



Safety and Efficacy of the CryoBalloon Ablation for Treatment of Sporadic and Familial Nonampullary Nonpolypoid Duodenal Adenomas

The C2D2 Trial

National Clinical Trial (NCT) Identified Number: NCT03847636

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Investigator Acknowledgement Signature

I have received and reviewed this investigational plan. The signature below provides the necessary assurances that this clinical study will be conducted according to all stipulations of the protocol and will be maintained as confidential.

Investigator Name (Print)

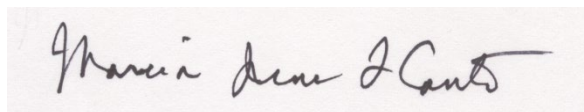
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Sponsor Signatures

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Clinical Study Synopsis

PRINCIPAL INVESTIGATOR	Marcia Irene Canto, MD, MHS; JHM gastroenterology (Investigator-initiated)
STUDY SPONSOR	Johns Hopkins University
PROTOCOL TITLE	Pilot Study of Nitrous Oxide CryoBalloon Ablation for Eradication of Non-Ampullary Sporadic and Familial Duodenal Adenomas (DAs) – “The C2D2 trial”
STUDY DEVICE	CryoBalloon™ Focal Ablation System (CbFAS) – first or second generation
STUDY DESIGN	Prospective multicenter non-randomized clinical trial
STUDY OBJECTIVE	To study the feasibility (technical success, safety, and efficacy) of completely eradicating nonpolypoid (flat) non-ampullary (not involving the ampulla) sporadic or familial DA with focal CryoBalloon ablation using the C2 system.
RATIONALE	Sporadic DA: 21% progress to HGD, even without change in size or appearance, and 54% of these have invasive cancer on resection, particularly those with HGD present in first biopsy and those ≥ 2 cm in size ¹ . DAs in familial adenomatous polyposis (FAP) may also precede cancer development in these high-risk patients. Endoscopic resection of large adenomas is associated with a significant risk of bleeding and perforation. This study may impact upon the management of patients with duodenal adenomas by demonstrating the potential for eradication with endoscopic cryoablation. The safety profile of endoscopic CryoBalloon ablation may be better than endoscopic resection or surgery.
STUDY POPULATION	Patients with benign sporadic or familial non-polypoid DAs >1-5 cm in size, ≤ 2 mm thick, and < 50% circumference of the first and second portion of the duodenum.

CryoBalloon™ Focal Ablation System

COMPARATOR	No comparison or control arm for this feasibility study
BASELINE ASSESSMENT	All eligible patients will undergo baseline EGD with HRE and targeted biopsy to assess Paris classification, size, thickness, circumferential extent. Local pathology review of the baseline or outside biopsies obtained within 12 months of enrollment will establish baseline histologic diagnosis and dysplasia grade.
INTERVENTION	The procedure will be performed on an outpatient basis according to the site's standards of care for anesthesia and sedation during esophagogastroduodenoscopy (EGD) procedures. Standard of care high resolution white light and enhanced imaging with NBI or IScan will be performed for lesion assessment with image and video documentation. Nitrous oxide focal CryoBalloon ablation of no more than 4 eligible targeted DAs in the first and second portion of the duodenum. In FAP patients, up to 4 largest eligible DAs will be treated. Target area(s) can be delineated first by argon plasma coagulation (APC) or snare tip with soft coagulation, with a 5 mm margin from the edge of the polyp. Cryogen dose will be 10 or 12 seconds for flat adenomas $\leq$ 2 mm in height from surface (Paris 2A, 2B) or a total of 14 seconds for thicker adenomas up to 4 mm or those not responding 1 prior cryoablation session . Assess for red color change of ablation effect, may re-treat with 5 seconds for skip areas without adequate red color change. After cryoablation and priming the submucosa with saline, a 1.0 ml submucosal injection of GI Spot will be applied to normal duodenum 2 cm proximal and 2 cm distal to the treated lesion(s) to tattoo the lesion for future assessment, similar to tattoo for marking the site of large colon polyps. Treatment sessions every 3 months +/- 8 weeks. Limit number of treatment sessions to 5 within 1 year.

PATIENT FOLLOW-UP	Follow-up with safety assessment: 1 day by phone call and 7 days +/- 7 days and 30 days +/- 14 days by phone call or electronic medical record messaging, and in person safety assessments at each scheduled visit. All patients undergo EGD with high resolution white light endoscopy and enhanced digital imaging, with image capture and video-recording to facilitate endpoint assessment every 3-6 months according to local standard of care. If the EGD exam at follow-up shows no evidence of residual adenomatous tissue in the treated area, at least 2 biopsies of the treated areas will be taken. If there is visible polyp tissue at any subsequent visit, endoscopic cryoablation may be repeated. A biopsy may be obtained according to physician discretion after ablation. All subjects will have EGD with biopsy at 12 months (± 12 weeks) from baseline CbFAS treatment, after which subjects will be followed for recurrence as part of standard of care, typically every 6-12 months with EGD and biopsy after treatment has been completed. All patients will exit study at an anniversary patient follow-up closure date of May 31, 2024, or 12 weeks after last study patient was enrolled. Patients with incomplete eradication of their polyp(s) at 12 months will be treated inside the study according to their physician's standard of care until May 31, 2023 but main study endpoints will be assessed at 12 months..
STUDY DURATION	3-year study, 1-1.5 years for subject enrollment, and at least 1 year for follow-up, or until the last enrolled and treated patients reaches 1 year follow-up.
PRIMARY ENDPOINTS	1. SAFETY: Safety: incidence of treatment-related adverse events, including, mean and median post-procedure pain scores ≥ 8 using Likert scale 1-10 at day 1, day 7, and day 30, proportion of patients requiring narcotic analgesics after treatment, hospitalization, ER visit, bleeding, and perforation, using per patient analysis
	2. EFFICACY: Clinical response ($>50\%$ reduction in baseline size) and Complete eradication (CE) rate of DAs by endoscopy and biopsy, at 12 months.

SECONDARY ENDPOINTS	EFFICACY: 3. Endoscopic assessment: percent reduction change in adenoma size by blinded review by 3-person expert panel of video and still images with region of interest marked by tattoo – per lesion analysis and per patient analysis, assessed at 1 year 4. In FAP patients, blinded scoring of treatment effect by 3 blinded reviewers: no change (zero), clinical improvement (1), or deterioration in adenoma size and number (-1)* - per patient analysis, assessed at 1 year. 5. In FAP patients: % change in Spigelman class from baseline 6. Progression rate: percentage of patients with progression of dysplasia grade to HGD or invasive cancer at 1 year. 7. Incidence of treatment-related acute pancreatitis in all treated patients. Risk factors for treatment failure or progression. Treatment failure defined as: a) progression of any treated DA to HGD or invasive cancer, b) alternative intervening therapy with endoscopic or operative resection, or c) < 50% polyp eradication at 12 months (by polyp analysis) 8. Median and mean number of CryoBalloon ablation treatments to achieve complete eradication by 1 year. 9. Treatment (technical) success rate (completion of all treatments to all targeted DAs). Technical failure is defined as failure to complete delivery of cryogen to target lesion, analyzed by procedure, and by patient. *similar to reported technique for blinded assessment of the efficacy of celecoxib for FAP duodenal polyposis 10. Duodenal adenoma recurrence rate at last follow-up. 11. Duodenal adenoma time to recurrence at last follow-up
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KEY INCLUSION CRITERIA	2 groups of patients will be studied: 1) sporadic duodenal adenomas between 1 to 5 cm in widest diameter; and, 2) FAP patient with Spigelman class 2, 3 or 4 (see definition below) Polyp characteristics: Non-polypoid lesions Paris 2A and 2B, histologically confirmed benign adenomas, occupying no more than 50% circumference of duodenum, and no more than 3 duodenal folds Because there is limited data on the safety and efficacy of this treatment, patients must be considered high risk for surgery or endoscopic resection, due to complication risk, or have declined standard therapies. Prior endoscopic mucosal resection (EMR) or saline-assisted polypectomy allowed if polyp characteristics meet inclusion criteria.
KEY EXCLUSION CRITERIA	1) Suspected or proven duodenal carcinoma 2) Paris 1p pedunculated, Paris 2c, 2a-c, 2c, or 3 lesions 3) Paris 1s lesion > 4 mm thick (estimated with closed biopsy forceps) 4) Ampullary lesion or lesion involving the ampulla 5) Prior failed ablative treatment in the duodenum with APC, laser, or cryotherapy 6) Cryoablation treatment of polyps located in third or fourth portion of the duodenum.

OTHER EXCLUSION CRITERIA	1. Pre-existing esophageal, gastric, pyloric, or duodenal stenosis/stricture preventing advancement of a therapeutic endoscope during screening/baseline EGD. Subjects are eligible if the stenosis/stricture is dilated to at least 15mm, but baseline treatment may need to be delayed. 2. Any endoscopically-visualized abnormalities such as ulcers, masses or nodules during screening/baseline EGD within 3 cm of the treatment area. 3. Subjects with nodular polyps or suspicion of invasive cancer by WLE/enhanced imaging/biopsy identified during screening/baseline EGD 4. Suspicion of malignancy by abdominal or EUS imaging based on malignant lymph nodes, invasion of lesion beyond mucosa. 5. EMR or polypectomy < 6 weeks prior to baseline treatment. 6. Untreated invasive duodenal malignancy, including margin-positive EMR. 7. Active duodenitis in treatment zone during screening/baseline EGD. 8. Severe medical comorbidities precluding endoscopy, or limiting life expectancy to less than 2 years in the judgment of the endoscopist. 9. Uncontrolled coagulopathy or inability to be off anticoagulation or anti-platelet medication (ASA, Plavix) for 1 week prior to and 2 weeks after each endoscopy, or according to site clinical standard care. 10. Known portal hypertension, visible esophageal, gastric, or duodenal varices, or history of varices. 11. General poor health, multiple co-morbidities placing the patient at risk, or otherwise unsuitable for trial participation. 12. Pregnant or planning to become pregnant during period of study participation. 13. Patient refuses or is unable to provide written informed consent.
SAMPLE SIZE	Up to 50 evaluable patients (estimate 1-4 patients enrolled per site per year x 6 sites), based on highly selected patient population and pilot study design
ENROLLMENT	Competitive enrollment, per patient payment, Johns Hopkins lead site and coordinating center
KEY DATA DELIVERY	Table 1 patient characteristics; Table 2 lesion characteristics, Table 3 complete eradication rates (endoscopy and histology) by polyp size Figure 1 blinded endoscopic improvement (physician review score) Figure 2 percentage change in size from baseline, by patient type
LIMITATIONS	No prior animal dosing studies to validate cryogen dose being used. However, the cryogen doses used in this trial have been used safely and effectively in 2 duodenal adenoma patients at JHH, BE early intramucosal adenocarcinoma, and gastric antral vascular ectasia (GAVE) patients.

PROPOSED TEAM	P.I. Mimi Canto Co-investigators: 1. Geisinger Medical Center – David Diehl/Harshit Khara 2. Long Island Jewish Medical Center, Long Island-(Northwell) – Arvind Trindade 3. University Medical Center New Orleans, New Orleans (Touro Infirmary)_– Virendra Joshi 4. Memorial Hermann Texas Medical Center, Houston – Nirav Thosani 5. Methodist Health – Paul Tarnasky, David Lee 6. Medical University of South Carolina – Pooja Elias, Brenda Hoffman
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Table of Contents

1.	BACKGROUND AND RATIONALE	12
2.	C2 CRYOBALLOON ABLATION SYSTEM DESCRIPTION	12
3.	STUDY OBJECTIVE	14
3.1	PRIMARY OUTCOMES	14
3.2	SECONDARY OUTCOMES	14
3.3	OVERVIEW	14
3.4	SAMPLE SIZE	14
3.5	STUDY POPULATION	14
4.	ELIGIBILITY CRITERIA	15
4.1	INCLUSION CRITERIA	15
4.2	EXCLUSION CRITERIA	15
5.	STUDY PROCEDURES	16
5.2.1	Screening	Error! Bookmark not defined.
5.2.2	Written Informed Consent	16
5.3	BASELINE	16
5.3.3	Baseline Pathology	17
5.4	TREATMENT	ERROR! BOOKMARK NOT DEFINED.
5.4.5	Treatment or Technical Failure	18
5.5	DISPOSAL OF INVESTIGATIONAL DEVICE	19
5.6	POST-PROCEDURE THROUGH DISCHARGE	19
5.7	FOLLOW-UP AFTER COMPLETION OF ABLATION	19
5.10	STUDY EXIT	22
6.	STATISTICAL METHODS	22
6.1	PRIMARY OUTCOMES:	22
6.2	SECONDARY OUTCOMES	22
7.	DATA AND SAFETY MONITORING BOARD	23
8.	RISK AND BENEFIT ASSESSMENT	24
8.1	POTENTIAL RISKS	24
8.1.1	Potential Risks to Subject Confidentiality	Error! Bookmark not defined.
8.2	MINIMIZATION OF RISK	24
8.3	POTENTIAL BENEFITS	24
8.4	SAFETY MONITORING	25
9.	DATA MANAGEMENT	ERROR! BOOKMARK NOT DEFINED.
9.1	DATA COLLECTION	26
10.	MONITORING PROCEDURES	26
10.1	PROTOCOL DEVIATIONS	26
11.	CLINICAL EVENTS	26
11.1	DEFINITIONS	26
11.1.1	Adverse Event	26
11.1.2	Serious Adverse Event	26
11.1.3	Unanticipated Adverse Device Effect	27
11.2	ADVERSE EVENT REPORTING	27
11.3	ADVERSE EVENT REVIEW AND RELATIONSHIP DETERMINATION	27
11.4	SEVERITY OF ADVERSE EVENTS	28
11.5	SUBJECT DEATH	28
11.6	TREATMENT FAILURES AND DEVICE OBSERVATIONS	27

12.	STUDY ADMINISTRATION	28
12.1	INSTITUTIONAL REVIEW BOARD (IRB) APPROVAL	27
12.2	AMENDING THE PROTOCOL	27
12.3	PATIENT INFORMED CONSENT	27
12.4	PRIVACY AND CONFIDENTIALITY	27
12.5	PRE-STUDY DOCUMENTATION REQUIREMENTS	27
12.6	RECORD RETENTION	28
12.7	CRITERIA FOR TERMINATING THE STUDY	28
12.8	LEAD INVESTIGATOR RESPONSIBILITIES	28
12.9	INVESTIGATOR RESPONSIBILITIES	28
13.	PUBLICATION	30
14.	SCHEDULE OF VISITS	31
	BIBLIOGRAPHY	35

1. BACKGROUND AND RATIONALE

Duodenal adenocarcinomas are rare but 21% of sporadic duodenal adenomas may progress to HGD, even without change in size or appearance. Moreover, 54% of these have invasive cancer on resection, particularly those with HGD present in first biopsy and those ≥ 2 cm in size¹.

Familial adenomatous polyposis (FAP) is due to a mutated APC gene. MYH polyposis is due to a mutated MUTYH gene. These polyposis syndromes are associated with duodenal adenomas and carcinoma. After colon cancer, the cumulative lifetime risk of progression to duodenal cancer is high (18% by age 75). The risk for progression is associated with Spigelman class² (see Supplementary table 1 with class 4 having the highest risk of 35-50%^{3,4}).

Treatment of duodenal adenomas is problematic. Some adenomas are flat and difficult to snare using standard polypectomy technique, particularly FAP adenomas. Endoscopic mucosal (EMR) resection and endoscopic submucosal dissection (ESD) have been performed, but these are associated with a high overall complication rate of 26%, including a high rate of bleeding (up to 22-40%, may be delayed). The bleeding risk is associated with size of the polyp, with < 3 cm bleeding rate of 19%, > 3 cm bleeding rate of 33-58%⁵⁻⁷. Closure of the large defect after resection is not possible (cannot approximate edges). Perforation rate is 2%. The duodenum in particular is at risk for endoscopy-related thermal injury or perforation due to its thin wall, and at risk for bleeding due to rich vascular supply.

Surgery (Whipple operation) is an option for treatment of duodenal adenomas, particularly large lesions, but it has a mortality rate of 1-4%, high morbidity rate of 20-30%.

Non-resection techniques for treatment, such as ablation with PDT and APC have had disappointing results. Celecoxib, a cyclooxygenase 2 inhibitor, has been used as a chemopreventive agent for FAP patients with Spigelman class 3 and 4 to decrease the number of polyps, but it has not been proven to prevent cancer⁸.

Cryoablation is an option for treating duodenal adenomas. Its mechanism of action lies in the delivery of extremely cold temperatures (-40°C to -60°C) to the target tissue. The tissue is rapidly frozen, creating a cryolesion, undergoing necrosis, and eventually healing. At a molecular level, the freezing causes direct cell damage through rapid extracellular and intracellular freezing and ice formation. Consequently, extracellular osmotic concentrations change, causing cell membranes to become dysfunctional through disrupted cell integrity.^{9,10} Argon, carbon dioxide or nitrous oxide are the cryogens most commonly employed.

CryoBalloon™ Focal Ablation System

C2 Therapeutics, Inc. (Redwood City, CA, USA) develops balloon-based cryotherapy devices for the removal of unwanted tissue, specifically for endoscopic applications. The C2 Focal CryoBalloon™ Ablation System provides controlled cryotherapy ablation for the treatment of BE. Deployed through the working channel of an endoscope, the Balloon is simultaneously inflated and cooled with an inert cryogen (nitrous oxide) delivered from the Controller (Handle) that ablates the BE cells upon contact with the esophagus. After ablation, the Balloon is repositioned for additional ablation or withdrawn. The CryoBalloon Focal Ablation System ablates an area of approximately 3cm by 2cm of the esophagus. The System may allow for potential benefits to the patient, the physician, and hospital, including a shorter and safer procedure, an easier deployment reducing the need for anesthesiology, and smaller device inventory with no capital equipment requirement.

High safety and efficacy have been demonstrated in published clinical trials on Barrett's esophagus¹¹⁻¹⁴, esophageal squamous dysplasia¹⁵ and gastric antral vascular ectasia (GAVE)¹⁶.

Recently, a retrospective review of a small number of patients treated in academic centers suggests use of CryoBalloon ablation for duodenal adenomas may be safe and effective (see Appendix for preliminary results submitted in abstract form to conference Digestive Disease Week 2019, unpublished)

2. DEVICE DESCRIPTION

The CryoBalloon Ablation System is an FDA 510K cleared device (K131523) being used in accordance with its label for this study (destruction of unwanted gastrointestinal tissue). The CryoBalloon Ablation System is comprised of three (3) components: 1) the disposable Catheter and the Controller (Handle), and the Cartridge containing the cryogen (nitrous oxide). The CryoBalloon Ablation System is deployed through the working channel of an endoscope.

The Catheter consists of a proximal connector, catheter shaft, balloon, and distal atraumatic tip and is provided with the balloon constrained within a protective sheath. The Balloon is a low pressure design constructed of highly-compliant material such that its contour accommodates the esophageal lumen and achieves apposition with the esophageal wall upon inflation. The nitrous oxide from the Cartridge exits the distal diffuser inside the Balloon. The Controller contains the Cartridge holder and refrigerant delivery valve, which is controlled by the trigger.

First Generation C2 CryoBalloon Ablation System

Figure 1 CryoBalloon Ablation System. The Cap is removed to insert the Cartridge. The Connector connects the Catheter Shaft to the Controller (Handle). The Trigger is pressed to inflate the Balloon (6) and ablate unwanted tissue.



The Second Generation C2 CryoBalloon System.

The second generation C2 CryoBalloon system was released this year after FDA clearance (K163684). The main difference of this system is the foot pedal, which attaches to the hand-held controller and allows the treating physician greater control over the targeting and application of the cryogen through controls on the pedal. The focal balloon also was modified so that the diffuser opening can be moved proximally and distally, in addition to clockwise and counterclockwise rotation. The handheld controller can be cleaned and reprocessed in the endoscopy unit according to local standards as specified in the IFU and re-used.



Once positioned within the duodenum at the target location, the Balloon is simultaneously inflated and cooled with nitrous oxide. The nitrous oxide cools the inner Balloon surface to a temperature of approximately -40°C . The Balloon remains stationary during the delivery of the nitrous oxide. All procedures using the CryoBalloon™ Ablation System will be performed under anesthesia according to the institution standard of care and physician preference. However, general anesthesia will not be used for any ablation procedures. Refer to the Instructions for Use (IFU) provided with the System for specific instructions on its use.

3. STUDY OBJECTIVE

The objective of this study is to determine the feasibility (technical success, safety, and efficacy) of completely eradicating non-polypoid non-ampullary sporadic or familial DA with focal CryoBalloon ablation using the C2 CryoBalloon system.

3.1 Primary Outcomes (to be assessed at 1 year from initial treatment designated as day 0)

1. Safety: overall incidence of treatment-related serious adverse events using per patient analysis.
2. Efficacy: complete endoscopic and histologic eradication (CE) rate of duodenal adenomas (per patient and per lesion analysis)

3.2 Secondary Outcomes (to be assessed at 1 year from initial treatment designated as day 0)

1. Technical success rate: Technical failure will be defined as failure to complete treatment (treat all targeted areas) analyzed by procedure and by patient.
2. Progression rate: percentage of subjects with progression of dysplasia from LGD to HGD or cancer, or progression of HGD to cancer at 12 months.
3. Risk factors (by patient and by polyp) associated with failure to respond to CbFAS.
4. Median and mean number of CbFAS treatments required to achieve CE by 12 months.
5. Endoscopic assessment: percent reduction change in adenoma size by blinded review by 3-person expert panel of video and still images with region of interest marked by tattoo – per lesion analysis and per patient analysis, assessed at 1 year
6. In FAP/MAP patients, blinded scoring of treatment effect by 3 blinded reviewers: no change (zero), clinical improvement (1), or deterioration in adenoma size and number (-1)* - per patient analysis, assessed at 1 year.
7. In FAP/MAP patients: % change in Spigelman class from baseline
8. Failure rate: defined as: a) intervening EMR or surgery, or < 50% polyp eradication (per polyp analysis)
9. Adverse events: mean and median post-procedure pain scores using a Likert scale 1-10 at day 1, day 7, and day 30, proportion of patients requiring narcotic analgesics within 30 days after treatment
10. Duodenal Adenoma recurrence rate for study duration.
11. Duodenal Adenoma time to recurrence for study duration.

3.3 Overview and Study Design

This is a multicenter, prospective cohort study, with standard of care treatment and follow-up procedures performed for a highly select patient population.

3.4 Sample Size

Up to 50 patients will be enrolled in this pilot study due to highly select patient population.

3.5 Study Population

Patients with benign sporadic or familial non-polypoid in the first and second portion of the duodenum.

4. ELIGIBILITY CRITERIA

4.1 Inclusion Criteria

Patients must meet ALL of the following criteria to be eligible for participation in the study:

1. Biopsy-proven adenoma (tubular, tubulovillous, or villous) in the first or second portion of the duodenum, histology within 12 months
2. Size and morphology characteristics
 - 2.1. Maximum diameter between 1 and 5 cm
 - 2.2. Maximum height 2 mm, or 1 closed biopsy forceps width (Paris 2A, Paris 2B)
 - 2.3 Lesion involving < 3 duodenal folds
 - 2.4 Lesion involving no more than 50% circumference
3. Older than 18 years of age at time of consent.
4. Provides written informed consent on the IRB-approved informed consent form.
5. Willing and able to comply with study requirements for follow-up.

4.2 Exclusion Criteria

Patients must be excluded if ANY of the following criteria are met:

1. EMR < 6 weeks prior to baseline treatment.
2. Untreated suspected or confirmed invasive duodenal malignancy by endoscopy, imaging, biopsy (such as thickened duodenal wall, lymphadenopathy, metastatic lesions), including margin-positive EMR.
3. Paris 1p pedunculated, Paris 2c, Paris 2a-2c or Paris 3 lesions
4. Paris 1s lesion > 4 mm thick (estimated with closed biopsy forceps)
5. Ampullary lesion or lesion involving the ampulla
6. Prior failed ablative treatment in the duodenum with APC, laser, or cryotherapy
7. Cryoablation treatment of polyps located in third or fourth portion of the duodenum
8. Endoscopically-visualized abnormalities such as erosions, ulcers, masses or nodules within 3 cm of target lesion
9. Subjects with nodular polyps or suspicion of invasive cancer by WLE/enhanced imaging/biopsy identified during screening/baseline EGD
10. Pre-existing esophageal, gastric, pyloric, or duodenal stenosis/stricture preventing advancement of a therapeutic endoscope during screening/baseline EGD. Subjects are eligible if the stenosis/stricture is dilated to at least 15mm, but baseline treatment may need to be delayed.
11. Severe medical comorbidities precluding endoscopy, or limiting life expectancy to less than 2 years in the judgment of the endoscopist.
12. Uncontrolled coagulopathy or inability to be off anticoagulation or anti-platelet medication (ASA, Plavix) for 1 week prior to and 2 weeks after each endoscopy, or according to site clinical standard care.
13. Known portal hypertension or liver cirrhosis.

14. General poor health, multiple co-morbidities placing the patient at risk, or otherwise unsuitable for trial participation.
15. Pregnant or planning to become pregnant during period of study participation.
16. Patient refuses or is unable to provide written informed consent.

5. STUDY PROCEDURES

All procedures will be documented on study-specific electronic case report forms that are provided in addition to this protocol. Refer to Appendix A for a summary of study procedures.

5.1 Screening Visit

5.1.1 Subject Recruitment and Identification

Patients from the participating sites will be screened for potential eligibility according to local IRB approved screening methods.

5.1.2 Written Informed Consent

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator or designee will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

5.1.3 Pathology Confirmation

Pathology report documenting the histology diagnosis of duodenal adenoma must be within 12 months from planned treatment visit.

5.2 Baseline Visit

Potential subjects who agree to participate and provide written informed consent will be enrolled on the study.

5.2.1 Endoscopy

All potential subjects will undergo baseline high-resolution EGD with WLE and NBI or other standard of care enhanced electronic imaging to evaluate the duodenal adenoma(s) to assess all other inclusion

CryoBalloon™ Focal Ablation System

criteria to ensure subject is eligible for the study. EGDs will be performed on an outpatient basis using the institution's standard of care for anesthesia and conscious sedation.

Patients with nodular polyps (Paris 1s, or nodularity > 2 mm in the polyp) must be treated with Endoscopic Mucosal Resection (EMR) prior to enrollment. If intramucosal cancer is present, complete eradication of cancer should be achieved prior to enrollment to this study.

Since final eligibility cannot be determined until the endoscopy exam, it is expected that some patients may be excluded based on the endoscopy findings and will not undergo the cryoablation procedure (e.g., because of narrowing of the esophagus such that the endoscope cannot be advanced).

Patients are considered enrolled ("subjects") at the time the CryoBalloon™ Ablation System is inserted to the endoscope.

5.2.2 CbFAS Treatment

Treatment time begins when the first or second generation CryoBalloon Ablation Catheter is inserted into the endoscope and ends when the Catheter is removed from the endoscope. Actual treatment time will vary by subject and will be based on the adequacy of the tissue in the target treatment area(s). Actual treatment time by subject and treatment location will be documented and reported. If a concerning neoplastic-type lesion is noted during or after treatment, EMR or biopsy should be performed according to standard of care.

The treatment will proceed as follows and in accordance with the device instructions for use. The CbFAS device is FDA-approved for the destruction of unwanted tissue (FDA 510K K101825, 9/29/2010)

1. ASSESS LESION(S):
 - 1.1. Document the location of each duodenal polyp (first/second portion), (superior/inferior/medial/lateral wall), and distance from incisors after straightening the endoscope.
 - 1.2. Document the circumferential extent of the duodenal involvement ("C-value") and maximum diameter ("D-value") of the polyp. The latter may be estimated using an open biopsy forceps (with known diameter of maximum width), or an open polypectomy snare of known size (for example, 1, 2, or 3 cm).
 - 1.3. Determine the number of duodenal folds involved by each lesion.
 - 1.4. Locate the ampulla with a forward viewing endoscope with transparent cap, or with side-viewing endoscope, according to local standard of care. Assess relationship of the target polyp to the ampulla (must be 1 cm or more away).
 - 1.5. Each polyp to be treated will be recorded by digital image capture and/or digital video-recorded. Digital images should be acquired 2 cm distal and 2 cm proximal to each polyp treated and every 1cm proximal throughout the extent of the polyp, with a minimum of 6 images per polyp treated.
2. MARK: Mark the circumference of the target lesion with a 10 mm margin using APC or a snare tip with soft coagulation current, according to local standard of care. This is optional and usually not necessary because the polyp margins are highly visible.
3. May give IV glucagon 0.5 x 1-2 doses if intestinal peristalsis is excessive and interferes with endoscopic evaluation and/or treatment.

4. TREAT:
 - 4.1. Ablate using the CryoBalloon Focal Ablation System. For each targeted lesion, an ablation of 10 or 12 seconds duration will be performed using either the first or second generation CryoBalloon Focal Ablation System. The System will be used according to the Instructions for use for the product.
 - 4.2. Cryoablation will be initiated on the most distal polyp. Ice patches should have minimal overlap to avoid overtreatment. The ampulla must be avoided.
 - 4.3. Aim the cryogen to treat within the demarcated area, to minimize treatment of normal duodenal mucosa.
 - 4.4. Any subsequent retreatment(s) is to be performed in the same manner for other targeted polyps. No more than 4 polyps can be treated in any patient at baseline and follow-up
 - 4.5. After the first set of ablations, inspection for post-cryoablation mucosal color change (hyperemia) will be performed after at least three (3) minutes to assess for untreated or “skipped” areas. Treatment of skipped areas (mucosa without red color change) with 5 seconds (for areas <5 mm) or 10 or 12 seconds (for areas >5 mm) will be performed.
 - 4.6. Each ablation will be recorded by digital image capture and/or digital video-recorded. After completing ablation treatment, digital images should be acquired 2 cm distal and proximal to each polyp treated and every 1cm proximal throughout the extent of the polyp. These images and/or digital video-recordings may be used as part of this research study.
5. TATTOO: After ablation, delineate the proximal/distal extend of the treated zone by injecting GI Spot sterile tattoo marker into the submucosa 2 cm proximal and 2 cm distal to the treated polyps to allow future localization of the polyp site(s) and biopsy, similar to standard of care for tattoo of colon polyps. “If multiple polyps are treated, the tattoo should be placed proximal to the most proximal polyp, and distal to the most distal polyp treated to delineate the entire treated area in the duodenum and minimize multiple tattoo injections.”
6. DOCUMENT: Post procedure: on the Duodenal polyp map, draw each polyp treated on a study map, in relation to the ampulla and location in the wall (inferior, superior, medial, lateral) or clock face orientation. Indicate if the polyp was treated (green) or not (yellow), as described in the Investigator’s meetings. Indicate whether the ampulla was covered by the inflated CryoBalloon during any of the applied ablations. This will be part of the procedure documentation.
- 7.

5.2.3 Device Failure

In the event the procedure must be discontinued due to an adverse device effect or CryoBalloon Ablation System malfunction, it will be considered a Technical (Device) failure.

If there is a CryoBalloon Ablation System malfunction but the investigator is able to complete treatment using a second CryoBalloon System, this will be reported as a Device Malfunction.

If the procedure must be discontinued due to change in patient’s condition or any other factor that may impact patient safety, it will be considered a Treatment (Procedure) failure.

The evaluation of the System from the treatment or technical failure may provide valuable information concerning System safety and performance. Therefore, the System must be returned to the Manufacturer, C2 Therapeutics, Inc.

CryoBalloon™ Focal Ablation System

5.2.4 Disposal of Device

All used first generation CryoBalloon Ablation Systems can be returned to C2 Therapeutics for evaluation. Second generation CryoBalloon systems do not need to be returned.

5.2.5 Post-Procedure through Discharge

During the immediate recovery period, subjects will be observed for any adverse events. Subjects will complete the Likert Scale for pain in the area of the ablation treatment, and their dysphagia score will be assessed. Analgesics will be administered as needed for pain according to the institution standard of care.

At discharge, analgesics will be prescribed as per the investigator's standard practice. Subjects will be instructed to contact the investigator in the event of significant pain, abdominal discomfort, nausea or vomiting, or difficulty swallowing. Subjects will be provided with standard of care discharge instructions in accordance with the investigator's standard of care.

All subjects will take anti acid therapy such as once or twice a day proton pump inhibitors until the next visit, to optimize conditions for healing, according to local standard of care. Additional medications may be prescribed at the discretion of the physician as indicated to achieve optimal acid control.

5.2.6 Follow-up after CbFAS Treatment (1 day, 7 days, and 30 days)

Study site personnel will contact subjects (by telephone, e-mail or in person, as appropriate) at day 1 (+2 days if Friday procedure), day 7 +/- 7 days, and day 30 +/- 14 days post-procedure to inquire on any adverse events and use of analgesics since discharge. Subjects will be asked to indicate the level of pain they have on a Likert scale from 0 (no pain) -10 (worse pain ever) and to report if they are tolerating a regular diet.

5.3 3 Month Visit

At each visit, subjects will be assessed for any adverse events that may have occurred since the last follow-up.

5.3.7 Endoscopy

All subjects will return for a high-resolution EGD with WLE and NBI or other standard of care enhanced imaging at 3 months (+/-8 weeks). Digital imaging will be performed to evaluate for any residual adenomatous tissue. Standard endoscopic imaging and video recording will be performed similar to baseline. Investigators should begin video recording and photo-documentation from D3 and pull back proximally to D1. If treatment is performed at the visit, repeat video-recording and photo-documentation

5.3.8 Visible Polyp

If any residual polyp tissue is visible during the endoscopy, then repeat cryoablation will be performed using CbFAS (as described in section CbFAS Treatment 5.2.2). Biopsy of the residual polyp tissue is optional, and at the discretion of the physician. Additionally, subjects will have 1 day follow-up assessments performed by phone call following CbFAS (as described in section 5.2.6).

CryoBalloon™ Focal Ablation System**5.3.9 No Visible Polyp**

If there is no visible polyp, then at least 4 biopsies will be obtained from the treated polyp area delineated by the baseline tattoo.

All biopsies are taken as part of the subject's routine clinical care and will be processed and read by local pathologist.

Subjects will return in 3 months (+/-8 weeks) for the 6-month visit to either confirm CE of the polyp(s) treated, or receive additional treatment if CE was not achieved.

5.4 6 Month Visit

At each visit, subjects will be assessed for any adverse events that may have occurred since the last follow-up.

5.4.10 Endoscopy

All subjects will return for a high-resolution EGD with WLE and NBI or other standard of care enhanced imaging at 6 months (+/-8weeks). Digital imaging will be performed to evaluate for any residual adenomatous tissue. Standard endoscopic imaging and video recording will be performed similar to baseline. Investigators should begin video recording and photo-documentation from D3 and pull back proximally to D1. If treatment is performed at the visit, repeat video-recording and photo-documentation.

5.4.11 Visible Polyp

If any residual polyp tissue is visible during the endoscopy, then repeat cryoablation will be performed using CbFAS (as described in section CbFAS Treatment 5.2.2). Biopsy of the residual polyp tissue is optional, and at the discretion of the physician. Additionally, subjects will have 1 day follow-up assessments performed by phone call following CbFAS (as described in section 5.2.6).

Subjects will return in 3 months (+/- 4 weeks) for the 9 month visit.

5.4.12 No Visible Polyp

If there is no visible polyp, then at least 4 biopsies will be obtained from the treated polyp area delineated by the baseline tattoo.

All biopsies are taken as part of the subject's routine clinical care and will be processed and read by local pathologist.

If pathology is clear (CE confirmed) and the subject has had 2 consecutive clear pathologies, then subjects will return in 3 months for the 9 month visit (if baseline polyp pathology included HGD), or 6 months for the 12 month visit (if baseline pathology was LGD).

If pathology is not clear, or if this is the first clear pathology, then subjects will return in 3 months for the 9 month visit to receive additional treatment or to obtain confirmatory biopsies for CEIM and CED.

5.5 9 Month Visit (optional, only if there is residual polyp tissue and treatment is planned)

At each visit, subjects will be assessed for any adverse events that may have occurred since the last follow-up.

5.5.13 Endoscopy

Subjects may return for a high-resolution EGD with WLE and NBI or other standard of care enhanced imaging at 9 months (+/-4 weeks).

5.5.14 No Visible Polyp

If there is no visible polyp, then at least 4 biopsies will be obtained from the treated polyp area delineated by the baseline tattoo.

All biopsies are taken as part of the subject's routine clinical care and will be processed and read by local pathologist.

If pathology is clear (CE), and the subject has had 2 consecutive clear pathologies, then subjects may return in 3 months for the 12 month visit (if baseline pathology was HGD), or 6 months for a 15 month visit scheduled as close to 12 months as allowable per SOC (if baseline pathology was LGD).

If pathology is not clear, or if this is the first clear pathology, then subjects may return in 3 months for the 9 month visit to receive additional treatment or to obtain confirmatory biopsies for CE.

5.6 12 or 15 Month Visit

At each visit, subjects will be assessed for any adverse events that may have occurred since the last follow-up.

5.6.15 Endoscopy

Subjects will receive high-resolution EGD with WLE and NBI or other standard of care enhanced imaging at 12 months (+/-12 weeks). Photodocumentation and video recording will be performed similar to baseline visit. Investigators should begin video recording and photo-documentation from D3 and pull back proximally to D1. If treatment is performed at the visit, repeat video-recording and photo-documentation

5.6.16 Visible Polyp

If polyp tissue is still present at 12 months, then participation in the study is complete. Treatment can be performed as necessary and at the discretion of the physician.

5.6.17 No Visible Polyp

If there is no visible polyp, then at least 4 biopsies will be obtained from the treated polyp area delineated by the baseline tattoo.

All biopsies are taken as part of the subject's routine clinical care and will be processed and read by local pathologist.

Study participation is complete following completion of the 12 or 15-month visit.

5.7 Study Exit

Subjects may be followed in standard clinical care for duodenal adenoma surveillance or recurrent for duration of study, planned to end 30 June 2024. . Subjects previously enrolled and exited, may be reconsented and re-enrolled for study duration, then exited from the study.

6. STATISTICAL METHODS

Sample size: Up to 50 evaluable patients (estimate 1-4 patients enrolled per site per year x 6 sites), based on highly selected patient population and pilot study design. Since patients may be excluded from the study due to baseline polyp characteristics, up to 500 patients may be consented and screened for patient and polyp eligibility.

6.1 Primary outcomes:

The primary outcomes for this study are efficacy and safety of the CryoBalloon Ablation System for the treatment of nonpolypoid sporadic and familial duodenal adenomas.

Efficacy will be reported as:

The percentage of all treated subjects who have endoscopic and histologic complete eradication of all adenomas treated (CE)

Complete eradication or CE- stratified by type of polyp (sporadic versus familial), and size (< 3 and > 3 cm) will also be reported as an efficacy outcome.

Safety will be evaluated by the incidence of CryoBalloon-related serious adverse events.

6.2 Secondary Outcomes

Technical failure will be defined as failure to complete treatment (treat all targeted areas), and will be analyzed by procedure, and by patient.

Progression rate will be assessed as the percentage of subjects with progression of dysplasia from LGD to HGD or cancer, or progression of HGD to cancer at 12 months

Time to complete eradication of the duodenal adenoma(s) treated will be described as the time to progression and recurrence (survival curve analysis)

Risk factors (endoscopic, demographic, histologic) associated with failure to respond to CryoBalloon ablation will be identified.

Median and mean number of CryoBalloon ablation treatments required to achieve CE-D and CE-IM by 12 months

Incidence of all treatment-related and all serious, non-treatment-related adverse events

Post-procedure chest discomfort in the area of the cryoablation treatment (scored on a 0-10 Likert pain scale): proportion of subjects requiring narcotic analgesic; mean and median pain scores immediately post-procedure, at Day 1 and Day 7 post-procedure.

Recurrence rate and time to recurrence of duodenal adenomas for study duration.

7. DATA AND SAFETY MONITORING BOARD; CLINICAL TRIALS REGISTRATION

This study will be conducted as a small multicenter prospective cohort study, with treatment and follow up procedures to be performed according to local standard of care. A DSMB charter was established and will have the following responsibilities:

- The DSMB will review the serious adverse events reported at quarterly meetings. It will recommend the study be stopped at any time if the study-related serious adverse event rate of > 3% is observed.
- Review and evaluate ad hoc safety issues concerning the study at the request of the PI, IRB of any institution participating in the study and
- Make recommendations to the PI, investigators or to IRB of any institution participating in the study concerning continuation, termination, or other modifications of the study based on the observed beneficial or adverse effects of the study; and
- Operate according to the procedures described in this charter and all procedures of the DSMB.

The DSMB will routinely meet each quarter but all SAEs are to be reviewed monthly or on an ad-hoc basis.

A three-person independent panel (scientific advisory board) will assist in reviewing any safety concerns or protocol issues. The advisory board will consist of:

1. Olaya Brewer, MD, Clinical Associate, Johns Hopkins Medicine, Baltimore, MD
2. Eun Ji Shin, MD, PhD, Associate Professor, Johns Hopkins University School of Medicine, Baltimore, MD
3. Kerry Dunbar, MD, PhD – Associate Professor, UT Southwestern, Section Chief, Dallas VA Medical Center, Dallas Texas (email: Kerry.Dunbar@va.gov)

The primary charge of the DSMB is to monitor the study for participant safety. The safety and related data the DSMB will review includes:

- Number of enrolled patients
- All serious adverse events, including those related to standard-of-care testing and treatments, will be reported to the coordinating center by the study coordinators at each center. These adverse events include GI bleeding, perforation, sepsis, and death.
- Any other safety-supporting data requested by the DSMB

Registration on the NIH clinicaltrials.gov website has been completed (NCT03847636).

Stopping Criteria

The study will be stopped if any of the conditions below are met:

1. If the perforation meets or exceeds that for the alternative therapy for duodenal adenomas (endoscopic mucosal resection) or 5%,
2. If the bleeding rate meets or exceeds that for the alternative resection therapy (endoscopic mucosal resection), or 30%
3. If the overall adverse event rate meets or exceeds that for the alternative therapy (endoscopic mucosal resection), or 26%

8. RISK AND BENEFIT ASSESSMENT

8.1 Potential Risks

In this study, endoscopy is performed under local anesthesia with sedation in accordance with the standards at the institution. The risks of participating in this study are those associated with local anesthesia with sedation (allergic reaction to medications used, bruising or pain at the intravenous access site) and the passage of an endoscope (dysphagia, sore throat, pain).

Complications specific to the CryoBalloon Ablation System may include perforation of or laceration to the duodenal wall, or duodenal strictures in treated areas.

8.1.1 Potential Risks to Subject Confidentiality

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 CRFs or other study-related documentation to be provided to the Lead Investigator.

8.2 Minimization of Risk

All efforts will be made to minimize risk to subjects who participate in this clinical study.

Physicians selected to participate in this study will be experienced and skilled in endoscopic procedures; The protocol clearly defines 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.

Physicians and staff will receive appropriate training prior to using the CryoBalloon™ Ablation System. Each investigator is required to have experience treating a minimum of ten (10) procedures with the CryoBalloon Focal Ablation System prior to enrolling to the study cohort. Five of the 10 procedures must have been supported or supervised by Pentax clinical team, PI (M. Canto), or user with > 25 case experience.

Patients will be closely monitored during the procedure and at regularly scheduled intervals for the duration of the study.

Risks related to the System are minimized by:

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 on the System and the results were determined acceptable.

Pre-clinical evaluation including bench testing, analytical testing and animal studies.

The CryoBalloon Focal Ablation System which will be used in this study is 510k-cleared in the United States (K131523).

8.3 Potential Benefits

Although no assurances or guarantees can be made that there is a direct benefit of study participation, there is a reasonable expectation that treatment with the CryoBalloon Ablation System may be beneficial to the subject. At least one published study in a patient with a localized duodenal intramucosal carcinoma arising in a polyp and limited clinical experience among the participating investigators suggest that the CryoBalloon Ablation System may be safe and efficacious for the eradication of benign nonpolypoid duodenal adenomas. Preliminary data from a submitted abstract from a retrospective multicenter cohort study suggests high safety and efficacy (see Appendix below).

In addition, other patients may benefit in the future from what is learned as a result of this study.

8.4 Safety Monitoring

The following measures will be taken to minimize safety risks to the subjects participating in this clinical study:

All adverse events (whether or not device-related) must be reported to the Lead Investigator as soon as possible after knowledge of the event. The physician will describe the adverse event including their opinion of its relationship to the treatment, the Device, or the subject's pre-existing condition.

Any Serious Adverse Event (SAE) or Unanticipated Serious Adverse Device Effect (USADE) must be reported to the Lead Investigator and coordinating center at Johns Hopkins within 24 hours of knowledge of the event.

Previously reported adverse events will be re-evaluated at subsequent follow-up visits to determine if they are continuing or resolved. Copies of relevant documentation (such as procedure reports, physician/nursing notes, diagnostic study results, discharge summary) will be provided to the Lead Investigator and coordinating center for additional evaluation of the adverse event.

The Lead Investigator and coordinating center will provide a written report to all sites in the event of the occurrence of a serious, device-related adverse event.

The investigator is responsible for ensuring that adverse events are reported to their IRB in accordance with the site-specific IRB requirements.

Automated REDCap alerts are set-up to inform the project P.I. and site P.I. about registered serious adverse events.

Annual reports will be prepared by the Lead Investigator and coordinating center and provided to all sites. The annual report will include detailed information on the safety results.

The investigator will submit reports on study progress to the IRB in accordance with the site-specific IRB requirements.

8.5 Data Collection

Data will be collected on electronic secure REDCap data collection software provided by the Lead Investigator, hosted by Johns Hopkins ICTR/ REDCap server, and reported via secure electronic data capture.

9. MONITORING PROCEDURES

No monitoring visits are required.

9.1 Protocol Deviations

Patients are treated according to FDA-approved indication for eradication of unwanted tissue according to device IFU and local standard of care. Protocol deviations are not applicable.

10. CLINICAL EVENTS

10.1 Definitions

10.1.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in a subject that may or may not be considered device-related.¹

All treatment-related and device-related adverse events, regardless of relationship to the device, must be recorded on the case report forms provided. Adverse events that occur during this study should be treated by established standards of care that will protect the life and safety of the subject.

These treatment-related adverse events include: pain score ≥ 8 (using Likert scale 1-10), proportion of patients requiring narcotic analgesics after treatment, endoscopic dilation for duodenal stricture, hospitalization, ER visit, bleeding, and perforation.

10.1.2 Serious Adverse Event²

A Serious Adverse Event (SAE) is any untoward medical occurrence that:

- a. results in death
- b. is life-threatening
- c. requires in-patient hospitalization or prolongation of existing hospitalization
- d. results in persistent or significant disability/incapacity
- e. results in medical or surgical intervention to prevent permanent impairment to body structure or a body function.

¹ ISO 14155:2011(E),3.1

² E6 Good Clinical Practice: Consolidated Guidance. April 1996

10.1.3 Unanticipated Adverse Device Effect³

An Unanticipated Adverse Device Effect (UADE) is defined as any serious adverse effect on health or safety or any life-threatening problem or death cause by, or associated with, a device, if that effect, problem, or death were not previously identified in nature, severity, or degree of incidence in the investigational plan, or any other unanticipated serious problem associated with a device that related to the rights, safety, or welfare of subjects.

10.2 Adverse Event Reporting

The procedure for reporting any adverse event (whether or not device-related) is as follows: Any Serious Adverse Event or USADE must be reported to the Lead Investigator within 24 hours of knowledge of the event. Reporting to the IRB will be done by the site, in accordance with the site-specific IRB requirements.

Copies of all relevant documentation (e.g., procedure reports, physician/nursing notes, discharge summary) will be compiled and provided to the Lead Investigator for additional evaluation of the event.

10.3 Adverse Event Review and Relationship Determination

The Site Investigator will report their assessment of the adverse event with respect to severity and causality.

The Site Investigator will assess all Serious Adverse Events considered device-related for potential regulatory reportability. The following lists the potential event attribution categories:

Device-Related: An adverse event is considered to be device-related when it is reasonable to believe that the event may have been caused by or is related to the investigational device.

Procedure-Related: An adverse event is considered to be procedure-related when it is reasonable to believe that the event is not specific to the investigational device. Other products may have contributed to the occurrence of the event.

Pre-Existing Condition Related: An adverse event is considered to be related to a pre-existing condition when it is reasonable to believe that the event is associated with the subject's pre-existing condition and is not specific to the investigational device or procedure. Pre-existing conditions that are exacerbated after the index procedure and have been determined to not be device-or procedure-related will be categorized as related to a pre-existing condition.

Concomitant Medications: An adverse event that is associated with concomitant medications and is not otherwise specific to the investigational device.

Intercurrent Condition: An adverse event that is believed to be directly associated with a new medical condition/co-morbidity/diagnosis that arises following the index procedure.

Intercurrent Intervention: An adverse event that arises from, or is associated with, an intervention that was performed after the index procedure for reasons other than to address a device- or procedure-related complication.

³ 21CFR 812.3(s)

10.4 Severity of Adverse Events

The intensity of adverse events will be evaluated using the following criteria and will be documented on the case report forms.

- Mild – noticeable to the patient but does not interfere with routine activity
- Moderate – interferes with the patient's routine activity but responds to symptomatic therapy or rest

Severe – significantly limits the patient's ability to perform routine activities despite symptomatic therapy

10.5 Subject Death

Subject death during the clinical study must be reported to the Lead Investigator and Pentax Medical/ C2 Therapeutics within 24 hours of investigator's knowledge of the death. The Adverse Event form must be completed and include a brief description of the relevant details of the death. A copy of the death records, death certificate and an autopsy report (if performed) are to be sent to the Lead Investigator. In addition, subject death must be reported to the IRB in accordance with the site-specific IRB requirements.

10.6 Treatment Failures and Device Observations

All treatment failures and device malfunctions of the CryoBalloon™ Ablation System are required to be documented on a case report form. In the event of a suspected malfunction or device observation, the investigational device will be returned to the Manufacturer, Pentax Medical/ C2 Therapeutics, Inc. for analysis. Instructions for returning the investigational device will be provided by the Lead Investigator.

11. STUDY ADMINISTRATION

11.1 Institutional Review Board (IRB) Approval

The study protocol will be reviewed and approved by the investigator's IRB prior to subject enrollment. Proposed significant changes to the clinical study protocol must be reported to but not necessarily approved by the Lead Investigator prior to implementation. A significant change is one which may increase the risk or present a new risk to a subject, affects the subject's rights or welfare, or which may adversely affect the scientific validity of the Study.

11.2 Amending the Protocol

This protocol is to be followed as approved by the IRB and will only be altered by written amendments. Administrative changes that do not affect the subject benefit/risk ratio (e.g., editorial changes for clarity) may be made without any further approvals.

11.3 Patient Informed Consent

Prospective subjects are required to provide written informed consent before any study-specific screening tests are performed. The investigator must explain details of the nature of the study. Prospective subjects will be given the opportunity to ask questions and will be provided sufficient time for consideration of participation.

Each subject will receive a copy of their signed Informed Consent Form (ICF), and the original will be filed in their study file.

Changes to the ICF by the investigative site require review by the Lead Investigator prior to final approval by the IRB.

11.4 Privacy and Confidentiality

Subject data obtained in connection with this clinical study will be treated as confidential and will be disclosed only as required by law. Subjects will be identified by a number, not by name. The subject Informed Consent Form discloses how subject information is collected and used, and all subjects must consent in writing prior to any study-related treatments.

11.5 Pre-Study Documentation Requirements

Prior to study initiation, the following documents must be provided to the Lead Investigator:

- Signed and dated Investigator Agreement(s)
- A copy of the written IRB approval of the protocol and the ICF noting the protocol number and document versions approved
- Signed and dated *Curriculum Vitae* of the Investigator(s)
- Copy of the Investigator(s)' current medical license(s) or equivalent
- Protocol signature page, signed by the Investigator

11.6 Record Retention

The Investigator will maintain all essential clinical study documents and source documentation, in original format, that support the data collected on the study subjects in compliance with ICH/GCP guidelines. Documents must be retained until at least two (2) years have elapsed since the later of either the date the clinical study is completed or terminated or the records are no longer required to support a regulatory submission. These documents will be retained for a longer period of time by agreement with Lead Investigator or in compliance with other regulatory requirements.

The Investigator will take measures to ensure that these essential documents are not accidentally damaged or destroyed. The Investigator cannot destroy or dispose of clinical study documents without the prior written approval of the Lead Investigator. If for any reason the Investigator withdraws responsibility for maintaining these essential documents, custody must be transferred to an individual who will assume responsibility. The study Lead Investigator must receive written notification of this custodial change.

11.7 Criteria for Terminating the Study

Lead Investigator reserves the right to terminate the study but intends only to exercise this right for valid scientific or administrative reasons and reasons related to protection of subjects. Investigators and the IRB will be notified in writing in the event of termination.

11.8 Lead Investigator Responsibilities

The Lead Investigator (Marcia Irene Canto) is responsible for:

- Selecting qualified investigators and providing them with the information and materials necessary to conduct this study in compliance with the protocol and local applicable regulatory requirements
- Securing compliance with the clinical protocol, investigator agreement, and local regulations
- Obtaining legal agreements from the Investigator and the Institution
- Confirming that IRB review and approval is obtained prior to patient enrollment or conducting protocol-required testing
- Ensuring that the Investigators and the reviewing IRB are promptly notified of significant new information about this clinical study

- Supervising the overall conduct of the study: ensuring proper monitoring of the clinical study and data, analyzing data (including evaluations of adverse events) and preparing study reports

11.9 Investigator Responsibilities

The Investigator is responsible for the following:

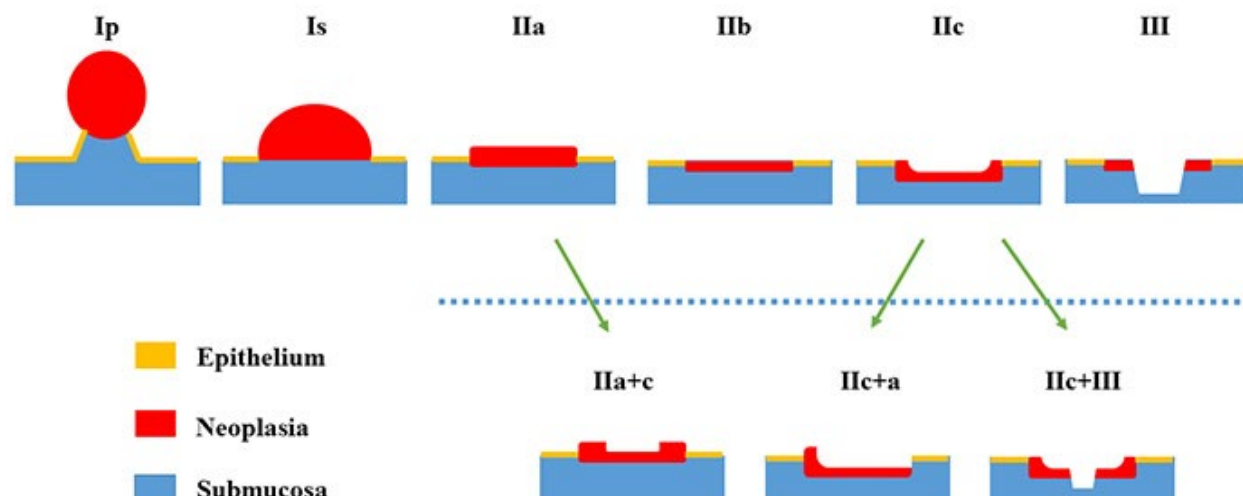
- Compliance with the statements specified in the Investigator Agreement and Certification
- Ensuring sufficient time and resources (including qualified staff and facilities) are available to manage the day-to-day conduct of the study, and ensuring data integrity and the rights, safety and well-being of subjects
- Obtaining approval from the IRB, including subsequent protocol amendments and changes to the ICF and obtaining renewals throughout the duration of the study
- Compliance with the clinical study protocol and applicable regulatory requirements, including but not limited to CFR Title 21: Parts 50, 54, 56; ISO14155: Clinical investigation of medical devices for human subjects – Good Clinical Practice; and World Medical Association Declaration of Helsinki (2008)
- Reporting all serious adverse events (SAEs) to the Lead Investigator and coordinating center as soon as possible after knowledge of the event, and to the IRB as specified in their requirements.

12. PUBLICATION

Authorship of publications will abide by the criteria established by the International Committee of Medical Journal Editors: Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (August 2013). Procedural details including rights of review and timing will be governed by the terms listed and agreed to by both parties in a separate clinical research agreement.

Appendix A

Paris Classification



Appendix B

Spigelman Classification

Variables	1 point	2 points	3 points
Number of polyps	1–4	5–20	>20
Polyp size (mm)	1–4	5–10	>10
Histology	Tubulous	Tubulovillous	Villous
Dysplasia	Mild	Moderate	Severe
Spigelman score	Stage	Surveillance	Risk of adenocarcinoma
0	0	Every 4 years	–
1–4	I	Every 4 years	–
5–6	II	2–3 years	2.3%
7–8	III	1–4 years	2.4%
9–12	IV	Surgery	36%

Note: Data from Spigelman et al.⁷⁴

Abstract below copied from manuscript published in Gastrointestinal Endoscopy (Elsevier)

INITIAL EXPERIENCE OF NITROUS OXIDE CRYOBALLOON ABLATION FOR ERADICATION OF NONPOLYPOID SPORADIC AND FAMILIAL ADENOMATOUS POLYPOSIS (FAP) DUODENAL ADENOMAS

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BACKGROUND AND AIMS: Endoscopic mucosal resection (EMR) is the preferred endoscopic therapy for duodenal adenomas (DA) but is associated with an overall adverse event rate of 26%. Cryotherapy using a CryoBalloon focal ablation system (CbFAS) can safely and effectively eradicate esophageal intestinal metaplasia. We report our first experience with CryoBalloon ablation for treatment of flat DA. **METHODS:** American, multicenter retrospective study involving 5 centers. DA (Paris 0-IIa and 0-IIb) were treated with 5-12 seconds of nitrous oxide using CbFAS. Follow-up EGD was performed at 3-12 months. **RESULTS:** Seventeen DA (mean size: 22.7mm ±14.3; 12 tubular, 5 tubulovillous) from 13 patients (mean age 66.5±9.99 years, 61.5% males) were included in the study. 13/17 (76.5%) of DA had failed prior treatment while 4/17 (23.5%) were treatment-naïve. All procedures were technically successful and had a >50% decrease in size after CryoBalloon ablation. There was no increase in size or progression of disease for any lesions. Overall, treatment was completed in 15/17 patients and recurrence-free survival was achieved in 12/17 (71%) after a median follow up of 15.5 months [IQR 6.8-19.4]. Median cryoablation time per polyp of 4 minutes [IQR 1-7.5] and median total procedure time 25 minutes [IQR 22-30.5]. There were no intra- or post-procedural adverse events. **CONCLUSIONS:** Nitrous oxide CryoBalloon ablation of non-polypoid DA is feasible, with promising safety and efficacy.

Schedule of Study Procedures and Assessment

	Baseline	1 Day call Follow- Up	7 Day Follow- up	30 Day Follow- up	3 Month	3 Month + 1 Day call Follow- Up ⁴	3 month + 7 Day Follow- up	3 month + 30 Day Follow- up	6 Month	6 Month + 1 Day call Follow- Up ⁴	6 Month +7 Day Follow- up	6 Month +30 Day Follow- up	9 Month	9 Month + 1 Day Follow- Up ⁴	9 Month +7 Day Follow- up	9 Month +30 Day Follow- up	12-15 Month SOC surveilla nce thru 5/31/24
		±2 days	±7 days	±14 days	+/- 8 weeks		±2 days	±2 days	+/- 8 weeks	±2 days	±2 days	±2 days	+/- 8 weeks	±2 days	±2 days	±2 days	+/- 12 weeks
Informed Consent	X																X as needed
Physical Exam and Medical History	X																
Likert Scale for Pain ¹	X	X	X	X		X ⁴	X ⁴			X ⁴	X ⁴	X ⁴		X ⁴	X ⁴	X ⁴	
EGD with CbFAS	X																
EGD with CbFAS and/or biopsy					X ³				X ³				X ^{3,5}				X ^{3,6}
Assessment of Adverse Events	X	X	X	X	X	X ⁴	X ⁴	X ⁴	X	X ⁴	X ⁴	X ⁴	X	X ⁴	X ⁴	X ⁴	X ^{3,6}

¹Pain score is assessed post treatment procedure and pre-discharge.

³If duodenal polyp tissue is visible, then CbFAS will be performed. If BE is not visible, then biopsies will be obtained. See section 5.3 for additional information. ⁴Only completed if CbFAS was performed at visit EGD.

⁵ If the duodenal adenoma treated has low grade dysplasia, follow-up at 9 months is optional and according to standard of care.

⁶ SOC surveillance for adenoma recurrence after treatment phase for duration of study.

CryoBalloon™ Focal Ablation System

	Screening/ Baseline/re -consent, as needed	Post- Proc/ pre-D/C	Tel/email follow-up at Day 1, 7, 30 days	12-wks (±8 wks)	24-wks (±8 wks)	36-wks (±8wks)	48-wks (±8 wks)	12-15 mo/SOC EGD thru 5/31/24 (±12wks) ⁶
Informed Consent,	✓							✓
Physical exam & Medical History	✓							
Likert Scale for Pain	✓	✓	✓					
EGD	✓			✓	✓	✓	✓	✓
Biopsy				○ ¹	○ ¹	○ ¹	○ ¹	✓
Assessment for Adverse Events	✓	✓	✓	✓	✓	✓	✓	✓
Ablation	✓			★ ²	★ ²	★ ²	★ ²	✓

¹ Biopsies will be obtained at the follow-up visit according to the physician's discretion, if there is residual polyp tissue present. If there is no visible residual polyp tissue, biopsies will be performed at the treatment site to document eradication.

²If repeat ablation is performed, the procedure and post-procedure assessments (pain Likert scale, telephone follow-up) will be performed as required at the Baseline ablation.

★ Repeat ablation will be performed as indicated by the EGD exam and/or histopathology results. If re-treatment is performed, the subject will return for follow-up at the next 12-week interval.

Appendix C

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