mm non-compliant balloon and inflates it to over 4 psi in the esophagus to estimate the esophagus diameter.

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Description of change

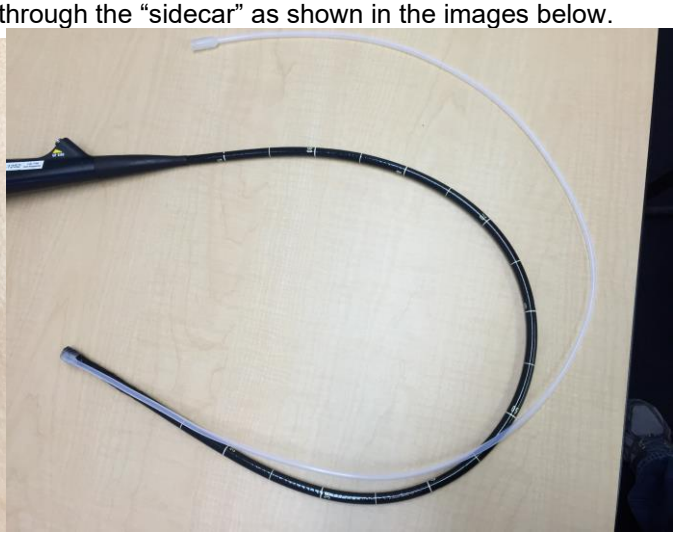
The system is composed of a catheter, a handle, and nitrous oxide cartridges – nitrous oxide is the cryogenic fluid used to ablate unwanted tissue. The design of the catheter and the cartridge remain the same. The handle design has been improved to reduce the pre-inflation pressure within the balloon from ~6 psi to ~3 psi for a 50% decrease. Pressures during ablation are ~3 psi.

The mechanism in the handle that controls the nitrous oxide exhaust gas has been modified from a mechanical valve to an electro-mechanical valve (solenoid). Due to the solenoid valve change, balloon pressure during pre-inflation and ablation can be more accurately controlled and decreased by the mentioned 50%.

Purpose of Change

The principle of operation of the C2 ablation system is to inflate the ablation balloon against the esophagus wall. The self-sizing capability of the system is enabled by the low operating pressure within the balloon. The pre-inflation pressure may reach ~6psi, while the pressure during ablation is ~3psi; therefore, it is desirable to lower the pre-inflation balloon pressure be similar to the ablation pressure, which also minimizes potential damage to the esophagus. A pressure reduction of approximately 50% is benefitted by the handle design change. An operating pressure of ~3 psi during pre-inflation and ablation is also lower than the 4+ psi, or approximately 25% lower than the Barrx™ Halo 360 Sizing Balloon procedure.

All procedures will be performed in the outpatient endoscopy unit by using monitored anesthesia care or conscious sedation, per standard institutional practice. The physician will perform an upper endoscopy using an endoscope with a therapeutic channel to accommodate the CbFAS balloon and to verify the treatment zone in the esophagus. Alternatively, a commercially available 510k-FDA approved accessory tube called the “sidecar”, can be attached to a standard non-therapeutic channel upper endoscope (more commonly available in endoscopy units compared to a therapeutic endoscope) and the cryoballoon advanced through the “sidecar” as shown in the images below.



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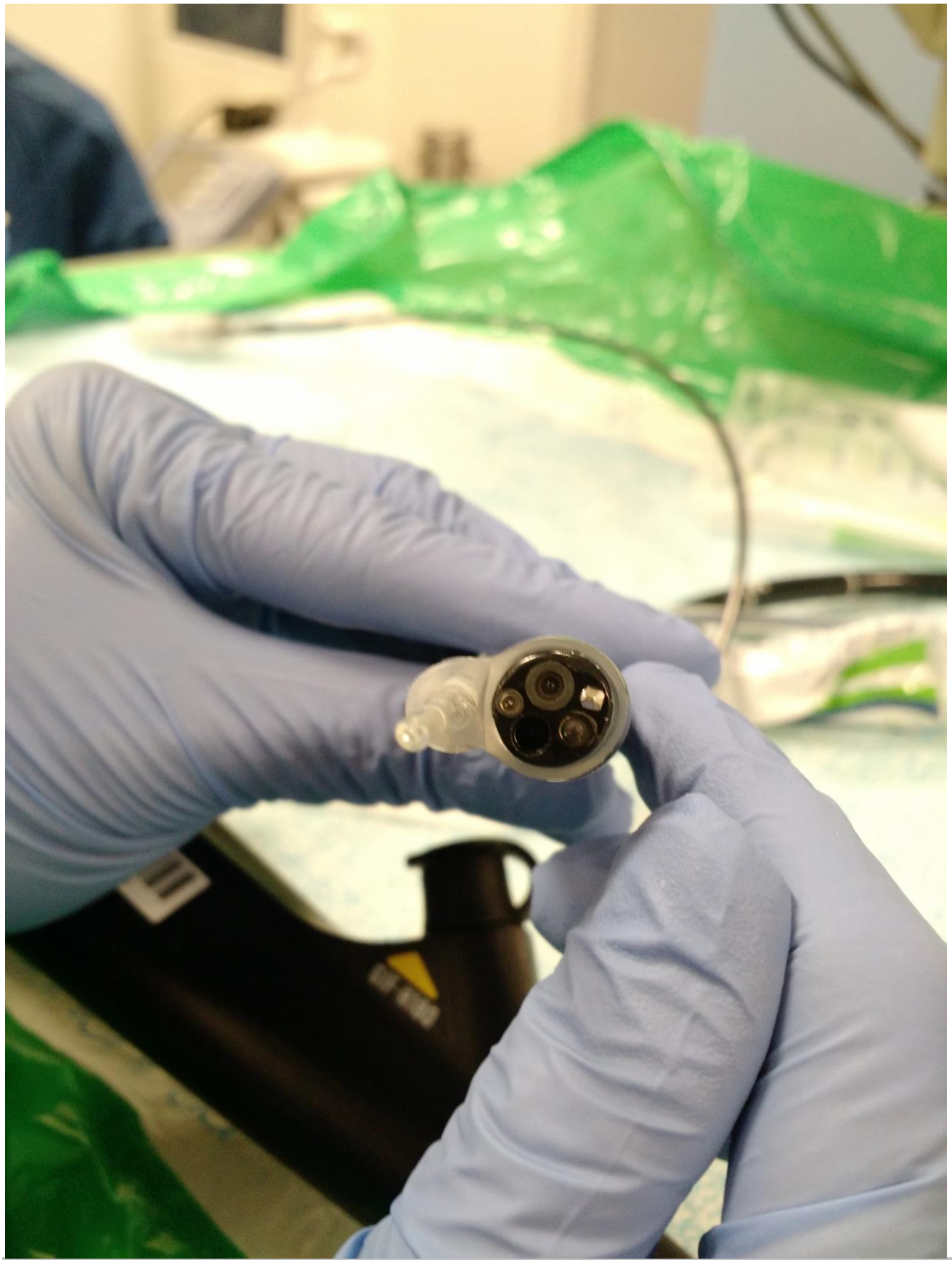

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The System is deployed under direct visualization.
Refer to the Instructions for Use (IFU) for specific device use instructions.

3.0 CLINICAL INVESTIGATIONAL PLAN (CIP)

3.1 CIP Summary

Title:	Evaluation of CryoBalloon Focal Ablation System on Human Esophageal Epithelium..
Name of Device:	Pentax C2 CryoBalloon Focal Ablation System (System) –
Indications for Use:	The System is intended to be used as a cryosurgical tool for the destruction of unwanted tissue, specifically for endoscopic applications. *Under this protocol, Cryoballoon ablation will be performed on patients with Barrett's Esophagus (BE) and localized esophageal squamous neoplasia.*
Patient Population:	Patients who have Barrett's Esophagus and esophageal squamous neoplasia and meet the protocol entry criteria.
Study Purpose:	The purpose of this study is to assess the safety, feasibility and performance of the C2 Focal Cryoablation System in patients with BE and esophageal squamous neoplasia.
Study Design:	This study is: • Prospective • Single center (Johns Hopkins Hospital) • Non-randomized
Sample Size:	It is estimated that up to 100 patients will be enrolled under this protocol.
Number of Sites:	One institution: Johns Hopkins Hospital
Study Procedures:	The study will involve100 subjects with each subject receiving up to 24 ablations (multifocal) using the C2 System Focal Ablation System per treatment session. Ablations will be at 5, 6, 8, 10 or 12 seconds. Up to 5 treatment sessions 8-12 weeks apart within 12 months may be performed, if there is residual BE or abnormal squamous mucosa, until all disease is eliminated, by endoscopic biopsy. The ablations will be performed in an area of BE that is $\geq$ 1cm 2 or recurrent BE at the gastroesophageal junction (GEJ). Prior and intervening endoscopic mucosal resection (EMR) may be performed for esophageal lesions, as part of standard care. After completing cryoablation treatment, subjects return for EGD with surveillance biopsy at every 2-3 months for first year and every 6 months for the second year as part of standard care then annually for next 3 years for

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	total of total of 5 years. Adverse events and pathology results will be recorded. At the time of endoscopic follow up, biopsies of the treated area will be taken and submitted for analysis according to standard care for analysis at the JHH Pathology lab.
Endpoints:	Primary Endpoints • Device Performance • Adverse events • Efficacy for complete elimination (CE) of all esophageal dysplasia • Efficacy for complete elimination of all BE All adverse events • Esophageal stricture formation[3] and assessment of clinical dysphagia. Secondary Endpoints • Patient Pain

3.2 Study Purpose and Objectives

The purpose of this study is to assess the safety, feasibility, performance and the short- and long-term effectiveness of the C2 Focal Cryoablation Device in patients with BE and esophageal squamous dysplasia. Three times of ablation (5, 6, 8, 10 or 12 seconds) can be used and post-ablation symptoms related to the Cryoballoon Focal Ablation will be recorded. At 6 to12 weeks, the patient will receive a follow-endoscopy to assess stricture formation along with biopsy samples taken.

The ultimate goal is to achieve 90% or greater ablation of BE and 100% ablation of all dysplastic areas.

Post-operative pain will be noted. Additionally, biopsy samples will be evaluated for the presence of residual Barrett's Esophagus. Through evaluation of the histological results, treatment parameters for the ablation of human esophageal epithelium will be better understood.

Evaluations include, but are not limited to the following:

- Deployment ease/scope compatibility.
- Device malfunctions.
- Time of catheter deployment.
- Adverse events.
- Stricture formation at 8 to 12 weeks.
- Patient Pain.
- Histological evaluation of treatment zone at 8 to12 weeks for presence of residual Barrett's Esophagus.

3.3 Study Population

The study population is patients who have BE or squamous dysplasia and meet the protocol entry criteria.

[3] Stricture formation is defined as none, mild (non-circumferential stenosis allowing easy passage of diagnostic endoscope), moderate (circular stenosis allowing passage of diagnostic endoscope), and severe (any stenosis preventing the passage of a diagnostic scope).

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3.4 Selection criteria

3.5.1. Patient Selection Criteria

Inclusion Criteria (Candidates for this study must meet ALL of the following criteria):

a) Patients scheduled for ablation, EMR, and/or surveillance for BE (with or without dysplasia) or esophageal squamous dysplasia. Patient is > 18 years of age at the time of consent (inclusive)

b) Patient has provided written Informed Consent (IC) using an Informed Consent Form (ICF) that has been approved by the Institution's reviewing IRB/EC.

c) Patient is willing and able to comply with all Clinical Investigation Plan (CIP) requirements.

d) Patient is deemed operable per standard institutional criteria.

Exclusion Criteria (candidates will be excluded if ANY of the following conditions apply):

a) Patient with endoscopically active inflammation in the treatment zone.

b) Esophageal stenosis preventing advancement of a therapeutic endoscope

c) Patient has a known history of unresolved drug or alcohol dependency that would limit ability to comprehend or follow instructions related to informed consent, post treatment instructions or follow-up guidelines.

d) Patient refuses or is unable to provide written informed consent.

e) Patients with uncorrectable coagulopathy, thrombocytopenia or on chronic anticoagulation unable to have "bridge" therapy for any standard endoscopy.

f) .

3.5.2. BE Selection Criteria

Inclusion criteria (BE areas in the treatment zone must meet ALL of the following criteria):

a) Each patient has pathologically confirmed BE with a C&M classification of C≥0 and/or M≥1.

b) BE lesion within the treatment zone should be flat.

Exclusion criteria (BE areas will be excluded if ANY of the following conditions apply):

Active esophagitis Grade B or greater.

a) Endoscopically visible abnormalities such as masses or nodules within 4 cm of the treatment zone. Neoplastic nodules or lesions must be treated first with EMR at least 4 weeks prior to planned treatment with the CbFAS.

3.5.3 Esophageal Squamous Neoplasia Selection Criteria

Inclusion Criteria (squamous esophagus areas in the treatment zone must meet ALL of the following criteria):

a) Each patient with esophageal squamous dysplasia must have dysplasia in the treatment area by prior Lugol's chromoendoscopy/narrow band imaging and targeted mucosal biopsies.

b) The squamous lesion should be flat.

Exclusion Criteria (squamous esophagus areas will be excluded if ANY of the following conditions apply

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a) Invasive squamous cell carcinoma

b) Endoscopically visible abnormalities such as masses or nodules within 4 cm of the treatment zone. Neoplastic nodules or lesions must be treated first with EMR at least 4 weeks prior to planned treatment with the CbFAS

3.5 Informed Consent, Screening, Enrollment, Treatment, and Follow-up

3.5.1 Stage 1) Patient Informed Consent

Written informed consent will be obtained prior to any study driven tests or procedures.

A member of the research team will approach the patient to obtain informed consent. The background of the proposed study, the procedure, the follow-up schedule and all potential benefits and risks will be carefully explained to the patient. The person obtaining consent shall:

- Avoid any coercion of or undue influence of subjects to participate.
- Not waive or appear to waive subject's legal rights.
- Use language that is non-technical and understandable to the subject or his / her legal representative.
- Provide ample time for the subject to consider participation.
- Include dated signatures of the subject or the subject's legal representative and of the clinical investigator.
- Ask whether the subject has any questions about the study.
- Copy signed ICF and provide to patient.

NOTE: The process of obtaining ICF will be documented.

The subject or his/her legal representative must sign the ICF approved by the study site's IRB/EC prior to enrollment. If the investigational procedure is to be filmed for educational purposes, this must also be carefully explained to the patient. An *example* ICF is included as **Attachment C**.

During the initial screening phase, the Investigator will perform an initial evaluation of potential study subjects for study eligibility. This initial screening phase may include review of existing patient information (review of medical history, medication, etc.).

For those patients that agree to participate in the study by signing the IRB/EC Approved ICF, a baseline evaluation will be performed (see **Table 2** for a schedule of assessments). If the baseline evaluation requirements listed in **Table 2** are available as part of the patient's routine examinations and medical history, they may not need to be repeated after the patient's IFC is obtained.

Subjects must undergo the investigational procedure within the timeframes indicated below:

-Review of Medical History (within 7 days)
-Review of medications taken (within 7 days)

Since final eligibility cannot be determined until the time of the procedure, it is expected that some study subjects may not undergo the cryoablation procedure (e.g. narrowing of the esophagus such that the endoscope cannot be advanced).

Subjects are considered enrolled at the time of introduction of the CryoBalloon Focal Ablation device.

3.5.2 Stage 2) The Treatment

The treatment stage begins at the time of the ablation (endoscopy) procedure. Actual treatment time will vary by patient and will be documented. The treatment stage will be as follows:

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1. The subject is evaluated for the presence of strictures (if present, the stenosis should not prevent advancement of a therapeutic endoscope or non-therapeutic endoscope with sidecar external channel). Locate and record the gastroesophageal junction (top of gastric folds) by depth of endoscope insertion.

2. For Barrett's esophagus patients:

3. Locate, measure, and record the depth of endoscope insertion at the most proximal circumferential extent of suspected columnar metaplasia. Locate, measure, and record the depth of endoscope insertion at the maximum extent of suspected columnar metaplasia. Document the number, and localization (insertion depth of the endoscope and orientation in the endoscopic field) of all BE islands. Document the level of the diaphragmatic pinch and size (length) of the hiatal/paraesophageal hernia.

4. Provide photo documentation of the entire Barrett's segment by taking one still image each centimeter of Barrett's epithelium. Endoscopic enhancements such as narrow band imaging should be performed to improve BE mapping and visualization.

5. For esophageal squamous dysplasia patients:

6. Locate, measure, and record the depth of endoscope insertion at the most proximal metaplasia. Document the number, size (maximum and minimum diameter) and localization (insertion depth of the endoscope and orientation in the endoscopic field) of all squamous dysplasia lesions. Document the level of the diaphragmatic pinch and size (length) of the hiatal/paraesophageal hernia.

7. Provide digital videorecording and photo documentation of the entire esophagus(performed part of standard procedure documentation on EndoPro software, Pentax Medical Corporation) involved by neoplasia by taking one still image each centimeter from the GEJ to the proximal level of the abnormal esophageal areas. When possible, high definition videoendoscopes should be used for treatment and follow-up procedures. Endoscopic enhancements such as narrow band imaging and Lugol's chromoendoscopy (standard image enhancement techniques) should be performed to improve mapping and visualization prior to ablation. When Lugol's chromoendoscopy is performed, the endoscope channel must be flushed with 60 cc of water before passage of the cryoballoon to minimize staining of the balloon with dye.

For all Subjects:

8. The cryoablation will be performed to potentially treat all BE and squamous dysplasia, preferentially treating distal to proximal in a circumferential fashion. The goal would be to treat the entire BE area with 24 ablations or less during outpatient endoscopic treatment session.

9. All cryoablations will be preferentially performed with 10 seconds per site, but dosing can be adjusted to 5, 6, 8, 10 or 12 seconds according to the physician's judgement based upon degree of overlap with other ablations.

   For BE patients: Additional "touch-up" 5 second ablations can be performed after endoscopic inspection of the treated areas show lack of typical post cryotherapy immediate tissue effects (erythema corresponding to vascular congestion/thrombosis).

   For esophageal squamous dysplasia patients, the unstained esophageal mucosa after Lugol's chromoendoscopy ("Lugol's voiding lesions or LGLs", which correspond to dysplastic mucosa not taking up the dye) will be targeted.

10. The Cryoballoon Ablation Catheter is introduced inside either the accessory channel of the therapeutic endoscope, or the external working channel. The catheter is positioned and the

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ablation performed inside the identified area of BE. The use of CryoBalloon Focal Ablation System is in conformance to the IFU.

11. Document the location of the top and bottom of the ablation zone (insertion depth of the endoscope and orientation in the endoscopic field). This is done by videorecording, photo documentation and/or by recording the localization (of the ablated area). Placing a tattoo at the top of the ablation zone is optional to further identify the exact location of the ablation zone. Document the location of the tattoo (insertion depth of the endoscope and orientation in the endoscopic field).

12. Up to 5 outpatient ablation endoscopic procedures can be performed 8-12 weeks apart per patient (up to 24 per procedure) to achieve complete ablation of dysplasia. This treatment schedule is comparable to that for other standard endoscopic ablative techniques, such as radiofrequency ablation and carbon dioxide cryotherapy

3.5.3 Stage 3) Patient Follow-up

The follow-up period begins immediately post-procedure. The patient will undergo a follow-up assessment immediately post-procedure / prior to discharge. The patient will undergo a safety phone contact at 1 days (±1 days) , 8 (±1 days) days, and 1 month post treatment. Assessment of pain score (0-10 Likert scale), dysphagia score (0-4), odynophagia, diet, medications (including narcotic analgesics) will be performed using a standardized questionnaire.

3.5.4 Stage 4) Follow-up Endoscopy (8 to 12 weeks)

A high definition diagnostic scope can be used during the Follow-up Endoscopy stage unless warranted by institutional guidelines. If there is endoscopically apparent residual BE or squamous dysplasia at the follow-up procedure, this visit will become a treatment visit and cryoablation can be performed.

The Follow-up Endoscopy stage will be as follows:

1. Locate and record the gastroesophageal junction (top of gastric folds) by depth of endoscope insertion. Locate, measure, and record the depth of endoscope insertion at the most proximal circumferential extent of suspected columnar metaplasia. Locate, measure, and record the depth of endoscope insertion at the maximum extent of suspected columnar metaplasia or squamous dysplasia. Document the number, localization (insertion depth of the endoscope and orientation in the endoscopic field) of all BE islands.

2. Document the location of the top and bottom of the ablation zone (insertion depth of the endoscope and orientation in the endoscopic field). This is done by videorecording or photo documentation and by recording the localization (of the ablated area).

3. Evaluate the presence of stenosis at the treatment zone.[4]

4. A biopsy (ies) will be performed according to standard of care, with targeted biopsies sampled from visible mucosal lesions and 4-quadrant random biopsies obtained from the 1 cm distal to the GEJ, the GEJ and every 1 cm proximal to the Z line, or the extent of the original BE or squamous dysplasia. The sample(s) will be collected and submitted as standard of care to Johns Hopkins pathology Thereafter, a representative photo of the biopsy site is taken.

[4] Stricture formation is defined as none, mild (non-circumferential stenosis allowing easy passage of diagnostic endoscope), moderate (circular stenosis allowing passage of diagnostic endoscope), and severe (any stenosis preventing the passage of a diagnostic scope).

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5. Treatment of residual BE and dysplasia with cryoballoon ablation using same technique, if indicated, will be performed, instead of endoscopic biopsy, if there is visible BE (for BE dysplasia patients) or unstained esophageal mucosa by Lugol's chromoendoscopy (for squamous dysplasia patients)

SCHEDULES OF ASSESSMENTS

An overview of the assessments to be performed along with the required timing is provided in **Table 2**. Visits occurring outside of the specified date range will be considered CIP deviations.

3.5.5 Stages 5 and 6 –12 24, 36, 48, 60 Month Assessments

1. As part of standard care, treated patients will be monitored by outpatient upper endoscopic procedures after completion of cryoballoon ablation (Stage 2), with EGDs every 3 months for first year (stage 5), then every 6 months for the second year to month 24 (stage 6). The endoscopic findings (detailed above) and pathology results will be documented at month 12,24, 36, 48 and 60 months for study endpoint analyses.

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Table 2 - Schedule of Treatments and Assessments
**Adverse events occurring prior to the procedure will be documented in the patient's medical record but will not count as related to the investigational device or procedure.*

*Treatments: Baseline or initial treatment visit = visit 0; subsequent treatments: 2nd to 5th treatment visits (10 weeks), as needed, up to 12 months from baseline. Treatments only if there is visible residual disease; no endoscopically- visible BE or abnormal squamous mucosa, biopsy will be performed using standardized protocol, beginning at the gastric cardia then the neo-Z line and every 1-2 cm proximal, to sample the previously treated BE areas (according to current standard of care).
Subjects can opt-out of participating in the surveillance phase of the trial after 12 months, if local endoscopic surveillance is preferred.

Adverse events: any patient with pain, dysphagia, fever, or other non-serious adverse event will be called weekly from day 8 to 1 month post-treatment or until AE resolution. Adverse events will be graded and recorded according to standard NIH definitions.

STUDY STAGES →	Stage 1	Stage 2	Stage 3				Stage 4	Stage 5	Stage 6
Assessments	Informed Consent/ Baseline Evaluation	Initial Treatment *	Immediately Post-/prior to discharge (up to 12 hours)	1-day Phone Contact (±1 day)	7-day Phone Contact (+/- 1 day)	30-day Phone Contact (+/- 1 day	EGD for treatment or biopsy (q 8-12 weeks)	EGD for biopsy (endpoint assessment) (12 months)	EGD (24, 36, 48 and 60 months)
Consent	**X**								
Review of Medical History	**X**								
Review of Medications	**X**	**X**	**X**	**X**	**X**	**X**	**X**		
Review of General Health	**X**	**X**	**X**	**X**	**X**	**X**	**X**		
Physical Exam	**X**								
Pain and dysphagia Assessment		**X**	**X**	**X**	**X**	**X**	**X**	**X**	

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Review of Adverse Events			**X**	**X**	**X**	**X**	**X**	**X**	**X**
EGD with cryoballoon ablation		**X**					**X***		
EGD with biopsy protocol							**X***	**X**	**X**

3.6 Treatment Procedure

3.6.1 Patient Preparation

The patient should be prepared for the planned endoscopy according to standard hospital procedures for endoscopy under conscious sedation or monitored anesthesia care.

3.6.2 Medication

Pain medication will be administered at the physician's discretion and the use of such medication documented. Acid suppression by double dose PPI will be administered between Informed Consent (Stage 1) and Follow-up Endoscopy (Stage 4,5,6). Other medications will be administered at the physician's discretion and will be documented.

3.6.3 Devices and Equipment

In addition to the Investigational System, the following may be required. All devices listed below are to be provided by the site and are available commercially for the indications for which they are proposed in this study.

- Endoscopic system with a therapeutic endoscope (3.7mm or 6.0 MM ID minimum; 100 cm length maximum) and a diagnostic endoscope.
- Materials and supplies used for standard endoscopy according to standard institution practice
- Materials to perform a biopsy.

3.6.4 Investigational Procedure

The investigational System must be used according to the manufacturer's IFU provided as **Attachment B**.

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3.6.5 Procedure Overview

A summary overview of the procedure is provided below; however, **refer to the attached IFU for specific details, warnings, precautions and details steps for system use.**

- Review the IFU document for any warnings, precautions or other cautionary statements.
- Prior to use, the investigational System should be examined for defects.
- Insert the balloon catheter into the working channel of the endoscope.
- Visualize and position the balloon in the target treatment zone.
- Prepare the handle per the IFU.
- Connect the balloon catheter to the handle.
- Depress the trigger on the handle for treatment.
- Disengage the trigger when prompted by the console.
- Reposition and retreat if necessary or allow complete balloon deflation and withdrawal the catheter.

3.6.6 Disposal of Investigational Device

The Investigational System should be returned to C2 for evaluation. For the return of biohazard product, C2 Therapeutics, Inc. must be contacted prior to product return for handling instructions.

3.6.7 Device Malfunction or Failure

A Risk Analysis was performed by C2 prior to the start of this study. Potential risks were identified and measures taken to reduce the chances for occurrence of each.

Additionally, Performance Testing of the System was conducted in accordance with C2's standardized testing procedures to demonstrate adequate device performance. The combination of the Risk Analysis and the Performance Testing allowed for risks to be identified, performance of the device confirmed, and potential device malfunctions minimized. Refer to the Investigator's Brochure **Attachment A** for additional details.

NOTE: All Adverse Events (AEs) will be followed to resolution. A patient may not be exited from the study until all AEs are resolved or stabilized and an outcome determined.

3.7 Study Withdrawal

Subjects may be terminated or withdrawn from the study for the following reasons:

- Subject death.
- Voluntary withdrawal – meaning that subject voluntarily chooses not to further participate in the study.
- Loss to follow-up – meaning that the subject is or does not return for further follow up.

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NOTE: For those patients considered lost to follow-up (meaning at least 3 attempts to contact the patient are documented and are unsuccessful), the site will, at a minimum, make a concerted effort to confirm that the patient is not deceased (e.g., active search of death indices will be performed to ensure the patient remains alive).

Any study subject who does not participate in a scheduled follow up assessment should be contacted by site personnel to determine the reason. The reason should be determined and documented in the patients study records. If the missed assessment was due to an adverse event (AE), an AE Case Report Form (CRF) must be completed and any reporting requirement met.

All subjects enrolled (including those withdrawn or lost to follow-up) shall be accounted for and documented.

4.0 ADVERSE EVENTS

Adverse events (AEs) may occur during the procedure or during the follow-up phase. **AEs occurring prior to the procedure will be documented in the patient's medical record but will not count as related to the investigational device or procedure.** See the Reporting of AEs section for reporting timelines.

Each AE will be recorded in the corresponding patient's Case Report (CRF). Each AE will be judged by the Investigator as to its relationship and level of relatedness to the investigational System and/or investigational procedure. In addition, the Investigator will identify the date of onset, severity and duration. All AE will be monitored until they are adequately resolved or explained.

5.0 RISK – BENEFIT ASSESSMENT

Upper endoscopy is standard of care for the evaluation of patients who present with foregut symptoms. The risk of participation in this study is similar to that which would be expected from these procedures were the patient not in the study. The most common risks are associated with the introduction of the endoscope and tissue sampling include sore throat, less commonly prolonged bleeding from the sampling site and very rarely injury to the esophagus or stomach that would require surgical repair. The risks associated with the sedation used during endoscopy include local bruising or pain at the IV site, allergic reaction to the medications and over sedation requiring sedation reversal medications and longer post-procedure observation. All patients undergoing endoscopy are monitored with continuous pulse oximetry and vital signs assessment during the procedure. Medications used for conscious sedation are carefully titrated and monitored based on the patients' arousal levels and vital signs.

Anticipated observations and complications are outlined in **5.2**.

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5.1 Potential Benefits

The simplicity of the Cryoballoon Focal Ablation System allows for potential benefits to the patient, the physician, and hospital including shorter or safer procedures, an easier deployment minimizing the need for anesthesiology, and smaller inventory requirements and no capital equipment improving capital resource utilization. The purpose of this study is to understand the effects of the system on BE epithelial tissue.

Therefore there is no direct benefit of study participation. However, other patients may benefit in the future from the study of the Cryoballoon Focal Ablation System resulting in the development of an easy-to-use alternative to current ablative technologies.

5.2 Potential Risks

The side effects listed below are associated with the investigational procedure as well as endoscopy performed under conscious sedation. The side effects have been categorized as either an observation or a complication. The observations are anticipated as a result of this procedure and are not considered an AE unless they are greater in severity or degree of incidence than anticipated.[5,6] The complications will be reported as an AE regardless of the degree of severity or incidence.

The observations and complications indicated by an asterisk are solely associated with the investigational procedure. The medical team in charge of this study is aware of these risks and, as with any endoscopy performed, all precautions are taken to avoid these risks. The patient will have the opportunity to discuss any concerns with the medical team.

Observations:

- Anxiety
- Bleeding
- Bruising / pain at the IV site
- Change in intestinal function (e.g., constipation)
- Abdominal or chest pain
- Depression
- Difficulty/painful swallowing
- Fever
- Headache
- Lethargy/disorientation/sleepiness
- Nausea and / or vomiting
- Numbness in the mouth, tongue or throat
- Pain
- Sore throat

[5] Sharma, et al, Balloon-based, circumferential, endoscopic radiofrequency ablation of Barrett's esophagus: 1-year follow-up of 100 patients, Gastrointest Endosc 2007;65:185-95.
[6] Dumot JA, et al, An open-label, prospective trial of cryospray ablation for Barrett's esophagus high-grade dysplasia and early esophageal cancer in high-risk patients, Gastrointest Endosc. 2009 Oct;70(4):635-44.

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- Bloated feeling

NOTE: If any of the above listed observations are greater in severity or degree of incidence than anticipated, the observation will be considered an AE.

Complications:

- Allergic reaction to the medications given
- Cardiac arrhythmia (irregular heartbeats which could be life-threatening)
- Inhalation of gastric secretions (breathing in of fluid in the stomach)
- Infection
- Hemoptysis (coughing up of blood)
- Over-sedation requiring sedation reversal
- Perforation or laceration (small hole or tear) of the esophagus
- Decreased respiration
- Stricture (narrowing of the esophagus)
- *Ingestion of the fluid in the cryoballoon which may result in discomfort/stomach bloating
- Death

5.3 Minimization of Anticipated Risks

All efforts will be made to minimize these risks by System design, selecting investigators who are experienced and skilled in endoscopic procedures, clearly defining eligibility criteria to ensure that only the appropriate patients are enrolled and by ensuring that the treatment and follow-up of the patient are consistent with current medical practice.

Risks are further minimized due to:

- The use of medical grade materials that have a long history of use and have been characterized and tested to assure biocompatibility.
- Biocompatibility testing has been performed and the results were determined acceptable.
- Pre-clinical evaluation including bench testing, analytical testing and animal studies.

5.4 Potential Risks to Subject Confidentiality

In all clinical studies, confidentiality of protected health information may be breached due to study-related activities beyond those of routine clinical care. This risk will be minimized by not collecting personally identifying information on data collection forms or other study related documentation to be provided to the study Sponsor.

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6.0 SAFETY MONITORING

Dr. Canto will oversee the single center clinical trial as the DMC. A Consulting Statistician may be consulted, if necessary. The DMC will review study progress and study conduct and will be responsible for the decision to stop recruitment or modify the study design, as necessary, throughout the course of the study. Refer to **Section 7.3** for Safety Related Stopping Rules.

7.0 STATISTICAL ANALYSIS PLAN

This is a feasibility study without a primary study hypothesis or statistical comparison.

7.1 Continuous Outcomes

Continuous outcomes will be summarized with mean, standard deviation and other relevant statistically summaries. When not normally distributed, medians and quartiles will be reported. A confidence interval approach may be used, if appropriate, to compare outcomes with historical data.

7.2 Binary Outcomes

Binary and ordinal outcomes will be tabulated. Confidence intervals for proportions will be calculated using the exact binomial distribution. A confidence interval approach will be used to compare outcomes with historical data.

7.3 Safety Related Stopping Rules

The principal investigator will be charged with monitoring the study for safety and for auditing the quality of the data. If there are any perceived safety concerns related to the system or therapy, a corrective action may be implemented or the trial may be terminated.

The principal investigator will meet periodically during the study, after every 10 patients are enrolled and treated, to review any safety concerns. After 20 patients are enrolled and treatments are completed, the DMC will meet to review preliminary efficacy data

Should any of the following occur within 30 days following treatment and be, in the opinion of the investigator, directly attributable to the Therapy or System, the DMC will be alerted *as soon as feasible* (not to exceed 48 hours of knowledge of the event) and study treatments will be suspended without delay.

- Death or an incident leading to death
- 5 consecutive patients with device malfunctions
- 5 consecutive patients with moderate or severe stenosis in treatment area.

The DMC will review the details surrounding the above-listed event(s) and will be charged with making one of the following decisions:

- Continue without modification to the study protocol or conduct
- Continue with modification

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- Stop the study

Should the study continue, but with modification (to the System or the study documentation) or if the decision is made to stop the study, the reviewing Ethics Committee(s) and where applicable, the governing agency(ies), will be alerted. Treatments will not commence until the necessary EC approvals for the modification have been obtained.

NOTE: If the decision is made to stop the study, treatments and patient screening activities will cease, however, patient follow up will continue as planned.

7.4 Missing Data

Missing values will generally be ignored in analyses. Imputation approaches, including last observation carry forward (LOCF) will not be used.

8.0 STUDY MANAGEMENT

As the study Sponsor, Pentax will provide study devices and supplemental funds to continue the trial as a single center study with expanded criteria and adjusted dosing schedule.
Dr. Canto is the principal investigator and has the overall responsibility for the conduct of the study.
Study data will be collected using a standardized Case Report Form. The CRF is designed to accommodate the specific features of the trial design.

To ensure proper tracking of Case Report Forms, a master tracking system will be utilized.

8.1 Data Management Responsibilities

The Data Management group will employ a relational database to manage and house the resulting study data as collected on the standardized CRF. Conventional data verification routines will be performed. Data Management will be performed according to data audit, data handling and other applicable SOPs.

8.1.1 Data Entry

Data entry will be performed as the completed CRF pages are received by Data Management. Data entry will be performed by qualified personnel that have undergone appropriate training. Data entry will be verified by a 2nd individual to ensure correct entry. Data entry and verification will be handled according to the applicable data handling and audit procedures.

8.1.2 Data Cleaning

All CRF pages will be subject to initial inspection for omitted data, gross data inconsistencies, illegible data and deviations. Any deficiencies or deviations will be reviewed and any necessary action determined (e.g., data query, communication to the study center).

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Intermittent data review (including crosschecks) will be performed and any discovered errors will be reported to the study site using the data correction and query process (as necessary). The study site will be expected to review the query, make any necessary corrections or comments, and return to Data Management where the correct response will be entered into the database. The data cleaning cycle will be repeated until all data are considered clean.

8.1.3Data Back-up

Incremental computer data back-up will be performed on a regular basis. All hard copies of Case Report Forms and media will be stored in a secure location.

8.1.4Confidentiality and Security

Passwords will be utilized by data entry, data verification and other personnel who have database access to insure confidentiality and protection of data.

8.1.5Data Retention

CRF pages, resolved queries as well as the locked database will be housed in a secure location and will be archived for a minimum of 2 years following commercialization.

CRF pages and queries will be scanned and housed in an organized fashion on a secure server which will be backed up intermittently. The original, hard copy CRFs will remain the original data.

The database will also be managed on a secure server and backed up intermittently per Data Management procedures.

8.2 Ethical Considerations

It is expected that all parties will share in the responsibility for ethical conduct in accordance with their respective roles in the study. The Sponsor and the Investigator(s) shall avoid improper influence or inducement of the subject, monitor, the fellow study Investigator(s) or other parties participating in or contributing to the study.

8.3 Data Management Responsibilities

The Investigator will be responsible for Data Management. Data Management will be handled by appropriately qualified personnel.

8.3.1 Maintenance of Study Records

The Investigator is responsible for maintaining medical and study records for every subject participating in the study (including information maintained electronically such as digital imaging). The Investigator will also maintain **original** source documents from which study-related data are derived, which include, but are not limited to:

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- Study progress notes recording subject's medical history and medications
- Medical charts with operative reports and the condition of subject upon discharge
- Medical records regarding AEs, including treatment and clinical outcome
- Results of diagnostic examinations
- Imaging (such endoscopic images), as well as the report of the reading/interpretation of the images
- Notes of phone calls and/or correspondence indicating investigational site's attempts to follow study subjects until subject's participation in the study is complete or terminated
- Records relating to subject death (e.g., death certificate, autopsy report)
- Print-outs of source data generated by technical equipment (e.g., x-rays, MRIs) must be filed with the patient's records.

The study Sponsor and Investigator must ensure that all study subject records are stored for at least 2 years since the formal discontinuation of clinical development of the investigational product. To avoid error, the study site should contact the Sponsor prior to the destruction of study records to ensure that they no longer need to be retained. In addition, the Sponsor should be contacted if the Investigator plans to leave the investigational site so that arrangements can be made for the handling or transfer of study records.

8.3.2 Required Documents from the Investigator

At a minimum, the following documents will be provided by the investigational site to the study Sponsor:

- Signed Investigator Agreement (for each investigator)
- Signed CIP Cover Page
- IRB/EC approval of the study
- IRB/EC approved ICF
- Investigator and Co-Investigator's current signed and dated Curriculum Vitae (CV) *

A site may not begin study participation until all of the above listed documents have been provided to the study Sponsor.

* The study may begin once one Investigator for the site has submitted their Investigator Agreement and signed CV. No additional Investigators may participate until a copy of their signed Investigator Agreement and CV has been provided to the study Sponsor.

8.4 Training

The System is intended for use by experienced Endoscopists. All Investigators will be provided training by Pentax personnel or designee in the use of the System using a bench top model or equivalent to familiarize them with the use of the System prior to their participation in the study.

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8.5 Protection of Patient Confidentiality

At all times throughout the study, confidentiality will be observed by all parties involved. All data shall be secured against unauthorized access. Privacy and confidentiality of information about each subject shall be preserved in the reports and in any publication. Each subject participating in this study will be assigned a unique identifier. All CRFs will be tracked, evaluated, and stored using only this unique identifier.

The Investigator will maintain a confidential study subject list identifying all enrolled subjects. This list will contain the assigned study subject's unique identifier and name. The Investigator bears responsibility for keeping this list confidential. This list will not be provided to the study Sponsor and is only to be used at the study center.

NOTE: The subject's name, medical record number or address will NOT be recorded in the sponsor's records, reports or the database; demographic data that may be recorded includes the date of birth, race, and gender.

Any source documents copied by the Sponsor will be identified using the assigned study subject's unique identifier in an effort to protect subject confidentiality.

8.6 Study Suspension or Early Termination

The study can be discontinued at the discretion of the Investigator or study Sponsor for reasons including, but not limited to, the following:

- Occurrence of AE unknown to date in respect to their nature, severity, or duration, or the unexpected incidence of known AE.
- Obtaining new scientific knowledge that shows that the study is no longer valid or necessary.
- Insufficient recruitment of subjects.
- Unanticipated adverse device effect (UADE) presenting an unreasonable risk to subjects (Sponsor may terminate the study immediately).
- Persistent non-compliance with the CIP.
- Persistent non-compliance with IRB/EC or other applicable laws or regulatory requirements.

If the study is discontinued or suspended prematurely, the Sponsor shall promptly inform all clinical Investigator(s) / study center(s) of the termination or suspension and the reason(s) for this. The IRB/EC shall also be informed promptly and provided with the reason(s) for the termination or suspension by the Sponsor or by the clinical Investigator / study center(s). Regulatory authorities and the personal physician's of the subjects may also need to be informed if deemed necessary.

8.7 Quality Assurance and Supervision by Authorities

The System conforms with applicable industry/international standards for performance testing, biocompatibility, packaging, labeling, sterilization as well as the applicable physical, mechanical and biological testing recommended in ASTM F 2096-04, ASTM F 88, ISO 10555-1, and ISO 10555-4.

All documents and data shall be produced and maintained in such a way to assure control of documents and data to protect the subject's privacy as far as reasonably practicable. The Sponsor and representatives of the FDA or other regulatory authorities are permitted to inspect the study documents (e.g., CIP, CRFs, and original study-relevant medical records/files) as needed. All attempts will be made to preserve subject confidentiality.

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All study sites are subject to audit by study Sponsor personnel or designee for CIP adherence, accuracy of reported data and compliance. Any evident pattern of non-compliance with may be cause for the site to be put on probation until appropriate corrective action is taken.

The CIP, data-recording procedures, data handling as well as study reports are subject to an independent study Quality Assurance audit by Pentax, its designee, or health authorities.

8.7.1 Approved Informed Consent

The reviewing IRB/EC must review and approve an ICF specific to this study. Each original, signed and dated ICF should be retained by the study site and a copy provided to the subject.

8.7.2 IRB / EC Approval

IRB/EC approval is required prior to study commencement. The Investigator must also obtain renewal of IRB/EC approval as dictated by local requirements during the entire duration of the study. The Investigator is responsible for fulfilling any conditions of approval imposed by the reviewing IRB/EC, such as regular reporting, study timing, etc. The Investigator will provide the study Sponsor with copies of such approvals and reports.

8.7.3 Reporting of Adverse Events

The Investigator will submit to the Sponsor a report of any SAE, including patient death occurring during an investigation as follows:

The Investigator will report all of the above to the reviewing IRB/EC (as applicable) according to the local reporting requirements.

8.8 Final Report

A final report will be completed even if the study is prematurely terminated.

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8.9 Publication Policy

At the conclusion of the study, an abstract reporting the results will be prepared and may be presented at a major meeting(s). A publication may also be prepared for publication in a reputable scientific journal. No publication will be made without the review of the Principal Investigator and of the Sponsor. Results will be published even if the trial is negative..

9.0 DEFINITIONS AND ACRONYMS

Adverse Events

Adverse Event (AE) - any untoward medical occurrence in a subject (ISO 14155)

NOTE: This definition does not imply that there is a relationship between the adverse event and the device under investigation.

Serious Adverse Event (SAE) - an adverse event that (ISO 14155):

- led to a death,
- led to a serious deterioration in the health of the subject,
- resulted in a life-threatening illness or injury,
- resulted in a permanent impairment of a body structure or a body function,
- required hospitalization or prolongation of existing hospitalization,
- resulted in medical or surgical intervention to prevent permanent impairment to body structure or function,
- Or led to fetal distress, fetal death, a congenital abnormality, or birth defect.

Adverse Device Effect (ADE) - any untoward and unintended response to a medical device (ISO 14155).

NOTE: This includes any event resulting from insufficiencies or inadequacies in the instructions for use or the deployment of the device. This definition also includes any event that is a result of user error.

Serious Adverse Device Effect (SADE) - an adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event or that might have led to any of these consequences if suitable action had not been taken or intervention had not been made or if circumstances had been less opportune (ISO 14155).

Unanticipated Adverse Device Effect (UADE) - any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21CFR812.3.s) and (ISO 14155).

NOTE: The occurrence of a diagnostic or elective surgical procedure for a pre-existing condition, unless the condition becomes more severe or increases in frequency, would not be considered procedure or device-related.

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Adverse Device Effect (ADE)
See Adverse Events.

Case Report Form (CRF)

Ethics Committee (EC)

Independent Review Board (IRB)

Informed Consent Form (ICF)

Instructions for Use (IFU)

Serious Adverse Device Effect (SADE)
See Adverse Events.

Serious Adverse Event (SAE)
See Adverse Events.

Source Data
All information in original and identified records and certified copies of original records of clinical findings, observations, or other activities in a clinical investigation, necessary for the reconstruction and evaluation of the clinical investigation (ISO 14155).

Source Documents
Original documents, data and records (ISO 14155).

NOTE: This may be, for example, hospital records, laboratory notes, pharmacy dispensing records, copies or transcriptions certified after verification as being accurate copies, photographic negatives, radiographs, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical investigation.

Standard Operating Procedure (SOP)

Unanticipated Adverse Device Effect (UADE)
See Adverse Events.

APPENDIX

Animal Data (prior to conduct of human clinical trials).

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The safety and effectiveness of the System used to deliver superficial freezing to the esophageal mucosal tissue was evaluated in a domestic swine (n=15, # of ablations = 47 for FG-1000 and n=10, # of ablations = 54 for FG-1003). The normal swine esophagus is very similar in dimensions to a human esophagus. The swine were treated with the System by exposure of the esophagus to short periods (3-15 seconds) of cryogen refrigerant (liquid nitrous oxide or N_2O) through the wall of the System balloon probe at low balloon pressure The N_2O is completely contained within the System balloon probe.

Additionally, C2 conducted animal experiments to study the safety, deliverability and performance characteristics of the System. The non-clinical studies were conducted using general animal facility protocols for the evaluation of devices in a normal pig esophagus at dimensions very similar to a human esophagus.

	5022	5018	5016	5055	5057	5058	5152	5153	5154	5157
Follow-Up (Days)	4	4	4	4	4	4	4	4	4	28
Top of Esophagus (cm)	30	30	30	24	28	28	26	27	29	29
GE Junction (cm)	63	62	62	65	67	62	65	63	64	63
Initial Weight	47.2	50.5	53.3	54.2	47.5	49.8	50.9	50.2	50.2	55
Final/ Weight	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	67.6
Location (cm)	57	57	57	60	55	55	60	60	60	57.5
Time (secs)	6	14	10	10	2	3	3	5	6	6
Size of ablation site (cm^2)	1.6	2.6	2.9	1.8	Negligible	0.6	0.4	0.5	1.1	No stricture
Location (cm)	52	52	52	54	50	50	55	57.5	57.5	55
Time (secs)	10	6	14	4	4	4	5	3	6	6
Size of ablation site (cm^2)	3.5	2.2	2.1	1.7	Negligible	1.0	0.5	0.8	1.0	No stricture
Location (cm)	47	47	47	48	45	45	50	55	55	52.5
Time (secs)	14	10	6	6	2	3	4	4	9	9
Size of ablation site (cm^2)	4.6	3.4	2.2	2.4	Negligible	0.8	0.4	1.2	1.4	No stricture
Location (cm)	42	42	42	42	40	40	45	50	50	47.5
Time (secs)	6	14	10	10	4	5	3	5	9	9
Size of ablation site (cm^2)	1.8	4.5	3.0	2.1	1.2	0.9	0.5	0.8	1.3	No stricture

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Location (cm)	37	37	37	36	35	35	40	47.5	47	N/A
Time (secs)	10	6	14	4	2	3	5	3	6	N/A
Size of ablation site (cm^2)	3.0	3.1	1.4	2.5	.76	0.9	0.6	0.4	1.8	N/A
Location (cm)	32	32	32	32	30	30	35	45	45	N/A
Time (secs)	14	10	6	6	4	5	4	4	6	N/A
Size of ablation site (cm^2)	5.6	5.2	3.5	2.5	1.8	0.9	0.4	Negligible	Negligible	N/A

Table Animal Study Summary, Cryoballoon Focal Ablation System (FG-1003)

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[i] Gage AA, Baust J. Mechanisms of tissue injury in cryosurgery. Cryobiology. 1998; 37: 171-86.

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